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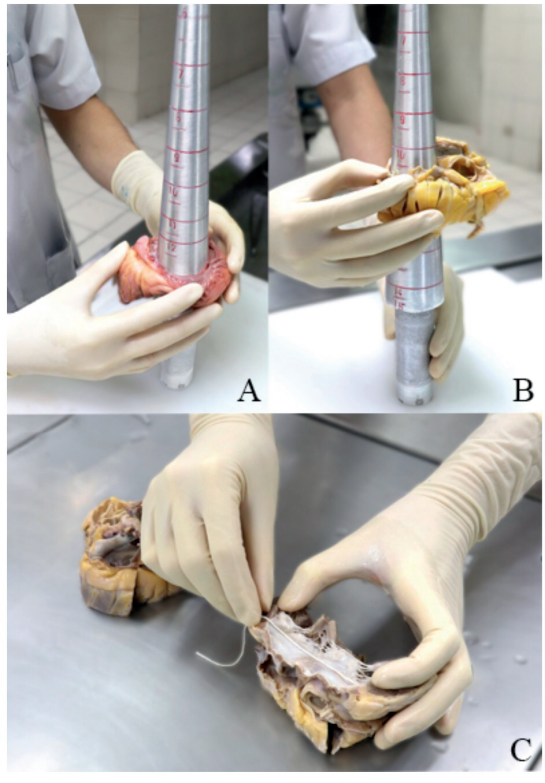

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COVID-19 Associated Mucormycosis in Head and Neck Region: Our Experiences at a Tertiary Care Teaching Hospital of Eastern India

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ABSTRACT

Objective: To study the COVID-19 associated mucormycosis in the head and neck region of the patients along with patient details, clinical manifestations and management.

Materials and Methods: This is a descriptive and retrospective study of COVID-19 associated mucormycosis (CAM) carried out at a postgraduate teaching hospital. This study was conducted between March 2020 to April 2021. A patient profile such as age, sex, comorbidities, clinical presentations, diagnosis and treatment of the CAM were analyzed.

Results: There were 11 patients of CAM were enrolled in this study. There were eight male and three female patients, aged from 3 years to 72 years. Out of the 11 patients, 8 were diabetic (72.72%). Three patients (27.27%) were taking prolonged systemic steroids with a long hospital ICU stay. One child (9.09%) was under chemotherapy for acute leukemia. The common clinical symptoms were facial swelling, facial pain, nasal block and nasal discharge. The diagnosis was confirmed by histological examination and fungal culture with Sabouraud dextrose agar (SDA) showing Rhizopus oryzae. All were treated with endoscopic surgical debridement and amphotericin B. One case died because of cerebral involvement.

Conclusion: Early diagnosis and prompt treatment for CAM are required. Aggressive endoscopic surgical debridement for local control and appropriate systemic antifungal treatment will help to improve the prognosis and survival of the patients.

Keywords: COVID-19; SARS CoV-2; COVID-19 associated mucormycosis; head and neck region; amphotericin B
(Siriraj Med J 2021; 73: 423-428)

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is caused by acute respiratory syndrome coronavirus 2 (SARS CoV-2), which has been considered a global public health emergency.[1] COVID-19 has rapidly spread to 212 countries and made approximately five million laboratory confirmed cases and more than 310,000 deaths worldwide by May 18th 2020.[2] The first case of SARS-CoV-2 infection was detected in Wuhan, China.[3] As this virus is a novel virus, data in relation to clinical manifestations of this COVID-19 disease are insufficient.[4,5] Mucormycosis is an invasive fungal infection caused by

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an opportunistic and ubiquitous fungus that belongs to the class Phygomycetes, subclass Zygomycetes, order mucrorales, family mucroraceae.[6] Histopathological study, direct, microscopy and culture from the clinical samples are the important diagnostic modalities for mucormycosis.[7] Early diagnosis and treatment with endoscopic surgical debridement are key for preventing this fatal clinical entity. COVID-19 associated mucormycosis (CAM) is less frequently documented in the literature. The aim of this study is to analyze the detail of patient profile and management of the CAM.

MATERIALS AND METHODS

This descriptive retrospective study was conducted at the otorhinolaryngology postgraduate department of a teaching hospital. This study was conducted between March 2020 to April 2021. Our Institutional Ethics Committee (IEC) accepted this study with the reference number IEC/IMS/SOA/12/08.03.2020. COVID-19 patients infected with mucormycosis during the treatment period at COVID hospital or after discharge from the COVID hospital were included in this study. All of the reverse transcription polymerase (RT-PCR) positive for viral RNA and diagnosed COVID-19 at the time of hospitalization. For RT-PCR testing, the nasopharyngeal swab was used and the sample was taken from nasopharyngeal secretions with wearing personal protective equipment. The COVID-19 patients without mucormycosis or Non-COVID-19 patients with mucormycosis were excluded from this study. All the patients underwent diagnostic nasal endoscopy for assessing the bilateral nasal cavity and nasopharynx. Computed tomography (CT) scan of the nose and paranasal sinus and magnetic resonance imaging (MRI) done to find out the extent of the diseases into orbit and brain. During nasal endoscopy, the tissue from the nasal cavity sent for microscopy, culture and histopathological examination showing broad non-septate hyphae with 900 branchings (Fig 1). Ophthalmological and neurological consultations were done in all cases to find the loss of vision or not and neurological involvement. There were 11 COVID-19 patients with mucormycosis enrolled in this study. Out of 11 patients, 7 was already discharged from COVID hospital attached to our Medical college and the rest 4 were diagnosed during the treatment at COVID hospital. Biopsy was taken from all the cases, which showed the picture of mucormycosis with some foci of non-septate fungal hyphae with right-angled hyphae branches. The diagnosis was based on histopathological examination and fungal culture. The fungal culture was done with SDA showing mycelia growth, features of Rhizopus oryzae. All patients underwent endoscopic debridement of the mucormycosis along with exenteration of the orbit in two cases, followed by parenteral infusion amphotericin B (1-1.5 mg/kg/day) and a total dose of 2.5-3 gm. Patient follow-up was done after 6 months' interval after surgery. SPSS Statistics for Windows, version 20, was used for all statistical analyses (IBM-SPSS Inc., Chicago, IL, USA).

RESULTS

Out of 11 patients with mucormycosis, there were 8 male (72.72%) and 3 female (27.27%) patients with a male to female ratio of 2.6:1. The age range of the patients was from 3 year to 72 years. Out of the 11 patients, 8 (72.72%) were diabetic. All 8 diabetic mellitus patients were under treatment with oral hypoglycemic agents/insulins regularly, but their blood sugar was poorly controlled. One child (9.09%) was diagnosed with acute leukemia and three patients (27.27%) were taking a high dose of steroids during the treatment of the COVID-19 infection. Out of the 11 patients, 6 (54.54%) were diagnosed with sinonasal mucormycosis, 2 (18.18%) had rhino-orbital mucormycosis, 1 (9.09 %) had sinonasal and palatal involvement of the mucormycosis and one had rhino-orbital-cerebral mucormycosis. All the patients presented with foul-smelling nasal discharge and nasal block. Out of the 11 patients, 9 (81.81%) of them were presenting with facial pain, but 6 (54.54%) were presented with orbital and facial swelling (Fig 2A&B). Three (27.27%) patients were presenting with headache, one (9.09%) had proptosis, one had nasal septal perforation and one had altered sensorium. (TABLE 1)

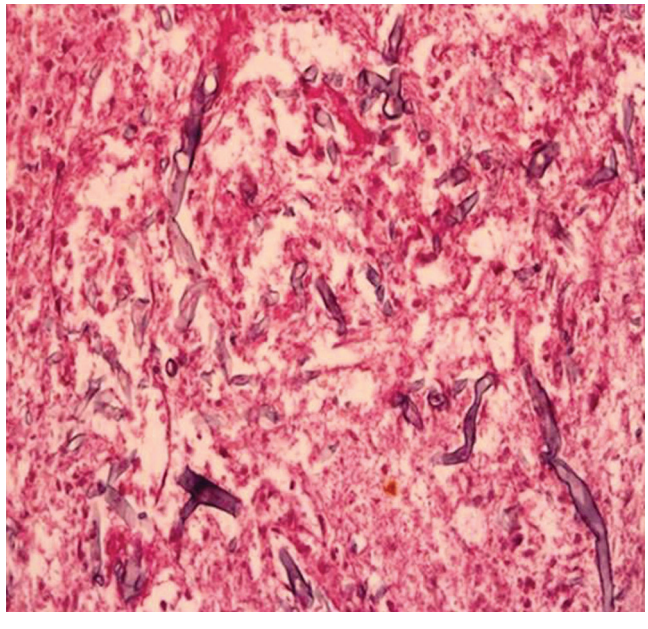

Fig 1. Histopathology microphotograph showing broad non-septate hyphae with 900 branching (Eosin stain and 400 X magnification).

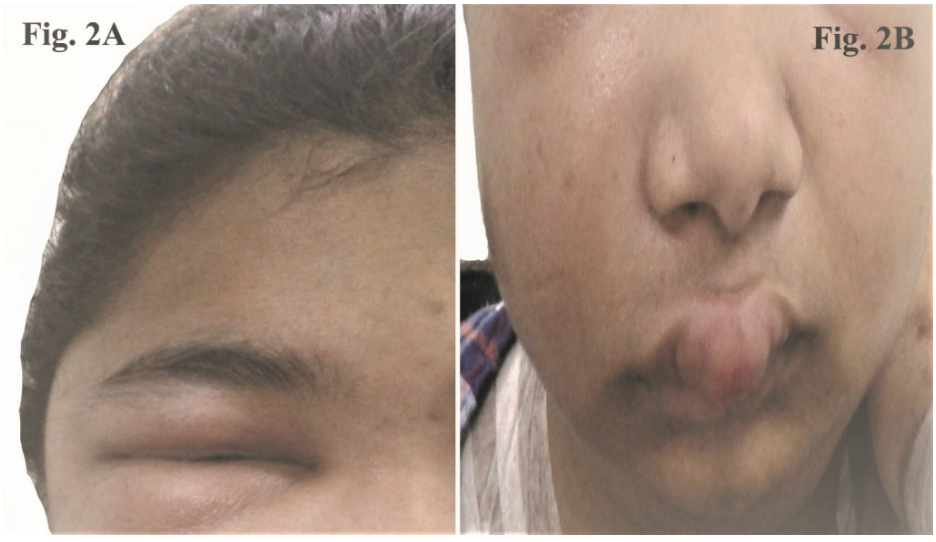

Fig 2. A 14-year-old girl of CAM presenting swelling at the right side orbit (Fig. 2A) and face (Fig. 2B).

Before the surgical debridement, the nasal swab sent for KOH mount where all patients showed aseptate hyphae. Culture of the nasal discharge showed Rhizopus oryzae in nine patients and the rest showed no growth. All cases underwent endoscopic surgical debridement under general anesthesia. All the patients were also administered an intravenous infusion of amphotericin B. One case (9.09%) was fatal due to cerebral involvement who died during the treatment period.

DISCUSSION

The ongoing COVID-19 pandemic started in Wuhan, China, in December 2019 and became a global pandemic because of its rapid spread.[8] The spectrum of clinical presentations of symptomatic COVID-19 patient ranges from mild to critical.[9] COVID-19 patients usually show higher levels of inflammatory cytokines (interleukin (IL)-2R, IL-6, IL-10 and tumor necrosis factor-alpha), impaired cell-mediated immune response, affect both CD4+ T and CD8+ T cells.[10] So, COVID-19 patients have susceptibility towards fungal co-infections such as mucormycosis is found.[11]

Mucormycosis is an uncommon opportunistic fungal infection characterized by infarction and necrosis of the host tissue by the invasion of the blood vessels by hyphae.[12] In the head and neck region of the body, the common clinical manifestations of the mucormycosis are due to rhino-orbital-cerebral infection, which secondary to inhalation of the spores into the nose and sinuses.[13] In this study, out of the 11 patients, 6 (54.54%) were diagnosed with sinonasal mucormycosis, 2 (18.18%) had rhino-orbital mucormycosis, 1 (9.09 %) had sinonasal and palatal involvement of the mucormycosis and one had rhino-orbital-cerebral mucormycosis. The predisposing factors for mucormycosis are diabetes mellitus, systemic corticosteroid use, hematological malignancies, neutropenia, stem cell transplant and immunocompromised persons.[14] The critical ill COVID-19 patients admitted to the intensive care unit (ICU) and required mechanical ventilation or had prolonged duration hospital stays, even as long as 50 days, are likely to get co-fungal infections.[15] In this study, the most comorbidity associated with COVID-19 patient was diabetes mellitus (72.72%). Rest three patients (27.27%) were taking a high dose of systemic steroids for reducing COVID-19 infections and one patient (9.09%) was a known case of acute myeloblastic leukemia.

Mucormycosis is a rapidly progressive fungal infection and often ended in a fatal outcome. Clinical presentations of mucormycosis depend on the site of the disease. The initial clinical symptoms of the CAM are nasal block or congestion, nasal discharge. The color of the nasal discharge appears as bloody or brown or black and facial pain. The patient may present with numbness over paranasal sinuses. Headache and orbital pain are also important features of the CAM. Many patients of CAM may present with fever, toothache, loosening of the maxillary teeth, blurring of vision or double vision. In this study, all the patients were presenting with foul-smelling nasal discharge and nasal block. Out of the 11 patients of this study, 9 (81.81%) of them were presenting with facial pain and 6 (54.54%) were presenting with facial swelling. Three (27.27%) patients were presenting with headache, one (9.09%) had proptosis, one had nasal septal perforation and one had altered sensorium in our study. Diagnostic nasal endoscopy shows black and necrotic tissue (eschar) inside the nasal cavity.

TABLE 1. Clinical profile of the COVID-19 associated mucormycosis patients.

Patient's serial	Age (years)	Sex	Affected part	Clinical presentations	Co-morbid diseases	Treatment	Outcome
1	3	M	sinonasal	Facial swelling, facial pain, nasal discharge	Acute lymphoblastic leukemia	Endoscopic surgical debridement plus amphotericin B	Cured
2	14	F	Naso-orbital	Facial pain, nasal block, nasal discharge, facial swelling, nasal septal perforation	Prolonged use of steroids	Endoscopic surgical debridement plus amphotericin B	Cured
3	37	M	Sinonasal	Facial pain, nasal block, nasal discharge	Uncontrolled Diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
4	38	M	Oronasal	Facial pain, palatal black eschar, nasal discharge, nasal block	Uncontrolled diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
5	41	F	Sinonasal	Facial pain, nasal discharge, nasal block	Uncontrolled diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
6	53	F	Naso-orbital-cerebral	Facial swelling, headache, altered sensorium, proptosis, nasal discharge, nasal block	Prolonged use of steroids	Endoscopic surgical debridement plus amphotericin B	Death due to rapid spread to brain
7	62	M	Naso-orbital	Headache, orbital pain, nasal discharge, nasal block	Uncontrolled diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
8	63	M	Sinonasal	Facial swelling, facial pain, nasal discharge, nasal block	Uncontrolled diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
9	65	M	Naso-orbital	Facial swelling, facial pain, proptosis, nasal discharge, nasal block	Uncontrolled diabetes	Endoscopic surgical debridement plus amphotericin B	Cured
10	68	M	Sinonasal	Facial swelling, numbness over face, nasal discharge	Uncontrolled diabetes mellitus	Endoscopic surgical debridement plus amphotericin B	Cured
11	72	M	Sinonasal	Headache,Numbness over face,nasal discharge	Uncontrolled diabetes mellitus and taken steroids	Endoscopic surgical debridement plus amphotericin B	Cured

Rhino-orbital-cerebral infection is a typical presentation of mucormycosis where fungi invade the paranasal sinuses to orbit and brain.[16] This clinical situation can result in orbital apex syndrome such as complete ophthalmoplegia with rapid loss of vision, involves cranial nerves such as II, III, IV, V and VI, which need urgent treatment with surgical intervention, antifungal drugs and control of risk factors for preventing such morbidity and fatal outcome.[17,18] Clinical suspicion and early diagnosis and prompt treatment are key steps for preventing the morbidity of the fatal condition like rhino-orbital-cerebral mucormycosis.[19] Proper history taking, physical examination and imaging are important components for the diagnosis of the suspected mucormycosis. In CAM, computed tomography (CT) scan will often show bone destruction. Brain magnetic resonance imaging (MRI) is helpful to rule out any involvement of the brain, sinuses and orbit.[20] An MRI of the brain may show multiple areas of infarction and ischemia, indicating invasive fungal disease. In case of unstable hemodynamic and poor respiratory status with the inability to keep the patient in a supine position without oxygen, desaturation made it unfeasible for performing MRI. Bedside diagnostic nasal endoscopy can be done in a timely manner and histopathological processing in case of active COVID-19 infection is useful for starting the treatment for rhino-orbital mucormycosis. Mucor is usually demonstrated via a nasal biopsy and subsequent culture. Tissue is sent for histopathological examination and KOH mount, which confirm the mucormycosis.[21] Direct microscopy, histopathology and culture from the clinical samples are important diagnostic modalities for mucormycosis.[22]

To avoid morbidity in this lethal clinical entity, clinical suspicion and early therapy, as well as endoscopic surgical debridement, is essential. The treatment of the CAM requires a team approach which includes otorhinolaryngologists, neurologist, ophthalmologist, dentist, microbiologist and infection disease specialist. The patient needs control of diabetes and diabetic ketoacidosis. The immunomodulating drugs, if they continue, should be stopped. Endoscopic surgical debridement should be done immediately after confirmation of the CAM. Then amphotericin should be started without delay. Liposomal amphotericin B (L-Amb) is a preferred medical treatment. The dose of the L-Amb is 5 mg/kg/day, diluted in 200 ml 5% dextrose over 2 to 3 hours infusion (avoid slow escalation; higher dose 10 mg/kg/day may be given in cerebral mucormycosis).[23] Amphotericin B deoxycholate (D-Amb) can be given if the cost and availability of L-Amb is an issue. D-Amb is given as 1 mg/kg/day in 5% dextrose, slow infusion for 6 to 8 hours. Premedication may be needed to avoid infusion reaction. Renal function and potassium levels should be monitored while treating amphotericin B. Patients who are intolerant to amphotericin B, alternative antifungals such as posaconazole or isavuconazole (injection/tablets) can be started. The dose of posaconazole is Tab. Posaconazole 300 mg twice daily a day on the first day followed by 300 mg once a day. The dose of the isavuconazole is 200 mg three times a day for two days, followed by 200 mg once a day.[24] The patients should be monitored clinically and radiologically for the response of the treatment or disease progression. After 3 to 6 weeks of amphotericin B therapy, consolidation therapy (posaconazole/isavuconazole) for 3 to 6 weeks. In this study, all had undergone radical debridement of the mucormycosis along with orbital exenteration in two cases, followed by parenteral infusion of amphotericin B (1-1.5 mg/kg/day) and a total dose of 2.5-3 gm.

Poorly controlled diabetes mellitus is a major issue while managing the CAM, so good glycemic control should be done during the management of COVID-19 patients. Systemic corticosteroids should be used only in case of hypoxemia.[24] Oral steroids should be avoided in patients with normal oxygen saturation on room air. If systemic corticosteroid is used, blood glucose should be monitored. The dose and duration of the corticosteroid treatment should be limited to dexamethasone (0.1 mg/kg/day) for 5 to 10 days. Patients should be advised to use a face mask for reducing the Mucorales. During discharge of the COVID-19 patients, the patient should be advised about the early symptoms or signs of mucormycosis such as facial swelling, facial pain, nasal blockage and excessive discharge, loosening of tooth, chest pain and respiratory insufficiency. The worldwide case fatality rate of mucormycosis is approximately 46%.[25] The diagnosis of mucormycosis is often difficult. However, the early diagnosis and prompt treatment are always important and late or even six days is associated with doubling of mortality rate from 35% to 66%.[25] A high suspicion of mucormycosis is considered in immunocompromised patients. In the case of a high-risk person, the diagnosis of mucormycosis can be anticipated if there is associated with one side facial swelling, pain over the face, orbital swelling or proptosis. The late sign is tissue necrosis which acts as a hallmark for mucormycosis, which occur due to vascular invasion and thrombosis.[26] If the diagnosis is confirmed, prompt surgical opinion is required, followed by antifungal agents. Early diagnosis and prompt treatment are necessary for the improvement of the outcome of mucormycosis in COVID-19 patients.

CONCLUSION

Mucormycosis is a dreaded fungal disease resulting in angio-invasion by the hyphae leading to thrombosis and necrosis of the host tissue. Patients with diabetes mellitus or taking systemic steroids or under any immunosuppressive medication with COVID-19 are at greater susceptibility for mucormycosis. In a COVID-19 patient, the severity of the mucormycosis is due to its rapid progression and angio-invasive nature. The clinician should act promptly to identify the mucormycosis, particularly in immunocompromised patients or poorly controlled diabetes mellitus. The widely accepted treatment for mucormycosis is amphotericin B, along with surgical debridement. The rising of mucormycosis or black fungus in COVID-19 patients can be managed effectively if identified early with adequate treatment with amphotericin B, surgical debridement and controlling of the associated risk factors.

Study limitation

This study has a rather small sample size and may restrict the outcome of the aforementioned interpretation. However, the conclusion of this study will undoubtedly inspire the future research effort in this catastrophic clinical entity called COVID-19 associated mucormycosis.

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Intervention Enhancing Medication Adherence in Stroke Patients: An Integrative Review

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ABSTRACT

Objective: This review aimed to systematically identify and analyze randomized controlled trials (RCTs) reported in the literature that were related to interventions targeted at enhancing medication adherence in stroke patients.

Materials and Methods: The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) system was applied to present the process flow, including study identification, screening, exclusion, and inclusion. The PubMed electronic database was searched, and the reference lists of relevant studies were reviewed from 2015 until 2020 to identify relevant RCTs.

Results: The results identified nine relevant RCTs, which included a medication-taking reminder mobile application (Medisafe), health empowerment interventions, telehealth education, and motivational interviews as the medication adherence enhancement interventions that have been most often used in the past five years. Furthermore, these RCTs mainly aimed to improve patients' medication adherence, physical activity, and clinical outcome, such as blood pressure and high-density lipoprotein cholesterol.

Conclusion: This integrative review has implications for the heightened recognition of the necessity for interventions aimed at enhancing patients' adherence to their medication, and that could be applied in clinical practice.

Keywords: Integrative Review; Medication Adherence Intervention; Stroke; Nurse (Siriraj Med J 2021; 73: 429-444)

INTRODUCTION

Stroke is a leading cause of mortality and disability globally.[1] In 2016, there were 5.5 million deaths and 116.4 million Disability-Adjusted Life Years (DALYs) lost due to stroke. Although age-standardized mortality rates have significantly fallen from 1990 to 2016, the stroke burden remains high.[2] Patients with a history of stroke have a risk of recurrence, ranging from 1.8% within one month to 43% within 5 years.[1] The mortality rate in the beginning stage of recurrent stroke is 56.2%, which is higher than for the first stroke.[1] Hence, critical improvements in the secondary prevention of stroke are required to decrease these risks, and there is a particular need to reduce the severity and mortality from stroke.[3,4] Adherence to stroke medication is considered critical to preventing the recurrence of stroke[5], but is often sub-optimal in many stroke survivors. Antihypertensive therapy, cholesterol reduction with statins, antiplatelet agents, or the treatment of atrial fibrillation with oral anticoagulants are all instances of evidence-based secondary stroke prevention medication.[6] Previous research has stated that management in combination with preventive medication could reduce the recurrence of ischemic events by about 75%.[7,8] However, approximately 50% of chronic disease patients do not adhere to their medical therapy[9,10], and this lack of adherence to medication

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dramatically impacts their health outcomes.[11] In the United States, general medication nonadherence is estimated to be responsible for roughly 125,000 deaths per year, at least 10% of hospitalizations, 23% of nursing-home admissions[11], and a substantial increase in morbidity and mortality.[12-14] For stroke patients, a previous study showed that about one-third of stroke survivors are considered to be nonadherent to their medication.[15]

Various interventions have been proposed and implemented to enhance patient adherence to medication, such as behavior therapy and the dissemination of information materials related to the importance of medication adherence.[16-20] Moreover, a previous study suggested that utilizing functional interventions in daily practice and management for both professionals and patients could be most promising for facilitating greater medication adherence.[21] Nevertheless, research, including stroke patients' preferences for medication adherence intervention, is profoundly lacking. A literature review was conducted on this subject, but we found no reported studies in which stroke patients were participants among the five meta-analyses and systematic reviews that we found and that covered 217 innovative studies.[9,11,14,18] Therefore, it was decided to perform an integrative review to identify and analyze medication adherence interventions in stroke patients. Specifically, it was decided to concentrate on summarizing these studies and the effect of different medication adherence interventions on the medication adherence of stroke patients. The following questions were identified to drive this integrative review:

1) What kinds of interventions and theoretical frameworks used for medication adherence interventions in stroke patients are reported across studies?

2) What effects do the different types of interventions have on the medication adherence of stroke patients?

MATERIALS AND METHODS

Identification of relevant studies

In this review, the PubMed electronic database was searched, and the reference lists in relevant studies were reviewed to identify randomized controlled trials (RCTs) reporting interventions for enhancing medication adherence in stroke patients. The following combined search terms were utilized: (***Stroke OR cerebrovasc* disorders OR cerebrovasc* disease OR cerebrovasc* accident OR brain isch?emi* OR isch?emi* cerebral attack OR brain attack OR intracranial h?emorrhage* OR CVA) AND (Medication Adherence OR Medication Nonadherence OR Medication Noncompliance OR Medication Persistence OR Medication Compliance OR Medication Non-Compliance)***. The detailed search strategy is shown in Table 1. The search phrases were used according to the fundamental guidelines of the database. Moreover, the authors reviewed the reference lists of the relevant literature, and one additional article was identified. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)[22] system was applied for the process flow, including the identification, screening, exclusion, and inclusion of the literature studies (Fig 1). The inclusion criteria, as well as exclusion criteria for eligible studies, are shown in Table 2.

Study selection

First, the researcher screened the titles and abstracts of the qualified studies. Subsequently, the full text was also assessed to decide whether or not it was relevant to the present study. Inclusion criteria were implemented to guarantee that only studies considered relevant to the study objective were included. Similarly, exclusion criteria were utilized to screen out literature not affiliated with a review (Table 2). A literature review paper matrix was designed (Supplementary Table 1), which included the following data for each study: references, countries, and duration of intervention, target population, sample size, problem and purpose, theoretical framework, intervention details, medication adherence measures (reliability, validity), methodological problems, key findings, and implications.

RESULTS

Search results and description of the studies

Fig 1 shows that 25 references were classified throughout the initial search (one was included through a list of references searched because of the study's relevance[23]), of which 17 articles were excluded in the title and abstracts screening phase by following the inclusion and exclusion criteria (Table 2), leaving 9 articles that qualified for the full-text screening.

Table 3 shows the included RCTs, which were published between 2015 and 2018 and were conducted in 4 countries, namely China (n = 3), United States (n = 3), Pakistan (n = 2), and New Zealand (n = 1). The research duration reported varied across the studies (from the enrollment to the final assessment of one participant), whereby 4 studies were performed over 3 months, 3 studies had a duration of between 3 months and 6 months, and 2 RCTs involved studies over more than 6 months. The target populations in the included studies were individuals with stroke, including ischemic stroke (n = 5), non-specified subtypes of stroke (n = 3), hemorrhagic stroke (n = 1), transient ischemic attack (n = 1), and hypertensive patients (n = 1), which were

TABLE 1. Search strategies.

Database	Search	Search String
PubMed	1	(((((((("Stroke"[MeSH Terms] OR "Stroke"[Text Word] OR "cerebrovascular"[All Fields]) AND ("disease"[MeSH Terms] OR "disorders"[Text Word])) OR "cerebrovascular"[All Fields]) AND ("disease"[MeSH Terms] OR "disease"[Text Word])) OR "cerebrovascular"[All Fields]) AND ("accidents"[MeSH Terms] OR "accident"[Text Word])) OR ("brain"[MeSH Terms] OR "brain"[Text Word])) AND (("cerebrum"[MeSH Terms] OR "brain"[MeSH Terms] OR "cerebral"[Text Word]) AND ("attack"[All Fields] OR "attacked"[All Fields] OR "attacker"[All Fields] OR "attacker s"[All Fields] OR "attackers"[All Fields] OR "attacking"[All Fields] OR "attacks"[All Fields]))) OR "Stroke"[MeSH Terms] OR ("intracranial"[All Fields] OR "intracranially"[All Fields]) OR ("Stroke"[MeSH Terms] OR "CVA"[Text Word])
	2	("medication adherence"[MeSH Terms] OR Medication Adherence[Text Word]) OR ("medication adherence"[MeSH Terms] OR Medication Nonadherence[Text Word]) OR ("medication adherence"[MeSH Terms] OR Medication Noncompliance[Text Word]) OR ("medication adherence"[MeSH Terms] OR Medication Persistence[Text Word]) OR ("medication adherence"[MeSH Terms] OR Medication Compliance[Text Word]) OR ("medication adherence"[MeSH Terms] OR Medication Non-Compliance[Text Word])
	3	1 AND 2

TABLE 2. Study criteria.

	Inclusion	Exclusion
1	Adult patients (18 years or older)	Stroke as a complication
2	Diagnosis of stroke (including ischemic stroke, hemorrhagic stroke, or transient ischemic attack)	Studies including children or adolescents under 18 years old, adults living in a nursing home, or the hospital who received the assistance with the medication adherence intervention
3	A randomized controlled trial aimed at improving medication adherence published from January 1, 2015, to December 31, 2020	Conference proceedings, abstracts, protocol, pilot study, and review articles
4	Described in the English language	Targeted only caregivers of stroke patients
5	Included an outcome measure of medication adherence such as adherence to combination therapy guide, elicitation of compliance and adherence behaviors questionnaire, medication adherence rating scale, medication event monitoring system, or patient medication adherence questionnaire	

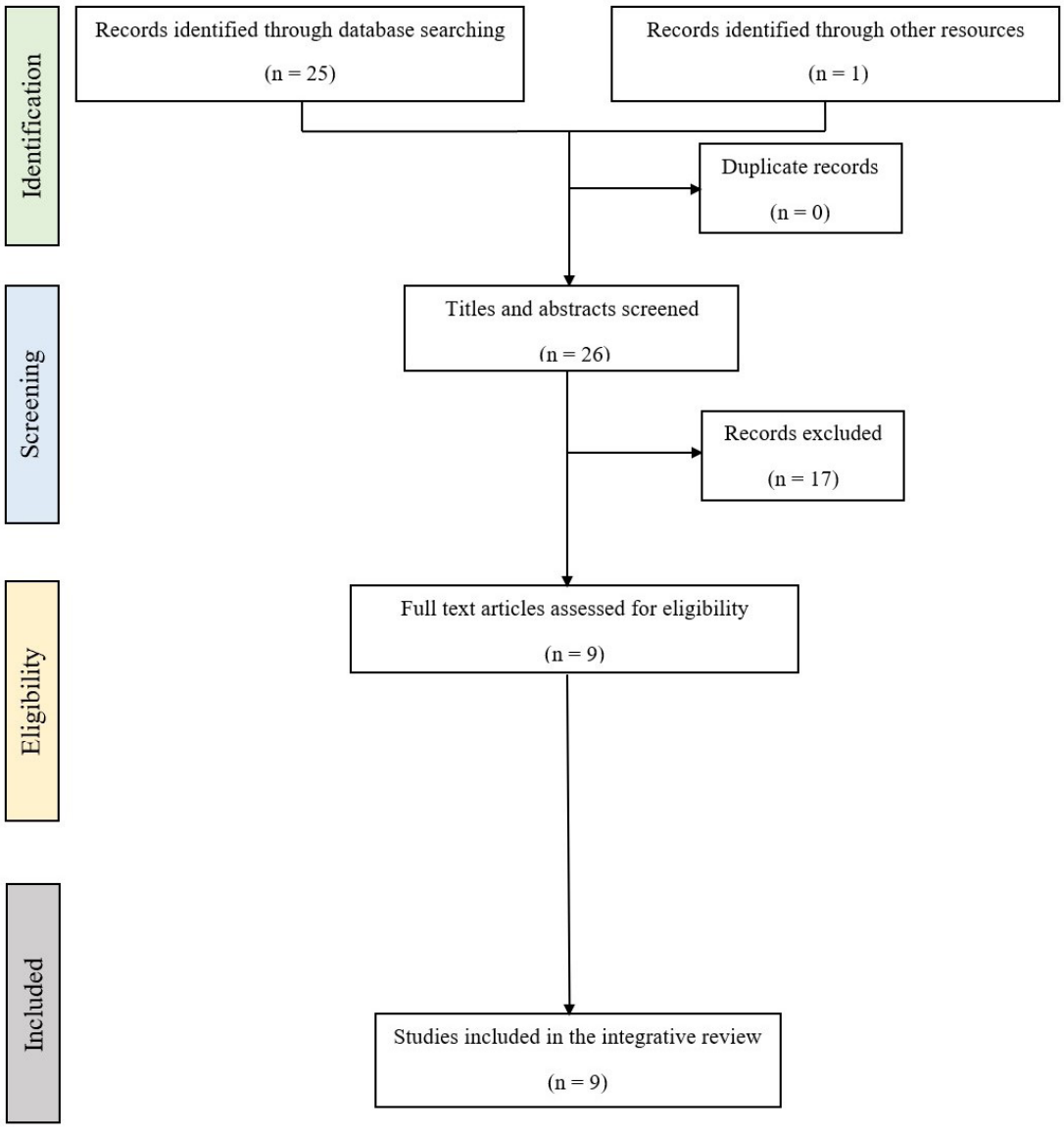

Fig 1. Flow chart diagram displaying the selection method of qualified studies.

included because of the study relevance from the bibliography search. The most commonly reported control and the experimental group sample sizes were >50 to 100 (n = 3).

Interventions enhancing medication adherence in stroke patients

Intervention and purpose

Medication-taking reminder mobile applications (e.g., Medisafe), health empowerment interventions, telehealth education, and motivational interviews are the medication adherence enhancement intervention that has been most often used in the past five years. Nevertheless, Medisafe has never been used in stroke patients before.[23] Furthermore, this review found that interventional studies mainly aim to improve medication adherence, physical activity, and clinical outcomes (blood pressure, high-density lipoprotein cholesterol, etc.).[23-31]

Theoretical framework

In this review, the theory of health empowerment[24], self-efficacy theory[24], guidelines for the secondary prevention of ischemic stroke and transient ischemic attack[25,26], the health belief model[27,30], and social cognitive theory[30] were applied to guide the interventions for enhancing medication adherence in stroke patients (Table 3), while 4 out of the 9 RCTs did not specify a theoretical framework.[23,28,29,31] The health belief model (2 out of 2) and guidelines for the secondary prevention of ischemic stroke and transient ischemic attack (2 out of 2) were identified as the most prominent and significant determinants of medication adherence compared to the other frameworks. Only 1 study used the social cognitive theory as a framework, and medication adherence and blood pressure as the clinical outcomes in this study were significantly improved after the intervention.[30] Regarding the theory of health

TABLE 3. Overall characteristics of the included studies.

Variables		Count	
Year			
2018[23,27,29,31]		4	
2017[26]		2	
2016[24,25]		1	
2015[28,30]		2	
Countries			
China[25-27]		3	
United States[23,24,31]		3	
Pakistan[29,30]		2	
New Zealand[28]		1	
Duration of intervention			
0 – 3 months[23,27,29,30]		4	
> 3 – 6 months[24,25,31]		3	
> 6 – 12 months[26,28]		2	
Target population#			
Ischemic stroke[24-27,30]		5	
Non-specified subtype of stroke[28,29,31]		3	
Hemorrhagic stroke[24]		1	
Transient ischemic attack[31]		1	
Hypertension*[23]		1	
Theoretical Framework##			
Unspecified[23,28,29,31]		4	
Health Belief Model[27,30]		2	
Guidelines for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack[25,26]		2	
The theory of health empowerment[24]		1	
Social Cognitive Theory[30]		1	
Self-efficacy Theory[24]		1	
Medication adherence measures			
The 8-item Morisky medication adherence scale (MMAS-8)[23,29,30]		3	
The 4-item Morisky medication adherence scale (MMAS-4)[24]		1	
Modified health behavior scale[25]		1	
The proportion of medication-taking was compared between the control and intervention groups[26]		1	
The Health Promoting Lifestyle Profile II[27]		1	
The Tablets Routines Questionnaire (TRQ)[31]		1	
Asking whether (in the past seven days) participants had taken all of their medication as prescribed. Moreover, patients were asked to indicate the number of doses/pills missed, if they just forgot (yes/no), the reason for the missed dose(s), and to provide detail if side effects were noted[28]		1	
Sample (n)			
Control (C)	**Experimental (E)**	**C**	**E**
0 – 25[31]	0 – 25[31]	1	1
> 25 – 50[25]	> 25 – 50[25]	1	1
> 50 – 100[27,29,30]	> 50 – 100[27,29,30]	3	3
> 100 – 200[24,28]	> 100 – 200[24,28]	2	2
> 200 – 300[23]	> 200 – 300[23]	1	1
> 300[26]	> 300[26]	1	1

#One study may consist of > 1 target populations
##One study may apply > 1 theoretical framework
*This study was included through other resources because of the study's relevance

empowerment and self-efficacy theory, although there was no statistical difference in medication adherence between the intervention and control groups in the studies that used these frameworks, other components, such as self-efficacy in illness management and self-management behaviors, were reported to be statistically significant.[24]

Medication adherence measurements

Six studies used different medication adherence report scales with acceptable reliability and validity to measure the medication adherence, while the other 3 RCTs did not provide this information (Table 3). The 8-item Morisky medication adherence scale (MMAS-8) (reliability: α = 0.83, validity: 93% sensitivity, 53% specificity) (n = 3) was often used to evaluate the medication adherence[23,29,30], followed by the 4-item Morisky medication adherence scale (MMAS-4) (reliability: α = 0.55, average item-test correlation: 0.65) (n =1)[24], modified health behavior scale (content validity index: 0.85, reliability: α = 0.878, the split-half reliability: 0.801) (n = 1)[25], and the Health Promoting Lifestyle Profile II scale (reliability: α = 0.853, split-half reliability: 0.781, test-retest reliability: 0.845) (n = 1)[27], respectively.

DISCUSSION

Although the prevalence of stroke is on the rise globally, medication can help prevent future strokes, and hence medication adherence is critical for preventing future strokes. However, the medication adherence rate is not at a satisfactory level.[28,29] The present integrative review found that medication-taking reminder mobile applications, such as Medisafe, have, to the best of our knowledge, not yet been used to improve medication adherence and clinical outcome in stroke patients.[23] Although the literature review revealed a previous interventional study that proposed the use of short message service (SMS) reminders to improve patients' medication adherence, there were limitations reported with this system; for example, SMS messages may not be received by patients if they provide an incorrect contact phone number, and also, as SMS is a one-way communication system, there is no guarantee that patients will rigorously comply with the treatment.[32,33] The use of reminders can though, provide a solution for some patients, mostly those with unintentional nonadherence, such as patients willing to take medicine but who may forget or miss the proper time.[34] The research explained that 22% - 73% of patients in diverse populations regularly reported forgetting to take medication, which is the most frequently cited reason for nonadherence in a number of studies.[35-44] Accordingly, a simple intervention such as Medisafe seems to perform well in daily practice and is comfortable for both patients and healthcare professionals to handle and adapt this intervention to prevent instances of nonadherence to medication.

The included studies' key strengths, namely for the RCTs, in this review, need to be reported. First, in this integrative review, a total of 9 RCTs were included. These studies had followed the design principles for randomized controlled trials, including randomly assigning participants to each group to ensure an equal chance of participation (minimized selection bias and sampling error). Moreover, RCTs display a higher degree of confidence in causal relationships than other research designs, thus increasing such a study's internal validity.[45-48]

Furthermore, the previous literature suggested that dropout rates can be expected to be less than 15% to 20% for RCTs.[49] In this review, 7 out of the 9 RCTs showed dropout rates of <20%, with the reasons and time of dropping out varying, including during the intervention (e.g., dead, declined to continue) and during the study assessment period (e.g., lost to follow-up). The dropout rate is an essential issue because it can threaten a study's' internal validity (e.g., by changing the random composition of the groups and their equivalence), external validity (e.g., by decreasing the generalizability of the RCT findings to only those who remained in the study), and statistical validity (e.g., by diminishing the sample size and the statistical power to detect differences between the intervention and control groups). Accordingly, most RCTs in this review seemed to have minimized these threats as acceptable dropout rates were reported.[50,51] However, another 2 RCTs had dropout rates of 39.38%.[28] and 26.32%[31], so improving the retention rates by following the appropriate guideline for additional investigation is required.

Nevertheless, there were also some limitations found in the included RCTs in this review that need to be noted. Self-reporting, the generalizability of the findings, and study recruitment issues need to be considered. First, 6 of the RCTs used various different medication adherence report scales with the reliability and validity reported, while the other 3 RCTs used medication adherence report scales without reporting the reliability and validity. Most of the included medication adherence reported scales in this review showed acceptable reliability and validity. However, most of them involved self-reporting, which is simple, inexpensive, and practically useful in the clinical setting, but is subject to certain limitations, such as being susceptible to errors, with the increase in time between visits particularly needing to be considered as this can threaten the study's internal validity.[52] On the other hand,

direct methods of measuring medication adherence, such as measuring medication concentration in the blood or urine and detecting biological markers in the blood, have several disadvantages too, such as typically being expensive and labor-intensive. Nevertheless, in some situations, this approach is practical; for instance, evaluating the serum level of antiepileptic drugs (phenytoin, valproic acid).[52] In summary, there is no gold standard to measure medication adherence[53,54], and the choice may depend on the research objective, funding, study population, and setting. Researchers need to consider the measurement carefully, and importantly, any error or bias that threatens the study validity should be minimized.

Second, the generalizability of the RCTs' findings should also be considered as a limitation since most of the RCTs were carried out in a specific unit and specific stroke population. For example, Wan et al.'s study[25] performed RCTs in 3 units of 2 major hospitals, thus limiting the generalizability of the findings to the whole population. Moreover, another study included only patients diagnosed with mild ischemic stroke, while severe stroke patients were excluded, which could have led to selection bias, thus limiting the generalizability of the results.[27]

Finally, study recruitment issues also need to be mentioned as a limitation of the included RCTs. With the ongoing development of technology, more people can now access the internet; however, some people still cannot or do not. In this regard, one study recruited participants entirely online[23], citing literature that indicated that more than 50% of patients use the internet for medical information.[23] Hence, the significant improvement in medication adherence or other related outcomes in either the control or intervention group may have been due to the interventional design itself or extraneous variables, like information obtained from the internet, and so this study's results may not be generalizable to other populations with different sociodemographic characteristics, such as those who cannot access the internet.

LIMITATIONS

There are several limitations in this integrative review itself to note. First, the researcher searched only the PubMed electronic database and relevant bibliographies, which could possibly lead to a limitation of this study's findings and generalizability. Additionally, this review's primary purpose was to systematically identify and analyze reported randomized controlled trials (RCTs) for interventions aimed at enhancing medication adherence in stroke patients. Therefore, the researcher solely included RCTs involving a medication adherence intervention. As it included only one type of study design, this may lead to a limitation of the research findings. In any further study, it is suggested that other types of study design that involve this kind of intervention should be included, such as quasi-experimental studies, which may increase the integrative review's uniqueness. Moreover, since the researcher did not include studies from the "gray" literature, such as conference proceedings or abstracts, this may introduce a publication bias. Finally, only English-language studies were included. Because of this, RCTs reported in other languages that also aimed to improve medication adherence in stroke patients may have been missed and hence omitted.

CONCLUSION

Medication adherence can lead to diminished healthcare service use, improved patient quality of life, and decreased healthcare expenses.[55-57] The present integrative review has implications for the heightened recognition of the necessity of interventions aimed at enhancing patients' adherence to their medication, and that could be applied in clinical practice. For example, there is the possibility of using medication-taking reminder mobile applications (such as Medisafe) to improve medication adherence and clinical outcomes among stroke patients, yet this innovation has never been used before among stroke patients. In the future, health care providers may utilize this innovation to promote high-quality stroke care in clinical practice. A previous systematic review revealed that four studies used such a reminder system, three of which reported a significant positive impact on medication adherence.[18] One study into the medication adherence of HIV patients receiving message reminders found a significant difference in favor of those receiving message reminders.[18] Likewise, one asthmatic study found that the adherence rate of asthma patients who received daily message reminders was higher than those who were not reminded.[58] Therefore, the use of Medisafe appears to be a potential tool for assisting medication adherence in patients with stroke, albeit some questions arise as an outcome of this integrative literature review related to its use, specifically: 1) Can Medisafe improve medication adherence in stroke patients? 2) Can Medisafe improve clinical outcomes, such as blood pressure, in stroke patients?

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SUPPLEMENTARY TABLE 1. The literature review paper matrix.

References, countries, and duration of RCTs	Target population, sample size (n)*	Problem & Purpose	Theoretical Framework	Intervention details	Medication adherence measures (reliability, validity)	Methodological problems: Threats to study validity (Critical Analysis)	Key Findings	Implications
(**1**)[1] The United States 3 months	Hypertensive **Experimental (E):** 209 **Control (C):** 202 **Note:** the author included hypertensive patients because it is the most relevant study; Medisafe never used in stroke patients. **- Dropout rate of 12%**	- Medication non-adherence estimates for 50% of uncontrolled hypertension. Smartphone applications (apps) that aim to improve adherence are broadly available but have not been rigorously evaluated. - This study aimed to determine if the Medisafe smartphone application increases self-reported medication adherence and blood pressure control.	Unspecified (Study protocol provided)	**E:** In this group, participants were guided to download and use the Medisafe application, including medication-taking reminder alerts, adherence reports, and optional peer support. **C:** N/A **Randomly assigned** The author attempted to enroll at least 390 patients to have 80% power to detect a 5 mmHg. the difference in systolic blood pressure between treatment arms, with an α of 0.05 (even with a 20% loss to follow up or a standard deviation of up to 17 mm Hg)	The 8-item Morisky medication adherence scale (MMAS-8) **Reliability:** Chronbach's alpha 0.83 **Validity:** (93% sensitivity, 53% specificity)	- The recruitment was performed entirely online, in which the literature showed that more than 50% of patients use the internet for medical information. These RCT results may not be generalizable to other populations of individuals with poorly controlled hypertension, who may have different sociodemographic and comorbidity characteristics than the patients in our study participants. - The researcher used home blood pressure monitors to evaluate blood pressure outcomes, in which different participant may measure their blood pressure differently. This can threaten internal validity (measurement). - Self-management	- After 12 weeks, the mean (SD) score on the **MMAS improved by 0.4 (1.5) among intervention participants** and remained unchanged among controls (between-group difference: 0.4; 95% CI, 0.1-0.7; P = 0.01). ^ - After 12 weeks, the mean (SD) systolic blood pressure decreased by 10.6 (16.0) mm Hg among intervention participants and 10.1 (15.4) mm Hg among controls (between-group difference: −0.5; 95% CI, −3.7 to 2.7; P = 0.78). ≠	- Patients randomized to use a smartphone application had a small improvement in self-reported medication adherence among poorly controlled hypertension patients but no change in systolic blood pressure than controls. Hence, its advantage and other mobile health interventions on clinical outcomes remain to be established. - The recruitment method should be improved. - The way of blood pressure measuring should be improved.
(**2**)[2] The United States 6 months	Ischemic stroke, Hemorrhagic stroke **E:** 105 **C:** 105 **- Dropout rate of 16.7%**	- After a stroke, self-management is a challenge because of multifaceted care needs and complex disabling consequences that further barrier patient participation.	- The theory of health empowerment - Self-efficacy Theory	**E:** Usual care (the ambulatory rehabilitation schedule) + small group sessions (establish a partnership with the nurse facilitator for stroke self-management to begin personal goal setting and action planning) +	The 4-item Morisky medication adherence scale (MMAS-4) **Reliability:** Chronbach's alpha 0.55 **Average item-test correlations:** 0.65	behavior was assessed using self-report. This might have led to over-reporting of what was seen as desired behavior by the participants. - The baseline characteristics between	**- Medication adherence. ≠** - Self-efficacy in illness management 3-month and 6-month. ^ - Self-management behaviors at all follow-up time points. ^	Stroke patient empowerment intervention could be combined into the ambulatory rehabilitation phase. It would become more plausible for continuing professional support

SUPPLEMENTARY TABLE 1. The literature review paper matrix. (Continue)

References, countries, and duration of RCTs	Target population, sample size (n)*	Problem & Purpose	Theoretical Framework	Intervention details	Medication adherence measures (reliability, validity)	Methodological problems: Threats to study validity (Critical Analysis)	Key Findings	Implications
		- This study aimed to investigate the effects of the Health Empowerment Intervention for Stroke Self-management (HEISS) on stroke patients' self-efficacy, self-management behavior, and functional recovery.		biweekly telephone follow-up calls (encourage and commend participants on their actions for positive changes and to provide problem-solving skills to overcome any perceived barriers that participants encountered). **C:** Usual care only. **Randomly assigned**	(internal consistency reliability) **Validity:** good sensitivity and moderate specificity in identifying nonadherent individuals	those who have completed data collection and those who have dropped-out were nearly similar. The intervention's effects might be overestimated if dropped out cases had better outcome measures or vice versa.		to aid stroke patients in understanding responsibility for and participating in stroke self-management in a home setting.
(**3**)[3] China 6 months	Ischemic stroke **E:** 40 **C:** 40 **- Dropout rate of 12.1%**	- Adopting healthy behaviors is critical for secondary stroke prevention. However, compliance often decreases with time after hospital discharge, yet few studies have investigated programs promoting long-term adherence to health behaviors. - This research proposed to evaluate the effectiveness of a guideline-based, goal-setting telephone follow-up program for patients with ischemic stroke.	Guidelines for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack	**E:** The intervention group consisted of predischarge education and three goal-setting follow-up sessions conducted by telephone. **C:** The control group gained the usual stroke education, including freely available educational brochures on understanding stroke and cutting stroke risk. **Randomly assigned**	Modified health behavior scale **Content validity index:** 0.85 **Cronbach's α (reliability):** 0.878 **The split-half reliability:** 0.801 (a measure of internal consistency — how well the test components contribute to the construct that's being measured)	- The information regarding health behaviors and medication adherence was self-reported, so memory errors and expectation bias may have influenced the result. - The study was carried in 3 units of 2 major hospitals, limiting the generalizability of findings to the whole population. - Eleven patients meeting the inclusion criteria declined to participate, suggesting that better recruitment methods are expected.	- Six months after discharge, patients in the intervention group exhibited **significantly higher medication adherence** than patients in the control group. ^ - Physical activity, nutrition, low-salt diet adherence, blood pressure monitoring, smoking abstinence, unhealthy alcohol use, and modified Rankin Scale (mRS). ≠	The intervention improved only medication adherence at six months post-discharge. These results indicate a need for more effective strategies to help stroke patients achieve guideline-recommended targets for health behaviors

SUPPLEMENTARY TABLE 1. The literature review paper matrix. (Continue)

References, countries, and duration of RCTs	Target population, sample size (n)*	Problem & Purpose	Theoretical Framework	Intervention details	Medication adherence measures (reliability, validity)	Methodological problems: Threats to study validity (Critical Analysis)	Key Findings	Implications
(**4**)[4] China 1 year	Ischemic stroke **E:** 613 **C:** 574 **- Dropout rate of 6.9%**	- Antiplatelet is the treatment of the first choice for long-term secondary prevention of vascular events. Although ischemic stroke patients are still at significant risk for recurrence; roughly one-third of stroke survivors will have a recurrent vascular event within five years. - This RCT proposes to evaluate a health promotion program on medication adherence to antiplatelet therapy among ischemic stroke patients.	Guidelines for the Secondary Prevention of Ischemic Stroke and Transient Ischemic Attack	**E:** The daily 30 minutes of training sessions for three days aimed to develop patients' awareness and improve their medication adherence. The physicians called the patients at one, three, and six months after hospital discharge to monitor progress and offer secondary prevention guidance. **C:** Usual stroke management programs. **Randomly assigned**	The proportion of medication-taking was compared between the control and intervention groups. **Reliability:** N/A **Validity:** N/A	- The data collected are self-reported; participants likely over or underrated their skills and knowledge when responding to survey items. - No reliability and validity of medication adherence measurement were reported.	- After a one-year follow-up, **the proportion of patients who took the antiplatelet therapy increased significantly** in the intervention group, reaching 73.2%, with a pre-post difference between two arms of 22.9% ($P < 0.01$). ^ - The proportion of patients with an awareness of antiplatelet therapy significantly increase (24.4%, $P < 0.01$). ^	The health promotion program showed a positive impact on awareness of and adherence to antiplatelet therapy, which can be scaled up to other resource-limited areas.
(**5**)[5] China 3 months	Ischemic stroke **E:** 80 **C:** 78 **- Dropout rate of 9.2%**	- The health behaviors of hypertensive stroke patients in China are not satisfactory. Because unimodal programs were inefficient for improving blood pressure control in stroke patients, recent efforts are directed at multi-modal interventions.	Health Belief Model	**E:** Usual care + face-to-face and telephone health belief education, a patient calendar handbook, and weekly automated short-message services. **C:** Usual care only (health education during hospitalization, a stroke prevention handout, follow-up by doctors	The Health Promoting Lifestyle Profile II **Cronbach's α (reliability):** 0.853 **The split-half reliability:** 0.781 **Test-retest reliability:** 0.845	- The program is considered proper only for patients diagnosed with mild ischemic stroke. Severe stroke patients were excluded; this can lead to selection bias. - Health behavior information was self-reported and subject to expectation bias (Hawthorne effect).	**- Medication adherence. ^** - Better health behaviors for physical activity, nutrition, low-salt diet. ^ - Decreased systolic blood pressure and increased blood pressure control rate. ^	At three months, the Comprehensive Reminder System's use based on the Health Belief Model produced a positive outcome of most health behaviors and blood pressure control. Continued implementation of this intervention protocol

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		- This study aimed to test the effect of a Health Belief Model Comprehensive Reminder System on health behaviors and blood pressure control in hypertensive ischemic stroke patients after the occurrence and hospital discharge.		at the outpatient department, and telephone follow-up by nurses at one week and one month after discharge). **Randomly assigned**				is guaranteed to determine the long-term effect.
(**6**)[8] New Zealand 12 months	Unspecified (excluding subarachnoid hemorrhage) **E:** 119 **C:** 115 **- Dropout rate of 39.38%**	- The literature shows that stroke recurrence rates are high (20%–25%) and have not declined over the past three decades. - This study aimed to examine the effectiveness of motivational interviewing (MI) for reducing stroke recurrence. Medication adherence and lifestyle change will be measured.	Unspecified	**E:** The intervention group received four motivational interview sessions at 28 days, 3, 6, and 9 months post-stroke. Sessions were audio-recorded. The primary interview was administered face-to-face; subsequent interviews were conducted by telephone. **C:** Usual care only. **Randomly assigned**	- Asking whether (in the past seven days) they had taken all of their medication as pre-scribed. - Patients were asked to indicate the number of doses/pills missed, if they just forgot (yes/no), the reason for the missed dose(s), and to provide detail if side effects were noted. **Reliability: N/A** **Validity:** The validity of self-reports was cross-checked with electronic medication dispense records where available, which suggests accurate reporting.	- Although medication adherence measured by self-reports is inexpensive and straightforward, and validated for use in clinical settings, it may have led to an overestimation of adherence. - The motivational interview's nature was not plausible to blind participants, which may have influenced self-report outcomes.	**- Self-reported medication adherence** at six months and nine months post-stroke. ^ - The change measures blood pressure and cholesterol. ≠	Motivational interview (MI) developed self-reported medication adherence. Other effects were nonsignificant, though in the direction of a treatment effect. Additional research is required to determine whether MI leads to improvement in other essential functioning (e.g., caregiver burden).

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(**7**)[7] Pakistan 3 months	Unspecified (stroke) + coronary artery disease **E:** 99 **C:** 98 **- Dropout rate of 9.64%**	- Medications can diminish stroke risk by 30% and Myocardial Infarction (MI) by 15%. Nevertheless, adherence, even in developed countries, is only around 50%. Health-based procedures can be an inexpensive and efficiently accessible tool to increase compliance and bridge the communication gap between health care providers and users. - This RCT aimed to develop and examined the effectiveness of a tailored health information technology-driven intervention: "Talking Prescriptions" to increase medication adherence in patients.	Unspecified	**E:** This group received daily Interactive Voice Response (IVR) call services regarding specific statin and antiplatelet, daily tailored medication reminders for statin and antiplatelet, and weekly lifestyle modification messages. **C:** N/A **Randomly assigned**	The 8-item Morisky medication adherence scale (MMAS-8) **Reliability:** (α = 0.83) **Validity:** (93% sensitivity, 53% specificity)	- Medication adherence was assessed using self-report; this might have led to overreporting. - This RCT did not study the effect of an intervention on improving patient clinical outcomes targeted for future larger-scale clinical trials.	The 8-item Morisky **medication adherence** scale. ≠	A phone-based medication adherence program was possible in settings with high volume clinics and low patient knowledgeability. Due to limited follow-up, the program did not achieve any statistically significant differences in adherence behavior as self—reported by the MMAS-8 Scale.

SUPPLEMENTARY TABLE 1. The literature review paper matrix. (Continue)

References, countries, and duration of RCTs	Target population, sample size (n)*	Problem & Purpose	Theoretical Framework	Intervention details	Medication adherence measures (reliability, validity)	Methodological problems: Threats to study validity (Critical Analysis)	Key Findings	Implications
(**8**)[8] Pakistan 2 months	Ischemic stroke **E:** 83 **C:** 79 **- Dropout rate of 19%**	- Mobile technology's effectiveness in improving medication adherence via customized Short Messaging Service (SMS) reminders for stroke has not been tested in low resource areas. - This study aimed to test the effectiveness of SMS on developing medication adherence in stroke survivors.	- Health Belief Model - Social Cognitive Theory	**E:** Usual care + received reminder SMS for two months that contained personalized, prescription tailored daily medication reminder(s), and twice-weekly health information SMS. **C:** Usual care only. **Randomly assigned**	The 8-item Morisky medication adherence scale (MMAS-8) **Reliability:** (α = 0.83) **Validity:** (93% sensitivity, 53% specificity)	- Medication adherence was assessed using self-report; this might have led to overreporting. - Participants who only have a telephone can participate in this study. Those who do not have may underrepresent, and this leads to selection bias.	**- The mean medication adherence score ^** - The mean diastolic blood pressure ^	The SMS intervention seems possible for clinical use in stroke survivors for improving adherence. Further investigations are needed to report on meaningful biologic outcomes like recurrent stroke, death, and disability.
(**9**)[9] The United States 6 months	Unspecified (stroke) + Transient Ischemic Attack **E:** 14 **C:** 14 **- Dropout rate of 26.32%**	- Stroke is the leading cause of disability, death, and health resource use among Americans. Self-management is a caring procedure that allows individuals to solve problems as they arise, practice new health behaviors, and gain emotional stability. Although several articles support self-management training for stroke survivors, there is limited data specific to young African Americans men.	Unspecified	**E:** Participants obtain self-management training, delivered in 1 individual and 4 group sessions (over three months). **C:** Usual care only. **Randomly assigned**	The Tablets Routines Questionnaire (TRQ) **Reliability:** N/A **Validity:** N/A	- Small sample size, limited duration, and research staff were not blind to the intervention assignment. - Using a one-time blood pressure assessment, including the possibility of elevation when a measurement is done in a clinical setting (white-coat hypertension) and underdetection (masked hypertension).	**- Medication adherence ≠** - High-density lipoprotein cholesterol ^ - Glycosylated hemoglobin ^ - Systolic blood pressure ^	The intervention was not capable of engaging all patients. Qualitative findings suggest that while the group format was highly acceptable, aspects of the program might be improved. More sessions might have been accommodating, supporting telephone attendance for those with travel or logistic difficulties.

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		- This study proposed to compare a self-management intervention (TargetEd MAnageMent Intervention [TEAM]) versus treatment as usual (TAU) to reduce stroke risk in African American (AA) men.						

RCTs Randomized Controlled Trials
N/A Not applicable
*Final analyzed
^ Outcome significantly improved after the intervention
v Outcome significantly worsened after the intervention
≠ Outcome unchanged after the intervention

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The Performance of Real-Time Polymerase Chain Reaction in Patients with Scanty Positive Acid-Fast Bacilli Sputum Smear in Diagnosis of Pulmonary Tuberculosis: 5-Year Retrospective Study

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ABSTRACT

Objective: To assess the performance of real-time polymerase chain reaction (RT-PCR) to diagnosis pulmonary tuberculosis in patients with scanty positive acid-fast bacilli sputum smears, in a single hospital.

Materials and Methods: All patients, who had scanty positive AFB sputum smears in Songklanagarind Hospital; between 2015 and 2019 were included. Demographic data, clinical data, radiographic findings, RT-PCR and mycobacterial culture results were reviewed.

Results: From a total of 269 patients reporting scanty AFB smears, 116 patients (43.1%) had cultures confirmed as *M. tuberculosis*. From overall, samples from 92 patients with scanty AFB smear were processed for RT-PCR. There were 26 (28.3%) isolates having positive RT-PCR test results. Of these 26 isolates that RT-PCR positive, 25 (96.2%) were culture positive, while only 1 (3.8%) were culture negative. A remaining 66 samples that RT-PCR negative, 15 (22.7%) were culture positive for tuberculosis. Using mycobacterial cultures as the gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of RT-PCR were 62.5%, 98.1%, 96.2%, and 77.3%, respectively. Pulmonary consolidation and cavity on chest radiograph were associated with the growth of *M. tuberculosis*, with an OR of 2.3 (95% C.I. 0.26-0.73) and 3.4 (95% C.I. 1.2-9.9), respectively.

Conclusion: Less than half of the patients with scanty smears had culture-confirmed tuberculosis; RT-PCR also has low sensitivity. Consequently, a negative RT-PCR does not exclude tuberculosis; especially in cases of a high index for clinical suspicion. Radiographic findings; including pulmonary consolidation and cavities, are helpful predictors for supporting this diagnosis.

Keywords: Performance; scanty acid fast bacilli; pulmonary tuberculosis; polymerase chain reaction (Siriraj Med J 2021; 73: 445-450)

INTRODUCTION

Pulmonary tuberculosis remains a serious, worldwide public health problem.[1] Thailand remains on the list of the world's 14th highest burden country of tuberculosis.[2-3] The high number of tuberculosis cases and deaths indicates that actions are urgently needed to reduce tuberculosis incidence. Rapid identification and treatment of new cases is the keystone of tuberculosis control.[4-5] Acid Fast Bacilli (AFB) sputum smear microscopy is widely used as the diagnostic test for mycobacterial disease; as it is a simple, rapid and cost-effective method for diagnosing tuberculosis.[6]

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Ziehl-Neelsen (ZN) stained smear is a conventional method; however, auramine-staining has reported to have 10 percent more sensitivity with similar specificity.[7-12] Since 2006, the central laboratory of Songklanagarind Hospital has applied the auramine fluorescence microscopic technique as its screening test for all requested sputum acid fast smears, before being confirmed with ZN-stained. With this more sensitive staining technique, we found increasing reports of scanty AFB in sputum smears.

The scanty AFB results were defined as: the presence of less than 10 bacilli in the 100 oil-field on microscopy. The International Union Against Tuberculosis and Lung Disease (IUATLD), along with the World Health Organization (WHO), previously recommended that additional sputum AFB smears should be repeated in this degree of positivity, when reported as doubtful.[13-14] In 2013, WHO revised the case definitions of pulmonary tuberculosis.[15] Cases with scanty AFB on microscopy will be considered as sputum smear positive tuberculosis. This lowering diagnostic threshold thereby increases case detection rates and treatment initiation. However, this degree of positive acid fast smear might not well correlate with cultures, as it could reflect non-tuberculous mycobacteria, or contamination by environmental mycobacteria.[16-17] The nucleic acid amplification test (NAAT) is useful as it can rapidly detect *M. tuberculosis* bacteria in specimens within hours, while the turnaround time of mycobacterial cultures requires 2 to 6 weeks for reporting. However, the NAAT performance to detect *M. tuberculosis* in scanty positive AFB sputum smears remains a challenge, because of low bacillary loads.[20-22] Hence, the aim of this study was to explore the yield of scanty positive acid fast sputum smears, and the multiplex Real Time-Polymerase Chain Reaction (RT-PCR) Anyplex™ II MTB/MDR Detection technique to diagnose pulmonary tuberculosis.

Objectives

The purposes of this study were:

1. To determine the performance of real-time polymerase chain reaction in patients with scanty positive acid-fast bacilli sputum smear in diagnosis of pulmonary tuberculosis

2. To explore the factors that can predict the growth of *M. tuberculosis* in patients with scanty positive AFB sputum smears

MATERIALS AND METHODS

Study design and population

A retrospective review of medical and microbiological records of all suspected respiratory tuberculosis patients, whose sputum were sent for AFB staining, from January, 2015 to December, 2019 in Songklanagarind Hospital, an 800-bed, teaching-based, tertiary care hospital in Songkhla province, Southern Thailand. The investigational protocol was approved by the Institutional Review Boards of Faculty of Medicine, Prince of Songkla University: REC. 63-287-14-1. Enrollment criteria of the patients were: (1) aged > 15 years, (2) had at least one specimen showing a scanty positive acid fast smear, (3) having at least one sputum specimen sent for mycobacterial culture. Patients were excluded if they: (1) had recent or ongoing treatment with anti-tuberculous agents, (2) had no obtained mycobacterial culture, (3) had not undergone chest radiograph during sputum collection.

Data collection

The patient's demographic data, presenting symptoms, underlying medical illness (es), results of mycobacterial culture and multiplex RT-PCR, chest radiographic findings, rate of anti-tuberculosis treatment and adverse drug events were all recorded.

As the laboratory routine works, sputum specimens are processed and performed for decontamination and concentration via a standard method, preparation of slides and fluorochrome staining with auramine O. With auramine O staining, mycobacteria appear as bright, yellow fluorescent rods under a fluorescent microscopy. Slide with a presence of fluorescent rods are ZN stained, and evaluated by using a conventional light microscope. Performing a ZN stain, after initial auramine O staining, is accepted as standard practice in Songklanagarind Hospital. The number of acid-fast bacilli observed has been quantified according to the IUALD and the WHO scales. Mycobacterial cultures are the gold standard for bacteriological confirmed diagnosis of tuberculosis, and growing bacteria is required to perform drug-susceptibility testing. Sputum specimens were cultured in liquid (7H9 broth) medium, with use of the automated Mycobacterial Growth Indicator Tube system and solid Löewenstein-Jensen medium.

Eligible patients were patients who had scanty positive acid fast bacilli of sputum smear, which was defined as: having presence of at least one of two, or three slides reported to have less than 10 acid fast bacilli (AFB) found in 100 oil fields; according to the WHO scale. Regarding the results of sputum mycobacterial cultures, they were assigned as either a positive culture for *M. tuberculosis* (positive C/S MTB) or negative culture for *M. tuberculosis* (negative C/S MTB). Negative C/S for MTB was defined by there being at least one or more sputum specimens sent for mycobacterial cultures

reported as growing non-tuberculous mycobacterial (NTM), or no mycobacterial growth.

As part of a diagnostic investigation, direct molecular testing was performed on 92 scanty sputum smears, submitted by physician request using the Anyplex™ II MTB/MDR Detection kit. This assay is a semi-automated system, and provides rapid results within 3-4 hours of sample receipt. This technique also has a lower rate of error and contamination [11], compared to the Line probe assay. Before performing the multiplex RT-PCR technique, DNA of *M. tuberculosis* was extract by using DNA-extraction solution, provided in the kits. Anyplex™ II MTB/MDR Real-time Detection was performed following the directions provided by the manufacturer. Amplification and detection were performed on a Rotor-Gene 3000 instrument, for all sample extracts. *M. tuberculosis* detection targeted the IS6110 and MPB64 genes. Result interpretation was performed automatically, using the instrument's software according to threshold and cutoff values outlined by the manufacturer (16). Overall, the Anyplex MTB/NTM assay demonstrated sensitivity, specificity, PPV, and NPV of 86%, 99% 96%, and 95%, for *M. tuberculosis* detection compared with mycobacterial cultures.

Statistical analysis

To describe the variables characteristics, these were expressed as mean with standard deviation, median with range, proportion in percentage and ratio. To examine differences between 2 groups of variables these were analyzed by Fisher's exact or χ^2 test. The categorical variables were analyzed by Student's t-test or Mann-Whitney U test for the continuous variables, according to types of its distribution. Statistical analysis was performed with SPSS software version 23 (SPSS Inc., Chicago, IL, USA). P-values <0.05 were considered statistically significant.

RESULTS

During the five-year study period, 416 patients met criteria for inclusion in the study. From this, 147 patients (35.3%) were excluded for the following reasons: 126 patients had recent or ongoing treatment with anti-tuberculous agents, 2 patients underwent no chest radiograph during sputum collection, and 19 had no obtained mycobacterial culture. In total, 269 patients were enrolled in this study (Fig 1).

Of the 269 patients with scanty positive AFB smears, 116 patients (43.1%) had positive culture for *M. tuberculosis*, while the remaining 153 patients (56.9%) had negative

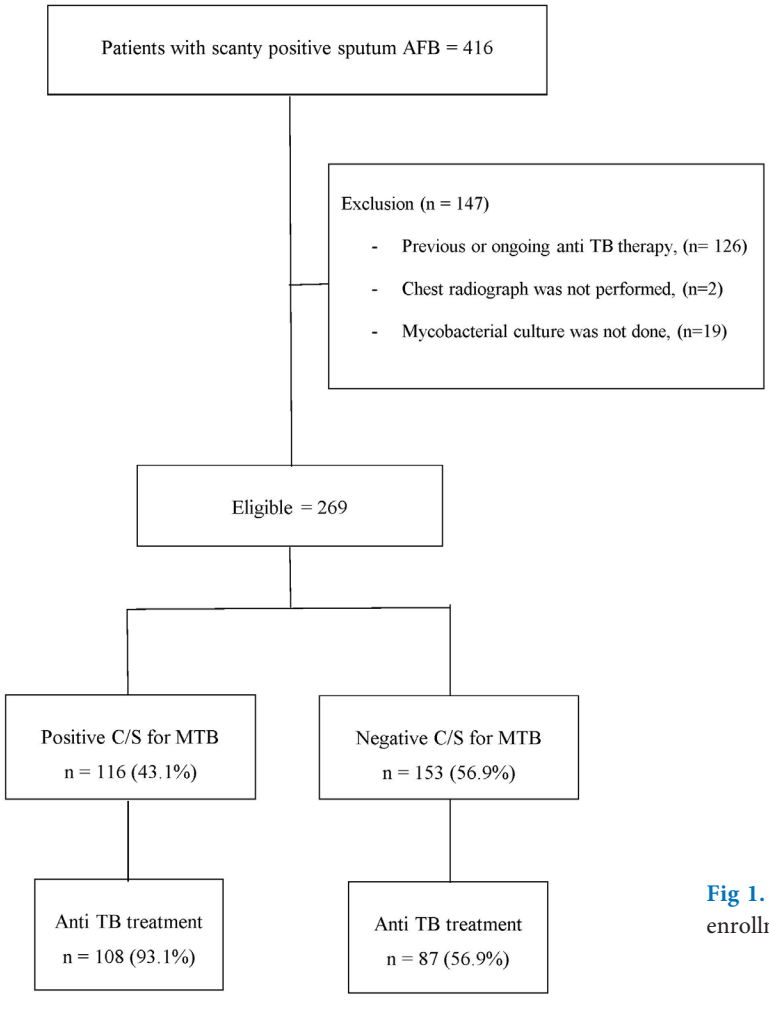

Fig 1. Flow diagram for patient enrollment.

cultures. There were no statistically significant differences between these 2 groups; in terms of: patient characteristics, presenting symptoms or comorbidities, as shown in Table 1. Means age of positive and negative mycobacterial culture groups were 55.6±18.7 years vs. 61.7±17.1 years, respectively (p=0.134). About two-thirds of patients in both groups were male. Pulmonary consolidation and cavity on chest radiograph were frequently detected in the culture positive group (44.8% vs. 26.1%, 10.3% vs. 3.2%, respectively, P<0.05), while normal chest radiograph favored the culture negative group (1.7% vs. 24.8%, P<0.05). Reticular lesions were commonly found in active respiratory tuberculosis; however, there was no statistically significant difference between positive and negative mycobacterial culture groups (P=0.676). From this, 93.1% of patients in the culture positive group received anti-tuberculosis treatment, compared to 56.9% of patients in the culture negative group (P<0.05). The percentage of adverse drug events were 26.7% in the positive culture group and 9.8% in the negative culture group (P =0.135)

There were 92 sputum scanty AFB smears sent for multiplex RT-PCR, and overall there were 26 (28.3%) cases having positive RT-PCR test results. Of these, 25 (96.2%) were culture positive, while only 1 (3.8%) were culture negative. A remaining 66 samples that RT-PCR negative, 15 (22.7%) were culture positive for tuberculosis. Using mycobacterial cultures as the gold standard, the sensitivity, specificity, positive predictive value and negative predictive value of RT-PCR were 62.5%, 98.1%, 96.2%, and 77.3%, respectively (Table 2). Overall, the diagnostic yield of multiplex RT-PCR in scanty AFB was relatively low. However, anti-tuberculous treatment should be initiated without delay in patients with positive RT-PCR, as it has a low, false positivity (3.8%).

DISCUSSION

Scanty positive AFB reporting accounted for one-third from all sputum smear positives in our institute. Of these, 41.3% grew *M. tuberculosis*. Similarly, previous studies showed that fewer than half of the smears, with scanty AFB, yielded positive cultures.[23-25] The chance of scanty positive AFB to be a positive culture for *M. tuberculosis* is limited, and might be due to low bacillary loads. False positive scanty sputum smears are possibly from non-tuberculous mycobacteria[26]; particularly asymptomatic patients without chest radiological evidence suggesting active tuberculosis.

As for the results of this study; 195 patients (72.5%) who had scanty positive AFB smears were being treated with anti-tuberculosis treatment. Interestingly, 12 out of 38 patients (31.6%), who had scanty AFB smears and negative mycobacterial cultures, were receiving anti-tuberculosis treatment; despite normal chest radiograph.

Our study also revealed that RT-PCR had low sensitivity for patients who had scanty positive AFB smears. The results are supported by IUATLD/WHO recommendations, in that a single negative NAA test could not exclude the diagnosis of pulmonary tuberculosis; especially in cases of moderate to high index of clinical suspicion. Chest radiographic findings are helpful in terms of prediction the following growth of *M. tuberculosis*. Chest radiographic findings indicate active respiratory tuberculosis; including, consolidation opacities, and cavitation, which are related to the growth of *M. tuberculosis*. Only 2 of our 116 patients (1.7%) having normal chest radiographic findings had *M. tuberculosis* in their sputum. Our findings suggest empiric anti-tuberculous treatment for all scanty positive AFB patients with radiographic findings suggestive of active tuberculosis, as this might be appropriate; whereas, the follow up approach is considered in patients who have normal chest radiography. This strategy is to avoid overtreatment and drug related complications.

There are some limitations in this study. First, tuberculosis diagnosis is based entirely on the detection of *M. tuberculosis* via culture techniques. Failure to isolate *M. tuberculosis* cannot definitely exclude a diagnosis of active tuberculosis. Second, our retrospective study may have introduced a selection bias, because approximately two-thirds half of the patients with scanty positive AFB smears did not performed RT-PCR tests. Thus, the outcome might not reflect the true performance of RT-PCR in all patients with scanty positive results.

CONCLUSION

Presence of scanty positive AFB in sputum smear was common, while only 43.1% of them were finally confirmed as tuberculosis by mycobacterial culture. Also, the RT-PCR sensitivity in scanty acid fast sputum smears is very low. Hence, if clinical suspicion is high, tuberculosis should not be ruled out based solely on negative RT-PCR results. Chest radiographic findings are helpful in determining empirical anti-tuberculosis treatment.

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TABLE 1. Demographic, clinical manifestations and laboratory results of patients with scanty AFB, and results of their mycobacterial culture.

Characteristics	Patients with scanty positive AFB (n=269)		
	Positive C/S for MTB n=116 (43.1%)	Negative C/S for MTB n=153 (56.9%)	*p*-value
Age in years (mean ±S.D.)	55.64 ±18.68	61.66 ±17.09	0.134
Male gender (%)	65.5%	57.5%	0.183
Presenting symptoms*			
Cough	63 (54.31%)	66 (43.14%)	0.069
Fever	22 (18.97%)	20 (13.07%)	0.187
Weight loss	20 (17.24%)	26 (16.99%)	0.957
Hemoptysis	8 (6.89%)	12 (7.84%)	0.769
Pleurisy	3 (2.58%)	2 (1.30%)	0.442
Dyspnea	13 (11.2%)	15 (9.8%)	0.709
Underlying diseases*			
HIV/AIDS	6 (5.17%)	9 (5.88%)	0.802
Diabetes	14 (12.07%)	23 (15.03%)	0.485
Malignancy	23 (19.83%)	43 (28.1%)	0.118
Renal failure	3 (2.59%)	5 (3.27%)	0.744
Immuno-suppressive therapy	6 (5.17%)	7 (4.58%)	0.821
Chest radiograph finding*			
Normal	2 (1.72%)	38 (24.84%)	<0.05
Consolidation	52 (44.82%)	40 (26.14%)	<0.05
Reticular	22 (18.97%)	26 (16.99%)	0.676
Cavity	12 (10.34%)	5 (3.27%)	0.018
Miliary	6 (5.17%)	1 (0.65%)	0.021
Pleural effusion	15 (12.93%)	12 (7.84%)	0.169
Nodule/mass	28 (24.14%)	51 (33.33%)	0.101
Received Anti-TB treatment	108 (93.1%)	87 (56.86%)	<0.05

Plus–minus values are means ±SD

Abbreviations: AFB=acid fast bacilli, C/S= Culture, MTB= *Mycobacterium tuberculosis*, HIV/AIDS = Human immunodeficiency virus/ Acquired Immune Deficiency Syndrome

*Patients may have had >1 manifestation.

TABLE 2. The results of multiplex real-time polymerase chain reaction of patients with scanty positive AFB sputum smears and mycobacterial culture.

	Positive C/S for MTB (n=40)	Negative C/S for MTB (n =52)	Total (n=92)
Positive PCR for MTB (n= 26)	25	1	26
Negative PCR for MTB (n= 66)	15	51	66
	40	52	92

Abbreviations: AFB=acid fast bacilli, PCR = Polymerase Chain Reaction, C/S = Culture, MTB=*Mycobacterium tuberculosis*, scanty AFB= positive acid fast staining but < 10 AFB/100 OF

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Relapse Rate and Clinical Risk Factors Affecting the Treatment of Graves' Disease

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ABSTRACT

Objective: To determine the relapse rate of Graves' disease (GD) and identify important clinical risk factors for relapse.

Materials and Methods: This was a 10-year retrospective cohort study. Information was collected with ICD10 E050 codes for Graves' hyperthyroidism among Thai patients of both sexes and all ages with no history of pregnancy, thyroid storm or antithyroid drug (ATD) allergy.

Results: The 286 included GD patients had a relapse rate of 35% after ATD withdrawal for one year. The clinical risk factors associated with relapse were male sex (p = 0.014), smoking (p = 0.001), serum free T4 (FT4) levels > 2 times the upper normal range at diagnosis (p = 0.005), duration for maintenance treatment < 6 and 9 months (p < 0.005) compared with remission. A TSH level < 1 mIU/L (p = 0.060) and MMI > 2.5 mg per day before ATD withdrawal (p = 0.094) trended toward associations with relapse. The clinical factors that predicted GD relapse were serum FT4 levels at diagnosis (p = 0.006) and serum free T3 (FT3) levels before ATD withdrawal (p = 0.019).

Conclusion: Male sex, smoking and serum FT4 levels at diagnosis > 2 times the normal range were significant clinical factors for GD relapse in Thai patients. To reduce the relapse rate in the first year, MMI should be used in maintenance periods for 9 to 12 months with serum FT3 levels within low-normal ranges before ATD withdrawal. This would promote future guidelines for GD management in Thailand.

Keywords: Relapse; Graves' disease; antithyroid drugs; risk factors (Siriraj Med J 2021; 73: 451-461)

INTRODUCTION

Graves' disease (GD) is the most common cause of thyrotoxicosis. There are 3 modalities for management: antithyroid drugs (ATDs), radioactive iodine (RAI) and surgery.[1] According to survey results for the management of GD conducted by members of the Endocrine Society of the USA, the proportions of respondents with ATDs, RAI therapy and thyroid surgery as the preferred modes of therapy for uncomplicated GD are 53.9%, 45% and 0.7%, respectively.[2] However, among members of the Endocrine Society of Thailand, ATDs are the preferred choice of therapy (90.8%), with RAI therapy (9.2%) being the second most preferred.[3] In a corresponding survey of the members of the European Thyroid Association, ATDs were preferred by 83.8% of respondents and RAI therapy by 14.1% of respondents.[4] ATDs have been used to treat hyperthyroidism for more than 75 years.[5] In Thailand, 2 ATD types are used: methimazole (MMI) and propylthiouracil (PTU). Generally, the guidelines suggest MMI for treatment of GD or other causes of hyperthyroidism for all patients except patients with a first-trimester pregnancy, thyroid storm or minor reaction to MMI.[1,6] The treatment goal is restoration to normal thyroid function as soon as reasonably possible even though this mode is not curative among GD patients but suppresses their autoimmunity for only a short time.[7]

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The GD relapse and remission rates vary among geographical areas due to varying in the duration of treatment and different iodine status.[8-15] In the USA, the remission rate after ATD use for at least 12-18 months is 20-30%[10], while that in Europe is 50-60% after medication use for 5-6 years.[11] In Sweden, the relapse rate is 30.2% after stopping ATDs within 1 year.[12] In Japan, the remission rate is 68% for at least 2 years after treatment with maintenance doses of MMI for a minimum of 19 months.[13] There are many clinical factors for predicting GD relapse, such as male sex, smoking, a large thyroid goiter, the FT3/FT4 ratio, and continuous TSH suppression.[16-21]

In principle, ATDs are used in most patients with varying characteristics and are easy to access. However, the responses to ATD treatment differ according to race and continental regions worldwide.[10-14,18] The previous study in Thailand showed the low remission rate of children with GD only 18.8% after ATD treatment about 3.5 years.[22] A recent study in adult showed the early relapse of hyperthyroidism within a year after MMI withdrawal, 27.8% of median duration of treatment being nearly 2 years.[23] However, there is no clear guideline for the treatment of such patients. Current management practices are based on those of outside countries; thus, there are variations in remedies, especially regarding treatment dosages and durations. Thus, this study aimed to evaluate the GD relapse rate in Thailand and clinically relevant factors affecting the disease that can be used as basic information to properly set treatment guidelines and reduce the cost of screening for TRAb levels before discontinuing ATDs in some groups of patients. The primary objective was to determine the GD relapse rate. The secondary objective was to identify important clinical factors for GD relapse.

MATERIALS AND METHODS

Study population

This retrospective cohort study included consecutive searches of an electronic database with ICD10 E050 for new diagnoses of GD, was 526 patients, that had been referred to and/or treated in all clinics at Charoenkrung Pracharak Hospital, Bangkok, Thailand, between 1st January 2007 and 31st December 2017. The inclusion criteria were Thai patients of any age and gender. The exclusion criteria were patients with thyroid storms, major side effects of ATDs, a history of prior RAI treatment and thyroidectomy, loss to follow-up before remission, pregnancy and death before remission. Of those, 286 patients were eligible by reviewing all documentations carefully. GD was diagnosed based on clinical diagnosis based on indicators including high serum FT4 and/or serum FT3 with suppressed TSH accompanied by one of the following clinical characteristics for at least 3 months: symptoms and signs of hyperthyroidism (pulse >100 bpm and/or fine tremor and/or warm and moist skin and/or onycholysis and/or proximal muscle weakness), diffuse goiter with or without bruit, thyroid-associated ophthalmopathy (TAO), and thyroid dermopathy (pretibial myxedema and/or thyroid acropachy). Goiter was categorized by physical examination by a physician: normal gland (10-20 gm), mild-to-moderate goiter (25-60 gm) and large goiter (>60 gm). TAO was classified according to nonspecific signs, lid lag and retraction, and specific signs, including exophthalmos.[14] An ATD titration regimen was commonly used. The duration of ATDs was divided into two periods: initial treatment and maintenance treatment. The initial period consisted of MMI > 5 mg/day or PTU > 100 mg/day followed by a maintenance period that consisted of MMI ≤ 5 mg/day or PTU ≤ 100 mg/day until withdrawal therapy. This study was approved by the Human Research and Ethics Committee of Bangkok Metropolitan Administration, Bangkok, Thailand.

Laboratory measurement

Serum FT4, FT3 and TSH levels were measured by electrochemiluminescent immunoassay (ECLIA) methods using Roche Diagnostics with normal ranges of 0.93 - 1.70 ng/dL, 2.20 - 4.40 pg/mL and 0.270 - 4.200 mIU/L, respectively. If any result value is too large or too small to measure, I will display only the highest or lowest reading for analysis.

Definitions of clinical outcomes

Remission was defined as euthyroidism with normal FT4, FT3 and TSH maintained for at least one year after ATD withdrawal. Relapse was defined as recurrence of hyperthyroidism, including low TSH with or without high FT4 and/or FT3 during follow-up after withdrawal therapy for less than one year.

Statistical analyses

The relapse rate and remission rate are presented as percentages. Continuous variables are described as means with standard deviations or medians with interquartile ranges (IQRs) and were analyzed using Student's *t* test or the Mann-Whitney U test. Categorical variables are presented as percentages and were examined with the chi-square test or Fisher's exact test. The multivariate analysis included age, sex, smoking, the presence of TAO, goiter size, serum FT4 and FT3 levels at diagnosis and after ATD withdrawal, serum TSH levels after stopping ATDs and ATD dosage before withdrawal, which were

analyzed by using logistic regression; the data are presented as the odds ratio (OR), 95% confidence interval (CI) and *p*-value. All two-sided *p*-values <0.05 were considered statistically significant. Statistical analyses were carried out using SPSS version 26.0.

RESULTS

Baseline and clinical characteristics of patients with GD

The mean age at diagnosis of the 286 GD patients was 43.5 ± 13.6 years (minimum, 8 years; maximum, 80 years), and 77.3% were female. Most were non-smokers (94.1%), and 48.3% had no underlying disease. Among patients with underlying disease, the most common disease was dyslipidemia, 36% (Table 1).

Regarding the clinical characteristics, laboratory results and treatments, there was no TAO in 82.5% of cases and mild-to-large goiter at diagnosis in 68.5% of cases. The mean levels of serum FT4, FT3 and TSH at diagnosis were 5.38 ± 2.14 ng/dL, 18.30 ± 8.87 pg/mL and 0.0096 ± 0.023 mIU/L, respectively. The mean serum FT4, FT3 and TSH levels after ATD withdrawal were 1.22 ± 0.29 ng/dL, 2.88 ± 0.49 pg/mL and 3.235 ± 0.289 mIU/L, respectively. In total, 92.7% were treated with MMIs. The median initial treatment duration was 7 months (IQR 4 - 14.25 months). The median maintenance treatment duration was 19 months (IQR 12 - 27.25 months). The median follow-up duration was 32 months (IQR 16 - 55 months) (Table 1).

GD patient baseline and clinical characteristics in the relapse and remission groups

The relapse rate of GD was 35% after ATD withdrawal for one year. We divided patients based on clinical features into the relapse group (n = 100) and remission group (n = 186). The baseline and clinical features, including laboratory results and treatments, are shown in Table 2. The relapse group had a mean age at diagnosis of 45 ± 15 years, most patients had no TAO (84.4%), however there were more TAO in relapse group (21%) than in remission group (15.6%) statistical insignificantly and a majority had a goiter of approximately 25-60 gm (68.5%), which was not significantly different from that of the remission group.

Clinical factors, including male sex (OR 2.01; 95%CI 1.13 – 3.53; p = 0.014) and smoking (OR 6.80; 95%CI 2.15 – 21.46; p = 0.001), were significantly different between the two groups. There was more underlying diabetes mellitus, hypertension, dyslipidemia in relapse group than in remission group but not significantly. The mean serum FT3 and FT4 level at diagnosis in the relapse group was insignificantly higher than that of remission group. The mean serum FT4 level before stopping ATDs in the relapse group (1.17 ± 0.25 ng/dL) was significantly lower than that of the remission group (1.25 ± 0.31 ng/dL) (p = 0.047). The mean dose of MMI before stopping ATDs in the relapse group (3.4 ± 2.4 mg/day) was significantly higher than that in the remission group (2.8 ± 1.7 mg/day) (p = 0.014). The median duration of remission in the relapse group was 4 months, in contrast to 1 year and 11 months in the remission group (Table 2).

Further analyses of the factors expected to influence future treatment were conducted. This was partly based on a study of a predictive model for GD recurrence based on clinical features or the Graves' Recurrence Events After Therapy (GREAT) score[25] (Table 3). The relapse group had serum FT4 levels > 2 times the normal ranges at diagnosis (OR 2.49; 95%CI, 1.30 - 4.77; p = 0.005) and intervals of the maintenance periods of ATDs < 6 and 9 months, which were significantly different from those of the GD remission group (OR 0.4; 95%CI, 0.21 - 0.78; p = 0.006 and OR 0.56; 95%CI, 0.31 - 0.99; p = 0.043, respectively). The relapse group tended to have TSH levels < 1 mIU/L before discontinuation and MMI doses > 2.5 mg/day before discontinuation in contrast to those of the remission group, but the difference was not statistically significant.

Other factors considered included age < 40 versus > 40 years, the thyroid gland abnormality at diagnosis, serum FT3 three or more times the normal value, and an ATD maintenance period duration less than or equal twelve months, but the comparisons between relapse and remission groups revealed no statistically significant differences.

As shown in Table 4, there were three clinical factors that predicted the likelihood of GD recurrence, namely, serum FT3 levels at diagnosis, serum FT4 levels at diagnosis and serum FT3 levels prior to discontinuation, and differences were statistically significant (p <0.05). It was found that when serum FT3 at diagnosis was reduced by 1 pg/mL, there was a chance of reducing GD relapse by 0.84 (OR 0.84; 95%CI, 0.71 - 0.99; p = 0.036). When serum FT4 at diagnosis was increased by 1 ng/dL, there was an increased risk of GD relapse by 2.35 (OR 2.35; 95%CI, 1.28 -4.32; p = 0.006). When serum FT3 levels before discontinuation increased by 1 pg/mL, there was an increased risk of relapsed GD by 3.85 (OR 3.85; 95%CI, 1.24 – 11.93; p = 0.019), so serum FT3 was considered the most important variable. If the serum FT3 value changed, it might also change the course of the disease.

TABLE 1. Baseline and clinical characteristics, including laboratory results and treatments, of 286 patients with GD.

Characteristics	Total, *N* = 286 (%)
Baseline characteristics	
Age at diagnosis (years), min–max	43.5 ± 13.6, 8–80
Sex	
Female	221 (77.3)
Male	65 (22.7)
Smoking	
Never smoker	269 (94.1)
Smoker	15 (5.2)
Former smoker	2 (0.7)
Coexisting disease	
None	138 (48.3)
Hypertension	90 (31.5)
Diabetes mellitus	42 (14.7)
Dyslipidemia	103 (36.0)
Cardiac diseases	11 (3.8)
Cerebrovascular diseases	8 (2.8)
Renal diseases	2 (0.7)
Liver diseases	4 (1.4)
Others	57 (19.9)
Clinical characteristics	
Thyroid-associated ophthalmopathy at diagnosis	
Absent	236 (82.5)
Lid lag and/or lid retraction	25 (8.7)
Exophthalmos	25 (8.7)
Goiter at diagnosis	
Normal (10–20 gm)	83 (29.0)
Mild to moderate (25–60 gm)	196 (68.5)
Large (> 60 gm)	7 (2.5)
Laboratory results and treatments	
Mean levels of thyroid function at diagnosis, min–max	
FT4 (ng/dL)*	5.38 ± 2.14, 1.16–7.77
FT3 (pg/mL)**	18.30 ± 8.87, 3.40–32.55
TSH (mIU/L)	0.0096 ± 0.023, 0.002–0.300
Mean levels of thyroid function at ATDs withdrawal, min–max	
FT4 (ng/dL)#	1.22 ± 0.29, 0.25–2.94
FT3 (pg/mL)##	2.88 ± 0.49, 1.21–4.39
TSH (mIU/L)###	3.235 ± 0.289, 0.032–84.960
Type of ATDs	
MMI	265 (92.7)
PTU	21 (7.3)
Median initial duration of treatment (months), IQRs	7, 4–14.25
Median maintenance duration of treatment (months), IQRs	19, 12–27.25
Median total duration of treatment (months), IQRs	27.5, 24–38
Median follow-up duration of treatment (months), IQRs	32, 16–55
Median duration of remission (months), IQRs	14, 5–31

*N = 277, **N = 261, #N = 239, ##N = 202, ###N = 284 due to missing data or data not obtained
GD, Graves' disease; ATDs, antithyroid drugs; MMI, methimazole; PTU, propylthiouracil; IQR, interquartile range

TABLE 2. Baseline and clinical characteristics, including laboratory results and treatments, of the relapse group versus the remission group of patients with GD.

Characteristics	Relapse group, *n* = 100 (%)	Remission group, *n* = 186 (%)	*p*-value, odds ratio (95% CI)
Baseline characteristics			
Age at diagnosis (years), min–max	45.0 ± 15.0, 12–80	42.7 ± 12.7, 8–76	
15 or under	2 (2.0)	6 (3.2)	
16–25	10 (10.0)	15 (8.1)	
26–35	11 (11.0)	27 (14.5)	
36–45	30 (30.0)	60 (32.3)	0.187§
46–55	24 (24.0)	49 (26.3)	
56–65	15 (15.0)	23 (12.4)	
Over 65	8 (8.0)	6 (3.2)	
Sex			
Female	69 (69.0)	152 (81.7)	**0.014**†, 2.01 (1.13–3.53)
Male	31 (31.0)	34 (18.3)	
Smoking			
Never smoker	87 (87.0)	182 (97.8)	
Smoker	11 (11.0)	4 (2.2)	**0.001**†, 6.80 (2.15–21.46)
Former smoker	2 (2.0)	0 (0.0)	
Coexisting disease			
None	42 (42.0)	96 (51.6)	
Hypertension	40 (40.0)	50 (26.9)	
Diabetes mellitus	22 (22.0)	20 (10.8)	
Dyslipidemia	40 (40.0)	63 (33.9)	
Cardiac diseases	3 (3.0)	8 (4.3)	0.121†, 1.47 (0.90–2.41)
Cerebrovascular diseases	4 (4.0)	4 (2.2)	
Renal diseases	2 (2.0)	0 (0.0)	
Liver diseases	1 (1.0)	3 (1.6)	
Others	24 (24.0)	33 (17.7)	
Clinical characteristics			
Thyroid-associated ophthalmopathy at diagnosis			
Absent	79 (79.0)	157 (84.4)	
Lid lag and/or lid retraction	12 (12.0)	13 (7.0)	0.348†, 1.44 (0.77–2.68)
Exophthalmos	9 (9.0)	16 (8.6)	

TABLE 2. Baseline and clinical characteristics, including laboratory results and treatments, of the relapse group versus the remission group of patients with GD. (Continue)

Characteristics	Relapse group, *n* = 100 (%)	Remission group, *n* = 186 (%)	*p*-value, odds ratio (95% CI)
Goiter at diagnosis			
Normal (10–20 gm)	28 (28.0)	55 (29.6)	
Mild to moderate (25–60 gm)	69 (69.0)	127 (68.3)	0.882†, 1.08 (0.63–1.85)
Large (> 60 gm)	3 (3.0)	4 (2.2)	
Laboratory results and treatments			
Mean levels of thyroid function at diagnosis			
FT4 (ng/dL)*	5.66 ± 1.94	5.24 ± 2.24	0.155‡
FT3 (pg/mL)**	18.79 ± 8.48	18.06 ± 9.08	0.548‡
TSH (mIU/L)	0.01 ± 0.03	0.01 ± 0.02	0.372‡
Mean FT3/FT4 ratio at diagnosis (pmol/L)	0.38 ± 0.10	0.41 ± 0.10	0.317‡
Mean levels of thyroid function at ATD withdrawal			
FT4 (ng/dL)#	1.17 ± 0.25	1.25 ± 0.31	**0.047**‡
FT3 (pg/mL)##	2.94 ± 0.54	2.84 ± 0.47	0.423‡
TSH (mIU/L)###	4.45 ± 10.64	2.58 ± 1.83	0.317‡
Type of ATD			
MMI	93 (93.0)	172 (92.5)	0.871†, 0.93 (0.36–2.37)
PTU	7 (7.0)	14 (7.5)	
Mean dose of ATDs at diagnosis (mg/day)			
MMI	37.1 ± 9.7	49.5 ± 10.9	0.962‡
PTU	158.2 ± 107.9	204.3 ± 89.2	0.219‡
Mean dose of ATDs before withdrawal (mg/day)			
MMI	3.4 ± 2.4	2.8 ± 1.7	**0.014**‡
PTU	67.9 ± 42.6	41.1 ± 12.4	0.091‡
Median initial duration of treatment (months), IQR	8, 5–16	7, 4–13.25	0.249‡
Median maintenance duration of treatment (months), IQR	19, 8–31	19, 14–25.25	0.804‡
Median total duration of treatment (months), IQR	28, 24–43.5	27, 24–37	0.445‡
Median duration of remission (months), IQR	4, 3–7.75	23, 14.75–40.25	**<0.001**‡

§Independent t-test, †chi-square test, ‡Mann-Whitney U-test

*N = 277, **N = 261, #N = 239, ##N = 202, ###N = 284 due to missing data or data not obtained

GD, Graves' disease; 95% CI, 95% confidence interval; IQR, interquartile range

TABLE 3. Specific clinical risk factors that are expected to influence future treatment in the relapse group versus remission group of patients with GD.

Characteristics	Relapse group, *n* = 100 (%)	Remission group, *n* = 186 (%)	*p*-value, odds ratio (95% CI)
Baseline and clinical characteristics			
Age at diagnosis			
< 40 vs ≥ 40 years	39 (39.0) vs 61 (61.0)	74 (39.8) vs 112 (60.2)	0.897†, 1.03 (0.63–1.70)
Goiter at diagnosis			
Normal vs abnormal glands	28 (28.0) vs 72 (72.0)	55 (29.6) vs 131 (70.4)	0.780†, 1.08 (0.63–1.85)
Laboratory results and treatments			
Level of thyroid function at diagnosis			
FT4 < 2 vs ≥ 2 times the normal range	14 (14.6) vs 82 (85.4)*	54 (29.8) vs 127 (70.2)*	**0.005**†, 2.49 (1.30–4.77)
FT3 < 3 vs ≥ 3 times the normal range	23 (26.4) vs 64 (73.6)*	64 (36.8) vs 110 (63.2)*	0.095†, 1.62 (0.92–2.86)
Level of thyroid function at ATDs withdrawal			
TSH ≤ 1 vs > 1 mIU/L	22 (22.2) vs 77 (77.8)	25 (13.5) vs 160 (86.5)	0.060†, 0.55 (0.29–1.03)
Dose of MMI before withdrawal			
≤ 2.5 vs > 2.5 mg/day	63 (63.9) vs 37 (37.0)	135 (72.6) vs 51 (27.4)	0.094†, 1.56 (0.93–2.61)
Duration of maintenance treatment			
≤ 6 vs > 6 months	23 (23.0) vs 77 (77.0)	20 (10.8) vs 166 (89.2)	**0.006**†, 0.40 (0.21–0.78)
≤ 9 vs > 9 months	28 (28.0) vs 72 (72.0)	33 (17.7) vs 153 (82.3)	**0.043**†, 0.56 (0.31–0.99)
≤ 12 vs > 12 months	31 (31.0) vs 69 (69.0)	41 (22.0) vs 145 (78.0)	0.096†, 0.63 (0.36–1.86)

*Missing data or data not obtained, †chi-square test
GD, Graves' disease; 95% CI, 95% confidence interval; ATDs, antithyroid drugs

TABLE 3. Demonstrated clinical factors for prediction of relapsed GD by Wald (forward stepwise) logistic regression.

Influencing clinical risk factors (variables)	β	S.E.	Wald	*p*-value	Odds ratio	95% CI for odds ratio	
						Lower	Upper
Constant	-4.252	2.510	2.869	0.090	0.014		
Age	0.021	0.016	1.689	0.194	1.021	0.989	1.055
Sex	-0.136	0.549	0.061	0.805	0.873	0.297	2.562
Smoking	1.116	1.077	1.074	0.300	3.052	0.370	25.187
TAO	0.411	0.721	0.325	0.569	1.508	0.367	6.199
Goiter size	-0.543	1.221	0.198	0.656	0.581	0.053	6.360
Serum FT3 level at diagnosis	-0.181	0.086	4.399	**0.036**	0.835	0.705	0.988
Serum FT4 level at diagnosis	0.855	0.310	7.602	**0.006**	2.352	1.281	4.321
Serum FT3 level before ATDs withdrawal	1.349	0.577	5.465	**0.019**	3.852	1.243	11.933
Serum FT4 level before ATDs withdrawal	-2.141	1.326	2.607	0.106	0.117	0.009	1.581
Serum TSH level before ATDs withdrawal	0.000	0.050	0.000	1.000	1.000	0.907	1.103
Doses of ATDs before withdrawal	0.072	0.064	1.254	0.263	1.075	0.947	1.219

GD, Graves' disease; 95% CI, 95% confidence interval; TAO, Thyroid-associated ophthalmopathy; ATDs, antithyroid drug

DISCUSSION

This 10-year retrospective study of 286 first-diagnosed Thai patients with GD focused on ATD therapy only. The relapse rate was 35% among patients with GD after first-year ATD withdrawal therapy. Overall, this study had a median follow-up of 2 years and 8 months, the relapse group was found to comprise males and smokers, and the mean MMI doses before discontinuation were greater than those in the remission group (Table 2). From which the mean serum FT4 level before stopping ATDs in the relapse group was significantly lower than that of the remission group while the mean serum FT3 and FT4 level at diagnosis in the relapse group was higher than that of remission group but insignificantly (Table 2), probably due to T3 toxicosis was predominated in the relapse group. When subgroup analysis for each factor that was expected to affect future treatment was performed, the relapse group had a serum FT4 level at diagnosis ≥ 2 times the normal range, and the maintenance treatment duration, less than 6 and 9 months, was significantly different from that of the remission group. However, there was no significant difference in use of this treatment for up to 12 months compared with more than 12 months. Additionally, the relapse group tended to have serum TSH levels ≤ 1 mIU/L and doses of MMI before discontinuation of more than 2.5 mg/day compared with the remission group, but the difference was not statistically significant (Table 3). When important clinical factors were used in relation to GD relapse during the first year after discontinuation, elevations in serum FT4 levels at diagnosis of 1 ng/dL and elevations in serum FT3 levels before discontinuation of 1 pg/mL increased the risk of GD recurrence by 2.35 and 3.85, respectively (Table 4).

Compared to previous studies in Asia, the relapse rate was 32 - 52% after one to two years of ATD discontinuation.[13,14,23,26,27] The clinical factors related to relapse were younger age[27], large thyroid gland[14,27], TAO after drug treatment[14], higher serum FT3 and FT4 levels[27], FT3/FT4 (pmol/l) ratio[14], continued TSH suppression levels[14], TRAb levels[14] and a duration of ATD use in the maintenance period of less than 6 months.[26] The clinical factors related to remission were older age (45.6 ± 10.3 years)[14] and a minimum dose of MMI of 5 mg every other day for at least 19 months.[13] Compared with rates from previous studies in Europe and Africa, the relapse rate was 30 - 49% after one year of discontinuation.[28-30] The clinical factors related to relapse were < 40 years of age; smoking; TAO; thyroid size by ultrasound; serum FT4, FT3, and TRAb levels; and total T4 levels ≥ 2 times the upper normal range.[30] The clinical factors related to remission were female sex, nonsmoking status, no TAO and duration of treatment of more than 2 years.[28] This study showed a relapse rate similar to that of previous studies and confirmed that some clinical risk factors influence relapse in both Asian and Western countries. However, we found that ATDs should be taken as maintenance treatment for more than 9 months and a trend toward having TSH > 1 mIU/L before discontinuation medications to reduce relapse.

In Thailand, a recent study showed the relapse rate 37% within first year of ATD withdrawal as well as this study (35%). However, the factors associated with early relapse in that study showed patients 40 years of age or less and the highest quartile of serum T3 level at the time of diagnosis[23], in contrast with this study showed no statistically significant of those factors.

When statistically significant clinical factors associated with relapsed GD after one year of ATD withdrawal were assessed in the context of previous studies, we made several observations. First, male sex: many previous studies have shown that male sex was a factor associated with the GD relapse. One study showed that men usually have a larger thyroid gland and more of a family history of autoimmune thyroid disease than women.[31] Another study found that men have a larger thyroid gland and still smoked more than women.[32] This was consistent with the results of our study showing that males smoked more cigarettes (24.6%) than females (0.5%), but there was no difference in thyroid size in our study. Second, smoking: in addition to being a major risk factor for developing GD[33], studies comparing smokers to nonsmokers found that elimination of serum FT4, serum FT3 and TRAb among smokers was slow, causing antibodies to be present longer, leading to disease progression.[34] This support the results of our study showing that smoking is associated with relapse. Third, serum FT4 levels at diagnosis ≥ 2 times the normal range: a previous study showed a positive correlation between FT4 and TRAb levels, indicating that higher FT4 levels were also associated with a high TRAb level[35], thus promoting disease recurrence. This was consistent with our study results. Fourth, higher mean serum FT3 levels before discontinuation: a previous study found that the remission phase is related to normal thyroid hormone levels regardless of the doses received.[36] This supports the results of our study, which show that more of such factors are associated with a higher risk of relapsed GD. Fifth, administration of more than 2.5 mg/day MMI prior to discontinuation and a maintenance treatment duration less than 6-9 months: these findings are consistent with a recent study showing that a 2.5-mg daily dose of MMI for periods of less than 6 months is associated with relapse[28] and that treatment with MMI

5 mg every other day for no longer than 6 months has a higher rate of relapse.[13]

According to the American Thyroid Association[1] and the European Thyroid Association[37], it is recommended that the total dose of ATDs is given for approximately 12 to 18 months and discontinued after the TSH and TRAb levels are normal; the consequent remission rate is 20-30%. Based on studies in 1994[38] and 2004[39], treatment with ATDs for 18 months versus 6 months has lower rates of recurrence. However, in a meta-analysis published in 2005[40], the remission rate did not increase after taking ATDs for more than 18 months. Consistent with the results of this study, the median total duration of ATDs was 28 months, and no difference was found between the relapse and remission groups. However, this study provides additional data showing that low-dose ATDs should be taken for 9-12 months before stopping medications to reduce the relapse rate.

Benefits

1. Knowledge of the relapse rate and the important clinical factors for GD relapse in Thai patients.
2. Ability to provide ATD treatment with appropriate dosages and durations of administration to minimize the likelihood of GD relapse in Thai patients.
3. Usefulness as the basis for planning suitable guidelines in the management of GD in Thailand that lead to a reduction in costs associated with measuring TRAb levels before discontinuation of ATDs for patients among whom these measurements are unnecessary, such as females, nonsmokers, those with serum FT4 levels at diagnosis < 2 times the normal range, and those with low serum FT3 levels before discontinuation.

Limitations

This is a 10-year retrospective study using data from past medical records. Patients were examined by physical examination, which might lead to inaccuracies regarding certain clinical features. Some information was considered missing data, namely, in cases in which data was lost or cases that were not fully investigated, with data including serum FT4, FT3 and TSH levels at diagnosis and before ATD withdrawal. However, these cases were rare and comprised missing data for only select hormones in a few patients but not all hormones at once. Therefore, only the information contained in the dataset was analyzed.

Suggestions

Further studies should include a prospective trial with physical examination by any internist or endocrinologist and set the protocol for the laboratory test. For further reliability, a Hertel ophthalmometer and ultrasound may be used for the measurement of thyroid gland size and additional TRAb levels to help provide more accurate data.

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Lower Extremity Reconstruction with Vascularized Free-Tissue Transfer: 20 Years of Experience in the Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, Thailand

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ABSTRACT

Objective: The reconstruction of extensive soft-tissue defects in the lower extremity still poses a great challenge to plastic and reconstructive surgeons. The ideal approach is to achieve a proper soft-tissue coverage with a well-vascularized flap, which results in a durable weight-bearing surface and permits normal joint motion. This study aims to retrospectively analyze the outcomes of lower-extremity reconstruction with vascularized free-tissue transfer performed at our plastic surgery division.

Materials and Methods: A retrospective chart review was performed regarding 58 patients with defects in the lower extremity which were reconstructed with vascularized free-tissue transfers between 2000 and 2019. Forty-four of the patients were male, and 14 were female. The mean age was 44.4 years (range: 6-89 years). The most common indication for free-flap surgery was a secondary reconstruction after tumor eradication (23 cases, 39.7%), and 84.8% of the defects were exposed bare bones, tendons, or joints.

Results: In our 58 reviewed cases, the foot was the most common area requiring reconstruction with a free flap (68.9%), and the mean defect size was 12.5 x 8.1 cm. The most commonly used free flap was the Anterolateral thigh free flap (39.7%), followed by the Gracilis free flap (29.3%), and the Superficial circumflex iliac artery-perforator free flap (10.4%). The recipient vessels most frequently used were posterior tibialis vessels (53.4%). The overall flap-survival rate was 75.9%, though there was an increased survival rate of up to 85.7% in the last five years of the period studied. The flap-salvage rate was 40.9%, and arterial thrombosis was the major cause of flap loss (50%). Factors associated with free-flap failure were re-exploration and free flap surgery after tumor or cancer eradication. The most common post-operative complication was flap-wound dehiscence (10.3%). Two patients received a flap correction due to bulkiness, and three had recurrence of ulceration.

Conclusion: Microvascular free-tissue transfers for lower- extremity-defect reconstructions are reliable and valuable as a surgical technique. In 20 years of experience, we've had an overall flap survival rate of 75.9%. Factors associated with free-flap failure were re-exploration and free flap surgery after tumor or cancer eradication. And our flap of choice was the Anterolateral thigh free flap.

Keywords: Lower extremity reconstruction; foot reconstruction; free flap; microsurgery; flap surgery (Siriraj Med J 2021; 73: 462-470)

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INTRODUCTION

Microvascular free-tissue transfers have become the preferred reconstructive technique to manage complex wounds and surgical defects after an ablative procedure, as the result of trauma. It is also the preferred technique to deal with special-requirements situations such as facial reanimation with a functioning muscle transfer in facial-palsy patients. There have been numerous clinical reports on free-tissue transfer since the microvascular technique was introduced in the early 1960s.[1-5] Previous data demonstrate that microvascular free-tissue transfers allow for reliable, single-stage, and immediate reconstruction involving more complex defects from various etiologies.[6-17] Many case series have also reported a high flap-success rate.

To date, reconstruction of extensive soft-tissue defects in the lower extremity still poses a great challenge to reconstructive surgeons. A defect on the lower limb will require some form of reconstruction in the majority of cases. Also, permanent scar formation after tight closure or after skin grafting or inadequate local flap use can result in pain and unstable wounds when a person is bearing weight. Thus, the ideal approach to treating a lower-limb defect is to achieve proper soft-tissue coverage with a well-vascularized flap, which results in a durable weight-bearing surface and permits normal ankle motion.[18] There is no debate that only free flaps can cover extensive soft tissue defects and provide satisfactory functional outcomes without amputation.[19,20]

At our institute, we started performing free-flap reconstruction for defects in the lower extremities in1992, but we had not collected the data nor had we evaluated the final outcomes after surgery. This study aims to retrospectively analyze the outcomes of lower-extremity reconstruction with vascularized free-tissue transfer performed at the Division of Plastic Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University.

MATERIALS AND METHODS

A retrospective chart review was conducted regarding all patients who underwent free-flap surgery for lower-extremity defect reconstruction at the Division of Plastic Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital from January, 2000 to December, 2019. The age, gender, underlying disease, other risk factors (smoking, obesity, etc.), American Society of Anesthesiologists (ASA) classification, indication for surgery, location of defect, type of flap, operative time, inflow and outflow vessels, type of anastomosis, vein-graft usage, ischemic time, length of hospital stay, length of intensive-unit stay, flap-success rate, re-exploration surgery, salvage rate, and perioperative complications of all the patients were recorded. These data were collected in a database and were available for statistical analysis.

Data assessment

The total operative time was defined as the time between the first incision and the wound closure. The ischemic time was defined as the time from the transection of the vascular pedicle to a complete arterial anastomosis and then the release of the vascular clamp. The flap-success rate was defined as complete flap viability or partial flap loss that still achieved the primary indication of surgery.[18] Complications were divided between flap- related and general complications.

Statistical analysis of the data was performed using the Statistical Package for the Social Sciences (SPSS). The chi-square and Fisher's exact test were used to statistically compare variables that influence the flap-success rate and perioperative complications. A p-value of 0.05 or less was regarded as statistically significant.

RESULTS

Between January 2000 and December 2019, 58 microvascular free-tissue transfers for lower-extremity reconstruction were performed to cover and/or reconstruct various kinds of defects and diseases. There were 44 men and 14 women ranging in age from 6 to 89 years (mean: 44.4 years). Most of the cases involved a body mass index (BMI) from 18.5-22.9 kg/m^2 (32.7%). Ten of the 58 patients had underlying type 2 diabetic mellitus. The three most common areas in the lower extremity that needed reconstruction with a free flap were 1: a foot (40 of 58, 68.9%), 2: a leg (14 of 58, 24.1%) and 3: an ankle (5 of 58, 8.6%). Also, the plantar hind foot was the most common area of the foot that needed coverage (18 0f 58, 31.1%). Among the 58 patients, there were 23 defects (39.7%) caused from tumor eradication, 19 defects (32.8%) due to trauma, and 8 defects (13.8%) resulting from post-operative debridement due to severe soft-tissue infection. Fifty-five of the 58 cases (84.8%) were defects that exposed bare bones, bare tendons, or joints.

Seventy percent of the defects were between 5-15 cm. (41 of 58, 70.7%); the mean defect size was 12.5 x 8.1 cm. (a range from 3-26 cm.). The three most common flap types for reconstructing a lower-extremity defect were the Anterolateral thigh (ALT) free flap (23 of 58, 39.7%), followed by Gracilis free flap (17 of 58, 29.3%), and the Superficial circumflex iliac artery perforator (SCIP) free flap (6 of 58, 10.4%). The recipient arteries frequently

used for anastomosis were the posterior tibialis artery (31 of 58, 53.4%), the anterior tibialis artery (11 of 58, 19%), and the dorsalis pedis artery (8 of 58, 13.8%). More than 70% (45 of 58) of the cases were performed with one artery and one vein anastomosis, and 75.9% (44 of 58) of the patients had an arterial anastomosis with the end-to-end anastomotic technique (end to side, 24.1%). Most of the cases resulted, primarily, in a closing of the donor-site defect (42 of 58, 72.4%). (Table 1)

The mean duration of the total operative time (including surgical resection) was 520 minutes (range: 330-960 minutes). The mean flap harvesting time was 76 minutes (range: 40-120 minutes). The mean ischemic time was 59 minutes (range: 36-115 minutes). (Fig 1)

For post-operative outcomes, the overall flap-survival rate was 75.9% (44 of 58 patients). Re-exploration for anastomosis revisions was performed in 22 of 58 cases (37.9%), with a flaps-salvaging success rate of 40.9% (9 of 22 cases). The most common cause of flap revision was venous congestion (11 of 22 cases, 50%), but the most common cause of total flap failure was arterial thrombosis (7 of 14 cases, 50%). The recipient-site complications included wound dehiscence (6 of 58 cases, 10.3%), surgical-site infection (4 of 58 cases, 6.9%), and skin-graft loss (4 of 58 cases, 6.9%). Donor-site complications were found in 8.6% of patients, and the most common complication was wound dehiscence (2 of 58 cases, 3.5%). (Table 2)

Due to advances in microsurgical techniques and the better quality of microscopes and other instruments, the outcomes of lower-extremity reconstruction were significantly better than previous outcomes. In our institute, the overall free-flap survival rate rose from 66.7% (2000-2014) to 85.7% (2015-2019). Some microsurgeons have performed a more sophisticated flap to serve the specific requirements of the patient with the aim of restoring both the function and the appearance. (Table 4)

DISCUSSION

A major development in the reconstruction of defects in various locations was the introduction of free vascularized tissue transfer in the 1960s and 1970s, which enabled primary reconstruction of more complex and extensive defects. Our retrospective study presents our staff's experience in performing 58 microvascular free-tissue transfers for lower- extremity reconstruction over the last ten years, and it revealed a success rate of 75.9%. The majority of defects were connected to tumor ablative surgery and were between 5-15 cm. in the largest dimension. A gracilis muscle free flap with skin grafting was frequently done in the past, but the ALT free flap is our free flap of choice for lower-extremity reconstruction, due to its harvesting in a very large flap size, its long pedicle length, its low donor-site morbidity, and its modifications, such as enabling the inclusion of fascia or muscle into the flap.[21] The re-exploration rate in our series was 37.9%, with a salvage rate of 40.9%, which is quite low when compared to results from other studies.[22-24] In the last five years, the flap-salvage rate has significantly improved, from 16.7% (2000-2014) to 70% (2015-2019). (Tables 3 and 4)

Factors that influence flap failure and associated complications continue to be debated. Reported factors related to flap failure are the pre-operative status of the patient, his or her age, smoking, pre-operative radiation, flap type, surgical expertise, use of a vein graft, operative time, and re-exploration for anastomosis revision; however, we still do not have sufficient prospective data to definitively identify all of the significant causes. The factors related to wound complications and general complications were age, ASA class, diabetes mellitus, pre-operative radiation, smoking, and alcohol consumption, but there remains a lack of prospective data to definitively identify all of the significant causes.[7,14,25-29]

For our report, re-explorative surgery and free flap surgery after tumor or cancer eradication were significant factors vis a vis total flap failure. The failure rate of free-flap surgery significantly decreased commensurate with the increase in surgical expertise. (Tables 3 and 4)

CONCLUSION

Microvascular free-tissue transfers are reliable and valuable as a surgical technique in achieving successful lower- extremity defect reconstructions. At our institute, we began performing free-flap reconstruction for defects in the lower extremities in 1992. For the past 20 years, the overall flap-survival rate was 75.9% (44 of 58 cases); the re-exploration rate was 37.9% (22 of 58 cases); and the rate of successfully salvaging flaps was 40.9% (9 of 22 cases). The anterolateral thigh free flap was the flap of choice in our lower-extremity defect reconstruction. The most commonly used recipient vessels were the posterior tibialis artery and vein. In our institute's experience, the key factors associated with lower extremity free-flap failure were re-exploration and free flap surgery after tumor or cancer eradication. Finally, this study presents 20 years of experience and surgical outcomes in lower-extremity defect reconstruction with vascularized free-tissue transfers at the Division of Plastic Surgery, Department of Surgery, Faculty of Medicine Siriraj Hospital, Mahidol University. These results present a current baseline for lower-extremity free-flap surgery to which future advances in technique and practice may be compared.

TABLE 1. Patients' demographic and clinical data.

Characteristics	
Number of patients	58
Mean age, year (range)	44.4 (6-89)
Sex, male (%): female (%)	44 (75.9%): 14 (24.1%)
Body mass index, BMI (%)	
<18.5	11 / 58 (19.0%)
18.5 - 22.9	19 / 58 (32.7%)
23.0 - 24.9	12 / 58 (20.7%)
25.0 - 29.9	15 / 58 (25.9%)
30 - 40	1 / 58 (1.7%)
40.1 - 50	no
>50	no
ASA classification	
Class I	38
Class II	9
Class III	10
Class IV	1
Underlying disease	
Diabetic mellitus no. (%)	10 / 58 (17.2%)
Smoking no. (%)	7 / 58 (12.1%)
Peripheral arterial disease no. (%)	5 / 58 (8.6%)
Hypertension no. (%)	8 / 58 (13.8%)
Dyslipidemia no. (%)	3 / 58 (5.2%)
Paraparesis or paraplegia	2 / 58 (3.4%)
Hemiparesis or hemiplegia	1 / 58 (1.7%)
Tumor or cancer	2 / 58 (3.4%)
Chronic renal failure	1 / 58 (1.7%)
Defect site no. (%)	
Thigh	1 / 58 (1.7%)
Knee	3 / 58 (5.2%)
Popliteal fossa	no
Leg	14/58 (24.1%)
Proximal third leg	no
Middle third leg	1 / 58 (1.7%)
Distal third leg	2 / 58 (3.4%)
Proximal and middle third leg	no
Middle and distal third leg	3 / 58 (5.2%)
Proximal, Middle and distal third leg	3 / 58 (5.2%)
Ankle	5 / 58 (8.6%)
Foot	40/58 (68.9%)
Dorsal of foot	7 / 58 (12.1%)
Plantar forefoot	4 / 58 (6.9%)
Plantar midfoot	2 / 58 (3.4%)
Plantar hindfoot	18 / 58 (31.1%)
Plantar forefoot and midfoot	3 / 58 (5.2%)
Plantar midfoot and hindfoot	5 / 58 (8.6%)
Plantar forefoot, midfoot and hindfoot	1 / 58 (1.7%)

Defect size no. (%)	
Small defect (<5 cm)	3 / 58 (5.2%)
Medium defect (5-10 cm)	22 / 58 (37.9%)
Large defect (10.1-15 cm)	19 / 58 (32.8%)
Very large defect (15.1-20 cm)	8 / 58 (13.8%)
Giant defect (>20 cm)	6 / 58 (10.3%)
Etiology no. (%)	
Trauma	19 / 58 (32.8%)
Tumor or cancer eradication	23 / 58 (39.7%)
Infection	8 / 58 (13.8%)
Ischemic ulcer	2 / 58 (3.4%)
Venous ulcer	no
Diabetic ulcer or neuropathic ulcer	4 / 58 (6.9%)
Irradiation	no
Unstable scarring	2 / 58 (3.4%)
Exposure to bone, tendon, or joint no. (%)	55 / 58 (84.8%)
Mean defect size (Length x width, cm)	12.5 x 8.1 cm (Range 3-26 cm)
Flap source artery no. (%)	
Branch of lateral circumflex femoral artery	3 / 58 (5.2%)
Popliteal artery	no
Posterior tibialis artery	31 / 58 (53.4%)
Medial plantar artery	3 / 58 (5.2%)
Anterior tibialis artery	11 / 58 (19.0%)
Dorsalis pedis artery	8 / 58 (13.8%)
Peroneal artery	1 / 58 (1.7%)
Lateral supramalleolar artery	1 / 58 (1.7%)
Number of anastomosis no. (%)	
1 artery:1 vein	45 / 58 (77.6%)
1 artery:2 vein	13 / 58 (22.4%)
Type of arterial anastomosis no. (%)	
End to end	44 / 58 (75.9%)
End to side	14 / 58 (24.1%)
Free flap no (%)	
ALT free flap	23 / 58 (39.7)
VL free flap	1 / 58 (1.7%)
Latissimus dorsi free flap	3 / 58 (5.2%)
Gracilis free flap	17 / 58 (29.3)
MSAP free flap	4 / 58 (6.9%)
SCIP free flap	6 / 58 (10.4%)
Radial forearm free flap	1 / 58 (1.7%)
Parascapular free flap	1 / 58 (1.7%)
Internal oblique muscle free flap	1 / 58 (1.7%)
Temporoparietal fascial free flap	1 / 58 (1.7%)
Flap composition no. (%)	
Fasciocutaneous flap	34 / 58 (58.6%)
Muscle flap	18 / 58 (31.1%)
Musculocutaneous flap	4 / 58 (6.9%)
Fascial flap	1 / 58 (1.7%)
Osteocutaneous flap	1 / 58 (1.7%)
Donor-site closure no (%)	
Primary closure	42 / 58 (72.4%)
STSG	16 / 58 (27.6%)

Abbreviations: ASA = American Society of Anesthesiologists; ALT = Anterolateral thigh; VL = Vastus lateralis; MSAP = Medial sural artery perforator; SCIP = Superficial circumflex iliac artery perforator; STSG = Split-thickness skin graft

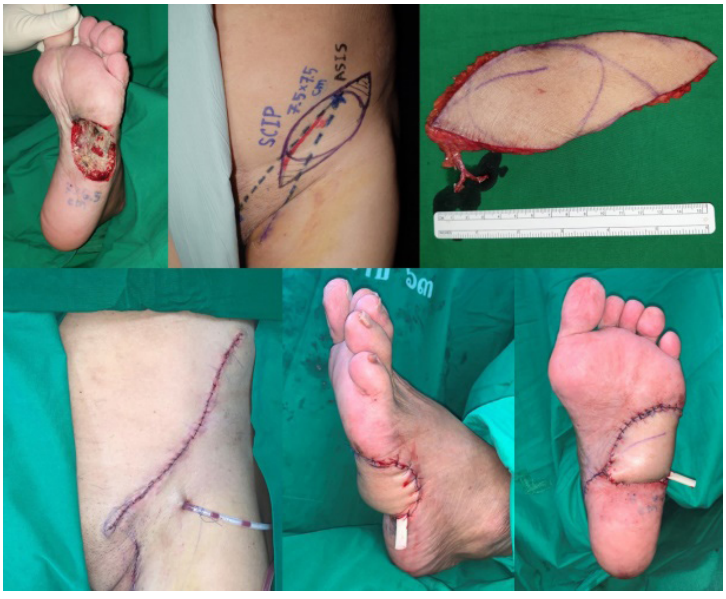

Fig 1. Left plantar midfoot defect after tumor eradication. The defect was reconstructed with superficial circumflex iliac artery perforator free flap which harvested from left groin area. Donor site defect was closed primarily. Inset flap was done and the microvascular anastomosis was performed with lateral plantar vessels.

TABLE 2. Post-operative surgical outcomes.

Flap survival no. (%)	44 / 58 (75.9%)
Flap loss no. (%)	
Partial loss no. (%)	11 / 58 (19.0%)
Complete loss no. (%)	14 / 58 (24.1%)
Arterial thrombosis no. (%)	7 / 14 (50.0%)
Venous thrombosis no. (%)	6 / 14 (42.9%)
Hypercoagulable stage no. (%)	1 / 14 (7.1%)
Flap revision no. (%)	22 / 58 (37.9%)
Arterial insufficiency no. (%)	10 / 22 (45.5%)
Venous congestion no. (%)	11 / 22 (50%)
Bleeding hematoma	1 / 22 (4.5%)
Flap salvage no. (%)	9 / 22 (40.9%)
Recipient site complication no. (%)	19 / 58 (32.8%)
Infection no. (%)	4 / 58 (6.9%)
Dehiscend wound no. (%)	6 / 58 (10.3%)
STSG loss no. (%)	4 / 58 (6.9%)
Hematoma no. (%)	2 / 58 (3.5%)
Seroma no. (%)	no
Donor site complication no. (%)	5 / 58 (8.6%)
Infection no. (%)	1 / 58 (1.7%)
Dehiscend wound no. (%)	2 / 58 (3.5%)
STSG loss no. (%)	1 / 58 (1.7%)
Hematoma no. (%)	no
Seroma no. (%)	1 / 58 (1.7%)
Flap correction	
Debulging no. (%)	2 / 44 (4.5%)
Recurrent ulcer no. (%)	3 / 44 (6.8%)

TABLE 3. Data comparisons between the group of flap success and flap failure.

Comparative data	Flap success (%)	Flap failure (%)	p-value
Case	44 (75.9%)	14 (24.1%)	0.165
Year 2000-2014	20 (66.7%)	10 (33.3%)	
Year 2015-2019	24 (85.7%)	4 (14.3%)	
Sex			0.931
Male	33 (75%)	11(25%)	
Female	11(78.6%)	3 (21.4%)	
ASA classification			**0.913**
Class I	29 (76.3%)	9 (23.7%)	
Class II	7 (77.8%)	2 (22.2%)	
Class III	7 (70%)	3 (30%)	
Class IV	1 (100%)	0 (0%)	
Cause			
Trauma	17 (89.5%)	2 (10.5%)	0.173
Tumor or cancer eradication	13 (56.5%)	10 (43.5%)	0.013*
Infection	7 (87.5%)	1 (12.5%)	0.701
Ischemic ulcer	2 (100%)	0 (0%)	0.977
Diabetic ulcer or Neuropathic ulcer	3 (75%)	1 (25%)	0.573
Unstable scarring	2 (100%)	0 (0%)	0.977
Underlying disease			
Diabetic mellitus no. (%)	7 (70%)	3 (30%)	0.944
Smoking no. (%)	6 (85.7%)	1 (14.3%)	0.858
Peripheral arterial disease no. (%)	4 (80%)	1 (20%)	0.749
Hypertension no. (%)	8 (100&)	0 (0%)	0.203
Dyslipidemia no. (%)	3 (100%)	0 (0%)	0.756
Paraparesis or paraplegia	2 (100%)	0 (0%)	0.977
Hemiparesis or hemiplegia	1 (100%)	0 (0%)	0.542
Tumor or cancer	1 (50%)	1 (50%)	0.977
Chronic renal failure	1 (100%)	0 (0%)	0.542
Flap revision			<0.001*
Yes (22 / 58, 37.9%)	9 / 21 (40.9%)	12 / 21 (59.1%)	
No (36 / 58, 62.1%)	35 / 37 (94.4%)	2 / 37 (5.6%)	

Abbreviation: ASA = American Society of Anesthesiologists

TABLE 4. Data comparisons between the group that had surgery from 2000-2014 versus 2015-2019.

Comparative data	Year 2000-2014	Year 2015-2019	p-value
Total cases	30 cases	28 cases	0.165
Flap success no (%)	20 / 30 (66.7%)	24 / 28 (85.7%)	
Flap failure no (%)	10 / 30 (33.3%)	4 /28 (14.3%)	
Flap revision no (%)	12 / 30 (40%)	10 / 28 (35.71%)	0.948
Arterial insufficiency no. (%)	6 / 12 (50%)	4 / 10 (40%)	
Venous congestion no. (%)	6 / 12 (50%)	5 / 10 (50%)	
Bleeding hematoma	0 / 12 (0%)	1 / 10 (10%)	
Flap salvage no (%)	2 / 12 (16.7%)	7 / 10 (70%)	0.036*

What is already known on this topic?

Based on the previous studies, the overall failure rate of microvascular free-flap reconstruction was 5 to 10%. Factors involved in free-flap failure and complications are still debated. Reported factors that relate to flap-failure include the patients' pre-operative status, age, smoking, pre-operative radiation, flap type, surgical expertise, use of vein graft, operative time, and re-exploration for anastomosis revision, but we still did not have sufficient prospective data to definitively identify all the significant causes.

What this study adds

Our institution has operated the microvascular free flap for lower-extremity reconstruction since 1992, but we had not previously collected data or had any long-term outcomes. This retrospective review presents our 20 years of clinical experience with 58 microvascular free-flap reconstruction from various kinds of indication. Our study is not only the first-ever data report from our institute, but may also be the first and largest report of lower-extremity free-flap reconstruction in Thailand.

Potential conflicts of interest

The author declares that there are no conflicts of interest related to this study.

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Social Skills Training Using the Thai Version of UCLA PEERS® in Thai Adolescents with Autism Spectrum Disorder

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ABSTRACT

Objective: To study the feasibility and effectiveness of the Thai version of UCLA PEERS® in Thai adolescents with autism spectrum disorder (ASD).

Materials and Methods: The UCLA PEERS® was modified to fit with Thai culture. Twelve adolescents, aged 11-19 years old, with ASD participated in this modified 10-session weekly group intervention during March to October 2015 at Siriraj Hospital, Bangkok, Thailand. Feasibility was assessed by parent satisfaction and session attendance rate. Effectiveness was assessed by social skills improvement rated by parents, Vineland Adaptive Behavior Scales (VABS), the Children's Depression Inventory (CDI), and the Clinical Global Impression-Improvement Scale (CGI-I).

Results: All enrolled participants completed the study. Parents' satisfaction with the program was 81.92%. The session attendance rates ranged from 83.3 to 100%. At the end of intervention, all of the skills trained in the program were rated as improved by at least half of parents. At 4-month follow-up, all but two skills (entering conversation and handling bullying) were still reported as improved by more than 50% of parents. VABS raw scores significantly increased in the domain of communication (95% confidence interval (CI): -2.25 to -0.89; p=0.036), daily living skills (95% CI: -3.70 to -0.47; p=0.016), and socialization (95% CI: -1.77 to -0.40; p=0.005), and significantly decreased in maladaptive behaviors domain (95% CI: 0.24 to 2.10; p=0.002). Six adolescents had CGI-I scores of very much improved or much improved.

Conclusion: The Thai version of UCLA PEERS® is a feasible and effective social skills intervention for Thai adolescents with ASD.

Keywords: Social skills training; Program for the Education and Enrichment of Relational Skills (PEERS®); Thai adolescents; autism spectrum disorder (Siriraj Med J 2021; 73: 471-477)

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by deficits in social communication and restricted, repetitive patterns of behavior and interests, as well as impairments in multiple other areas.[1] Social skills deficit is one of major areas of impairment in ASD, particularly in adolescence. Difficulty in achieving social competence can adversely impact peer acceptance, and

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may lead to anxiety, depression, and low self-esteem.[2] Therefore, an effective social skills training intervention should be part of a comprehensive treatment plan for adolescents with ASD.

While there have been no well-studied social skills training interventions available for ASD patients in Thailand, a multidisciplinary care team at Siriraj Hospital initiated a pilot program of social skills intervention for adolescents with ASD, using the UCLA Program for the Education and Enrichment of Relational Skills (PEERS®) developed by researchers from University of California, Los Angeles (UCLA). The program has been systematically proven in different cultural and linguistic contexts.[3] It is a manualized 14- weekly sessions treatment employing various evidence-based strategies to teach social skills to adolescents with ASD, and emphasizing parental involvement in coaching the adolescents.[4,5] Our group modified the UCLA PEERS® to suite with Thai culture. The objective of this pilot study was to assess the feasibility and effectiveness of this modified (Thai) version of UCLA PEERS® in Thai adolescents with ASD.

MATERIALS AND METHODS

Participants

Twelve adolescents with ASD, aged 11-19 years old, and their parents participated in a 10-week Thai version of UCLA PEERS® at the Division of Child and Adolescent Psychiatry, Department of Pediatrics, Faculty of Medicine Siriraj Hospital, from March to October 2015. All participants were previously given a diagnosis of ASD by a certified child and adolescent psychiatrist. To be eligible to the program, participants must have an ability to communicate verbally, an intelligence quotient (IQ) above the intellectual disability level (>70), and no comorbid severe psychiatric or medical conditions. This study was approved by the Siriraj Institutional Review Board (Si 267/2017).

Intervention

Prior to the intervention, the researchers conducted a brief survey in the participating parents to explore the participants' social skills deficits related to the skills listed in the original UCLA PEERS® manual.[6] Modifications were then made according to the deficit social skills in the participants and Thai cultural context. The number of intervention sessions was changed from the original 14 to 10, accordingly.

Some of the modifications made were as follow: (1) in the session focusing on electronic communication, didactic content related to making phone calls and leaving voice messages was substituted with communication networks popular among Thai adolescents (e.g., Facebook, LINE, and Instagram); (2) the homework assignment to have a get-together, possibly in one's home, was changed to a "going out" with friends in settings outside of the home, since home-based get-togethers are less common in Thailand; and (3) the period of time conducting the intervention was changed from during school days to during summer vacation in order to circumnavigate problems associated with school schedules and transportation difficulty in Bangkok. A description of the intervention content in targeted lessons is outlined in Table 1.

The adolescents were participating in this modified 10-session weekly group intervention led by the investigators, one of whom (NS) received a UCLA PEERS® provider certification. Each weekly session consisted of a 90-minute adolescent group and a separate 60-minute parent group. The session included a lesson on targeted social skills, and a homework assignment to promote the generalization of the learned skills to real-life settings. After the first week, each session started with a review of the assigned homework from the previous session, followed by didactic teaching and demonstrated role playing, and behavior-oriented rehearsal exercises to practice newly learned skills. The investigators met after each weekly session to review the intervention process, and to make minor adjustments to the intervention manual according to the observed responses of the participants.

Measurements

Demographic and clinical data were collected from medical records and parent intake records.

Participation and parent satisfaction

Participation in the program was abstracted from weekly participation logs. After the intervention was completed, parents were asked to rate their satisfaction with the program on a 5-point Likert scale (5 indicating the most satisfaction).

Parent report of changes in social skills

Parents were asked to rate the changes in their child's social skills relative to each of the 10 targeted lessons (as improved, unchanged, or worse) at the end of intervention and at 4-month follow-up.

Vineland Adaptive Behavior Scales (VABS)

The VABS measures adaptive behavior skills needed for everyday living in the domains of motor skills, communication skills, daily living skills, and socialization, and a separate domain of maladaptive behaviors, with test-retest reliability of 0.8-0.9.[7] Higher scores represent better adaptive functioning. The VABS was administered through a parent interview by a clinical psychologist at baseline and at the end of intervention.

TABLE 1. Topics and Abbreviated Content of the Thai PEERS®

Session	Targeted Lessons	Contents	Homework
1	Sharing of information	Sharing information with peers to find a common interest	Practicing sharing information on the phone with an assigned group member
2	Two-way communication	Key elements of having a two-way conversation with peers Parents identify teen activities that could lead to potential sources of friendship	Practicing sharing information on the phone with an assigned group member
3	Electronic communication and choosing appropriate friends	Appropriate use of electronic and online communication (e.g., telephone, email, LINE, Facebook, Twitter, Instagram, and Skype) Parents and teens identify interest-based extracurricular activities that could lead to potential sources of friendship	Beginning to pursue extracurricular activities, and sharing information with members of this group
4	Peer entry I: Entering conversations	Precise steps to entering conversations with peers	Practicing entering conversations with peers
5	Peer entry II: Exiting conversations	Assessment of peer receptiveness when entering a conversation, and how to exit a conversation when not being accepted	Practicing entering and exiting conversations with peers
6	Good sportsmanship	The rules of good sportsmanship	Practicing good sportsmanship at home
7	Rejection I: Teasing and embarrassing feedback from peers	How to appropriately respond to teasing How to differentiate between teasing and embarrassing feedback, and how to modulate your response	Practicing coping with teasing appropriately in relevant situations
8	Rejection II: Physical bullying and changing a bad reputation	Strategies for coping with physical bullying and how to change a bad reputation	Practicing new strategies for coping with bullying and physical threats in relevant situations
9	Good sportsmanship practicum: Playing chairball	Good sportsmanship rehearsal	Practicing good sportsmanship
10	Coping with disagreements & program conclusion	Elements necessary for resolving arguments and disagreements with peers	Practicing coping with arguments with parents and peers via the role-playing exercise in relevant situations

Children's Depression Inventory (CDI)-Thai version

The Children's Depression Inventory (CDI) consists of 27 self-reported items measuring symptoms of depression in children and adolescents aged 7-17 years.[8] Each item is scored from 0 to 2 to define the severity of depressive symptoms within the past two weeks. The higher the scores indicate more severe depressive symptoms. In this study, the participants completed the Thai version of the CDI[9], at baseline and the end of intervention.

Clinical Global Impression (CGI) scale

The CGI is a clinician-rated 7-point scale for rating global improvement in the patient's illness. The CGI-I rates the patient's illness improvement or decline relative to the patient's baseline, as follows: 1 = very much improved; 2 = much improved; 3 = minimally improved; 4 = no change; 5 = minimally worse; 6 = much worse; and, 7 = very much worse[10]. The CGI-I was administered after the intervention was completed.

Statistical analysis

Demographic and clinical data were analyzed and described using descriptive statistics. Pre- and post-intervention scores were analyzed using either paired t-test or a Chi-square test, and results are shown as either number with percentage or mean ± standard deviation. Data analyses were performed using PASW Statistics version 18.0 (SPSS, Inc., Chicago, IL, USA). A p-value less than 0.05 was regarded as being statistically significant.

RESULTS

Participant's mean age was 14.8 years (range: 11-18), and 83.8% were male (Table 2). Nine participants had comorbid psychiatric diagnoses (4 ADHD, 4 anxiety disorders, and 2 mood disorders). Participants had a mean IQ of 94.7±20.21 and a baseline VABS score of 58.75±16.90. The three most common reported social

TABLE 2. Demographic and Clinical Characteristics of the Enrolled Adolescents (N=12)

Characteristics	
Age (yrs), mean±SD (range)	14.8±1.99 (11-18)
Male gender, *n* (%)	10 (83.8%)
Living with biological parents, *n* (%)	12 (100%)
Father's education, *n* (%)	
High school or lower	4 (33.3%)
University	8 (66.7%)
Mother's education, *n* (%)	
High school or lower	3 (25.0%)
University	9 (75.0%)
Family monthly income (Thai baht), *n* (%)	
10,000-50,000	6 (50.0%)
>50,000	6 (50.0%)
Number of siblings, *n* (%)	
0	3 (25.0%)
1	6 (50.0%)
2	3 (25.0%)
Number of years receiving treatment, mean±SD	6.80±4.93
Educational stage, *n* (%)	
Elementary	3 (25.0%)
Secondary	9 (75.0%)

Note. SD = standard deviation; CGI-S = Clinical Global Impression-Severity scale.

skill problems were peer rejection, inability to handle disagreements with peers, and difficulty developing friendships.

Participation and parent satisfaction

All participants completed the program. The session attendance rates ranged from 83.3% to 100%, and the individual participant attendance rates ranged from 70% to 100%. The rate of homework completion was 60%. The average parent satisfaction score was 4.10 out of 5. Common reasons for the satisfaction included applicability of the program content, structured home practice, and parent coaching guidance.

Outcome measurements

Data specific to social skills reported as improved by the parents are shown in Table 3. All of the 10 social skills trained in the program were rated as improved by at least half of the parents. The skills rated as improved by the highest percentage of parents (83.3%) included trading information, two-way communication, and good sportsmanship. At the 4-month follow-up, all but two skills (entering conversation and coping with physical bullying) were still reported as improved by more than 50% of parents.

Post-intervention CGI-I was rated as very much improved or much improved in 6 adolescents (50%). Mean CDI scores decreased from 18.08 at baseline to 16 at post-intervention; however, the change was not statistically significant (p=0.345).

Significant improvements were observed in the raw scores of the VABS in the daily living skill domain, the socialization domain, and the communication domain (p's < 0.05). The maladaptive behavioral domain score decreased significantly from 5.33 to 4.17 (p< 0.05). Changes in the VABS domain scores after the intervention are shown in Table 4.

DISCUSSION

This study examined the feasibility and effectiveness of the Thai version of UCLA PEERS®, a parent-assisted social skills intervention, in 12 Thai adolescents with

TABLE 3. Social Skills Reported as Improved by Parents of Adolescent Participants

Skills	After 10th session (N=12) *n* (%)	At 4-month follow-up (N=9) *n* (%)
Sharing of information	10 (83.3%)	5 (55.6%)
Two-way communication	10 (83.3%)	7 (77.8%)
Electronic communication and choosing appropriate friends	8 (66.7%)	5 (55.6%)
Peer entry I: Entering conversations	6 (50.0%)	2 (22.2%)
Peer entry II: Exiting conversations	6 (50.0%)	6 (66.7%)
Good sportsmanship	6 (50.0%)	5 (55.6%)
Rejection I: Teasing and embarrassing feedback from peers	9 (75.0%)	5 (55.6%)
Rejection II: Bullying and changing a bad reputation	9 (75.0%)	4 (44.4%)
Good sportsmanship practicum: Playing chairball	10 (83.3%)	7 (77.8%)
Coping with disagreements	9 (75.0%)	5 (55.6%)

TABLE 4. Pre- and Post-Intervention Domains on the Vineland Adaptive Behavior Scales

Measurement	Pre-intervention	Post-intervention	*p*-values	95% CI
Daily living skills domain	131.83	133.92	**.016**	[-3.697, -0.470]
Personal	71.67	71.92	.082	[-0.537, 0.037]
Domestic	21.83	22.33	**.026**	[-0.928, -0.072]
Community	38.33	39.67	**.031**	[-2.525, -0.142]
Socialization domain	89.50	90.58	**.005**	[-1.772, -0.395]
Interpersonal relationships	37.75	37.83	.777	[-0.716, 0.550]
Play and leisure time	26.25	26.83	**.027**	[-1.087, -0.080]
Coping skills	25.83	26.08	.082	[-0.537, 0.037]
Communication domain	116.08	117.25	**.036**	[-2.245, -0.89]
Adaptive behavior composite	58.75	59.67	.255	[-2.597, 0.763]
Maladaptive behavior domain	5.33	4.17	**.019**	[0.235, 2.099]

Note. CI = confidence interval.
p-values < .05 are in boldface, indicating statistical significance.

ASD. To our knowledge, this is the first study of parent-assisted social skills training program in adolescents with autism in Thailand. We found a high attendance rate (>80% attendance with no dropouts) and high parent's satisfaction. We also found improvements of the participant's social skills after the intervention, measured by parent report, VABS, and CGI-I.

The high participation rate and high parent satisfaction demonstrates feasibility of the Thai version of UCLA PEERS® in Thai adolescents with ASD. This might be due to the fact that this intervention emphasizes parental involvement in the social skills training process in everyday living, and the skills taught in the program address the common social problems reported by parents. The program includes several activities that have been proven effective for teaching social skills to children and adolescents with ASD. Moreover, the evidence-based strategies used in the program were modified to fit with Thai culture and social context.

Effectiveness of this intervention is demonstrated by the improvement of the participant's social skills reported by parent and the CGI-I rated by treating psychiatrists. It is also supported by more objective measures of adaptive functioning on the VABS, which revealed improvements in socialization and other adaptive domains, as well as a decrease in maladaptive behaviors. Our findings are consistent with other studies on effectiveness of the PEERS® intervention in the USA and other countries.[11-14] While other studies demonstrated a decrease in depression score[3], post-intervention CDI scores did not decrease significantly in the present study, possibly due to lack of power related to small sample size. The participant's CDI scores were not significantly elevated at baseline. Furthermore, the commitment by parents to participate in the training and to coach their children was encouraging, and would be expected to have positively influenced the observed improvement in social skills in this study. Conversely, the effectiveness of Thai PEERS® for youth whose parents do not fully participate in treatment is unknown and requires further investigation.

It was also observed that the areas of social skills with the highest percentage of improvement according to parent reports, such as coping with rejection, good sportsmanship, and coping with disagreement, were the skills identified to be the most problematic by parents at baseline. This suggests that the contents of the intervention are well-suited to meet the needs of the parents. The difference in the percentage of parents

that reported improvement in each skill is likely due to session attendance. More specifically, parents and adolescents that didn't attend a certain session would presumably have been less likely to report improvement for that training topic. We found that more than 50% of the parents still reported improvements in most of the trained social skills at 4-month follow-up, suggesting this social skill intervention program has some long-term effects. However, since the percentage of improved cases decreased, continued parent coaching and/or boosting interventions may be required to enhance the sustainability of the social skills improvement.

This study has some limitations. First, the sample size was small and there was no control group. Second, the improvement in social skills reported by parents was subjective, which renders the present study vulnerable to some potential parent bias. Third, information from other sources, such as teachers or parents/caregiver unaffiliated with the program was not collected. Lastly, other co-occurring interventions that could have contributed to improvements in social skills were not controlled in the current study.

Despite these limitations, this study demonstrates that the Thai version of UCLA PEERS® is feasible in Thai adolescents with ASD and this intervention is effective in improving social skills in this population. Future studies using larger randomized controlled trials with independent raters, more objective measures, and longer follow-up assessment periods would further elucidate the effectiveness of this intervention.

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Comparison of Heart Valve Circumference Examined Before and After 10% Formalin Fixation

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ABSTRACT

Objective: To compare the heart valve circumference before and after 10% formalin fixation.

Materials and Methods: The study analyzed 63 Thai human cadaveric hearts. Each heart valve circumference was separately measured in the fresh state by specifically designed equipment. After that, the hearts were fixed in 10% formalin for 3 days. Then each heart valve circumference was measured by the same equipment and by the thread and ruler technique. The results were analyzed using SPSS package to find the association between the heart valve circumference before and after formalin fixation.

Results: This study showed that the average circumferences of the heart valve measured in the fresh state were 13.329 cm in the tricuspid valve, 10.617 cm in the mitral valve, 8.416 cm in the pulmonic valve, and 7.122 cm in the aortic valve. The average circumferences of the heart valve measured after 10% formalin fixation were 11.019 cm in the tricuspid valve, 8.714 cm in the mitral valve, 6.751 cm in the pulmonic valve, and 6.089 cm in the aortic valve. The average ratios of the heart valve circumference measured fresh and after 10% formalin fixation were 0.8267 in the tricuspid valve, 0.8235 in the mitral valve, 0.8050 in the pulmonic valve, and 0.8573 in the aortic valve. There were significant differences in the heart valve circumference between the fresh state and after formalin fixation ($p < 0.001$).

Conclusion: This study revealed important information on the dimensional changes of all the formalin-fixed heart valves. We found that the heart valve shrank after formalin fixation, with the formalin-fixed hearts an estimated 0.8 times smaller than the fresh cadaveric hearts.

Keywords: Heart valve circumference; formalin fixation (Siriraj Med J 2021; 73: 478-484)

INTRODUCTION

The number of people with cardiovascular disease is increasing globally and it is now one of the leading causes of death worldwide, not only in industrial countries but also in developing countries, such as Thailand.[1,2] Valvular heart disease represents an important public health problem and its rising incidence is leading to an increased mortality rate among the general population.[3,4] Acute rheumatic fever and chronic rheumatic heart diseases, for example, were responsible for 115 deaths in 2013, leading to a death rate of 0.2 per 100,000 persons per year in Thailand; while the number of deaths had increased to as many as 226 persons in 2017, leading to a death rate of 0.3 per 100,000 persons per year.[1]

The heart valves play a significant role in controlling blood circulation between the heart chambers and systemic circulation. The four heart valves are the tricuspid valve (TV), pulmonic valve (PV), mitral valve (MV), and aortic valve (AV). The cardiac valves are affected by various factors, including genetics, aging, sex, and lifestyle, as well as by infection. Pathological changes of the heart valves can affect the circulation, such as regurgitation

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and stenosis. People with severe heart valve disease may need to undergo a surgical procedure to replace the damaged valve with an artificial heart valve.

Knowledge of the cardiac anatomy plays an important role in cardiothoracic surgery as well as in understanding the pathophysiology of various cardiac diseases. Several studies from a variety of countries and based on different ethnicities have been performed to find the average heart valve circumference to aid more accurately estimating the circumference needed for an artificial heart valve.[5-15] In general, previous studies took measurements on formalin-fixed hearts. However, it is well known that formalin fixation can shrink fresh tissues in an unpredictable manner. Consequently, in this study we thought it would be interesting to study the comparison between the heart valve circumference before and after 10% formalin fixation. In addition, this study aimed to compare different methods of measuring the heart valve circumference.

MATERIALS AND METHODS

This study was approved by the Human Research Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University (Si 483/2020(IRB2) on July 20, 2020). This descriptive study included a total of 63 hearts, which were collected from Thai adult male and female cadavers at the Department of Forensic Medicine, Faculty of Medicine Siriraj Hospital from 1 August 2020 to 31 December 2020. Hearts with disease, trauma, or postmortem changes were excluded from this study.

The heart samples were collected from dead bodies with known age, sex, weight, and height. The heart was removed from the pericardial cavity. The great vessels connecting the heart with the other organs were separated and cut without damage to the valvular structure. Then, the heart chamber was cut in a horizontal line through the left and right ventricle, and blood clots were removed to show all the heart valves. Measurement of each valve circumference was performed using specially designed equipment (Method 1). This cone-shaped equipment (Fig 1) was fully accredited and designed to directly measure the exact size of a heart valve circumference.

Next, each heart was fixed in 10% formalin solution for three days. A thread was used to hold the heart within the formalin to maintain the normal shape of the heart. The circumference of all the formalin-fixed heart valves was observed again and measured with the same equipment (Method 2). Subsequently, the heart chambers were routinely opened to expose the opened valve annulus, then the thread was placed along the boundary of each valvular annulus and measured with a ruler (Fig 2) (Method 3). All the data were recorded in millimeters to 2 decimal points.

All the data were collected in Excel 2016 and analyzed using the SPSS package (PASW 18.0 for Windows). Quantitative data according to the average and the standard deviation of the data were calculated. To determine whether the fresh and formalin-fixed heart samples, as well as the measurements obtained by the direct and thread and ruler techniques, were statistically different ($p < 0.05$), and ANOVA or non-parametric Freidman tests were performed on the data. Lastly, assessment of the intra-observer and inter-observer variability in the measurements was also conducted with intra-class correlation coefficients.

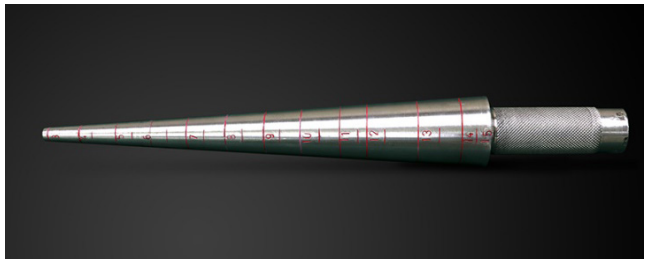

Fig 1. Specially designed cone-shaped equipment.

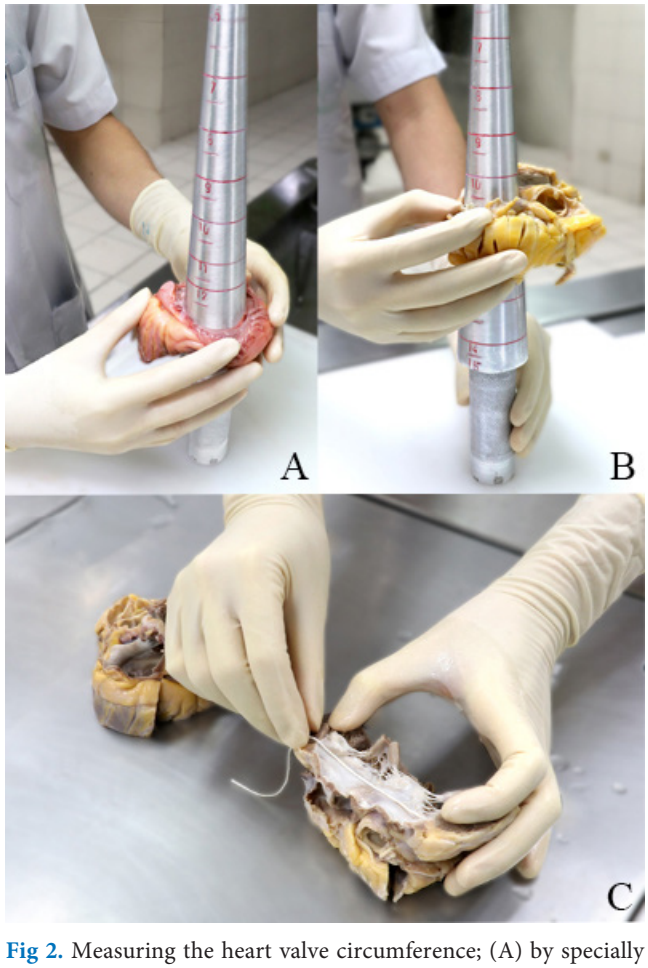

Fig 2. Measuring the heart valve circumference; (A) by specially designed equipment in the fresh state; (B) by specially designed equipment after formalin fixation; (C) by the thread and ruler method after formalin fixation.

RESULTS

In this study, 63 hearts were examined to observe the normal values regarding the heart valve circumferences. The males included in this study were aged between 14 and 64 years old, and the females between 16 and 68 years old. In the study sample, the mean age of the male and female cadavers was 34.31 ± 14.2 years old and 41.58 ± 15.85 years old, respectively. A summary of the descriptive data from the heart valve circumference of the mixed group (both male and female) is provided in Table 1. All the measurements were found to be normally distributed. This study showed that the average circumference of the tricuspid valve was the highest, while the average circumference of the aortic valve was the lowest. Among the measurements of the heart valve circumference, this study indicated that the heart valves after 10% formalin fixation were smaller than those before fixation.

Differences between the measurements were also explored. Comparisons of the measurements taken before and after 10% formalin fixation with the different methods are shown in Table 2. The circumference ratios of all the valves after 10% formalin fixation compared with those before 10% formalin fixation were less than 1 throughout all the valves ($p < 0.001$ by t-test). The average ratios of heart valve circumference measured by the specially designed equipment before and after 10% formalin fixation were 0.8267 in the tricuspid valve, 0.8235 in the mitral valve, 0.8050 in the pulmonic valve, and 0.8573 in the aortic valve (Table 2).

Different plots were conducted to explore the agreement between Method 2 and Method 3 (Fig 3). A Bland-Altman plot was used in this study since neither of the two methods are reference techniques. Almost all the measurement values were within a 95% confidence interval of the mean difference, implying that the variability of the measurements was low. In addition, intra-class correlation coefficients (ICCs) were determined to identify the relationship between the direct measurement and the measurement by the thread and ruler method. This study showed excellent reliability between the two types of measurement. The ICC of the tricuspid valve was 0.952, the mitral valve was 0.958, the pulmonic valve was 0.947, and the aortic valve was 0.956 (Fig 3).

The reliability of this study, particularly the intra- and inter-observer variations, was also evaluated to exclude the bias of heart valve circumference measurement by human error. Measurements were performed on 15 samples using all the measurement methods and the results compared with previous results. Based on Kappa statistics, the average values of ICC were more than 0.9 for both the intra- and inter-observer reliabilities. Therefore, this study showed excellent reliability of all the measurements.

TABLE 1. Descriptive statistics for all the heart valve circumferences; Method 1 = Measurement with specially designed equipment before 10% formalin fixation; Method 2 = Measurement with specially designed equipment after 10% formalin fixation; Method 3 = Measurement with the thread and ruler technique after 10% formalin fixation.

Heart valve (n = 63) Type	Method	Mean (cm)	Standard deviation (cm)	Minimum (cm)	Maximum (cm)
Tricuspid Valve (TV)	1	13.33	0.92	11.00	15.00
	2	11.02	1.10	8.60	13.70
	3	10.81	1.02	8.50	13.50
Mitral valve (MV)	1	10.62	1.13	8.20	13.50
	2	8.71	0.99	7.00	12.00
	3	8.65	0.91	7.20	12.20
Pulmonic Valve (PV)	1	8.42	1.01	6.80	10.70
	2	6.75	0.80	5.00	8.70
	3	6.66	0.70	5.30	8.10
Aortic Valve (AV)	1	7.12	0.79	5.50	9.00
	2	6.09	0.70	4.90	8.30
	3	6.00	0.69	5.00	7.80

TABLE 2. Descriptive data of the proportions among the different measurement methods.

Ratio of Different Methods	Mean	Standard Deviation	Mean of Standard Error	Sig. (2-tailed)	Mean Difference	95% Confidence Interval of the Difference	
						Lower	Upper
TV_2 / TV_1	0.83	0.06	0.01	0.00	-0.17	-0.19	-0.16
TV_3 / TV_1	0.81	0.06	0.01	0.00	-0.19	-0.20	-0.17
MV_2 / MV_1	0.82	0.07	0.01	0.00	-0.18	-0.20	-0.16
MV_3 / MV_1	0.82	0.06	0.01	0.00	-0.18	-0.20	-0.17
PV_2 / PV_1	0.81	0.07	0.01	0.00	-0.20	-0.21	-0.18
PV_3 / PV_1	0.80	0.07	0.01	0.00	-0.20	-0.22	-0.19
AV_2 / AV_1	0.86	0.07	0.01	0.00	-0.14	-0.16	-0.13
AV_3 / AV_1	0.85	0.07	0.01	0.00	-0.15	-0.17	-0.14

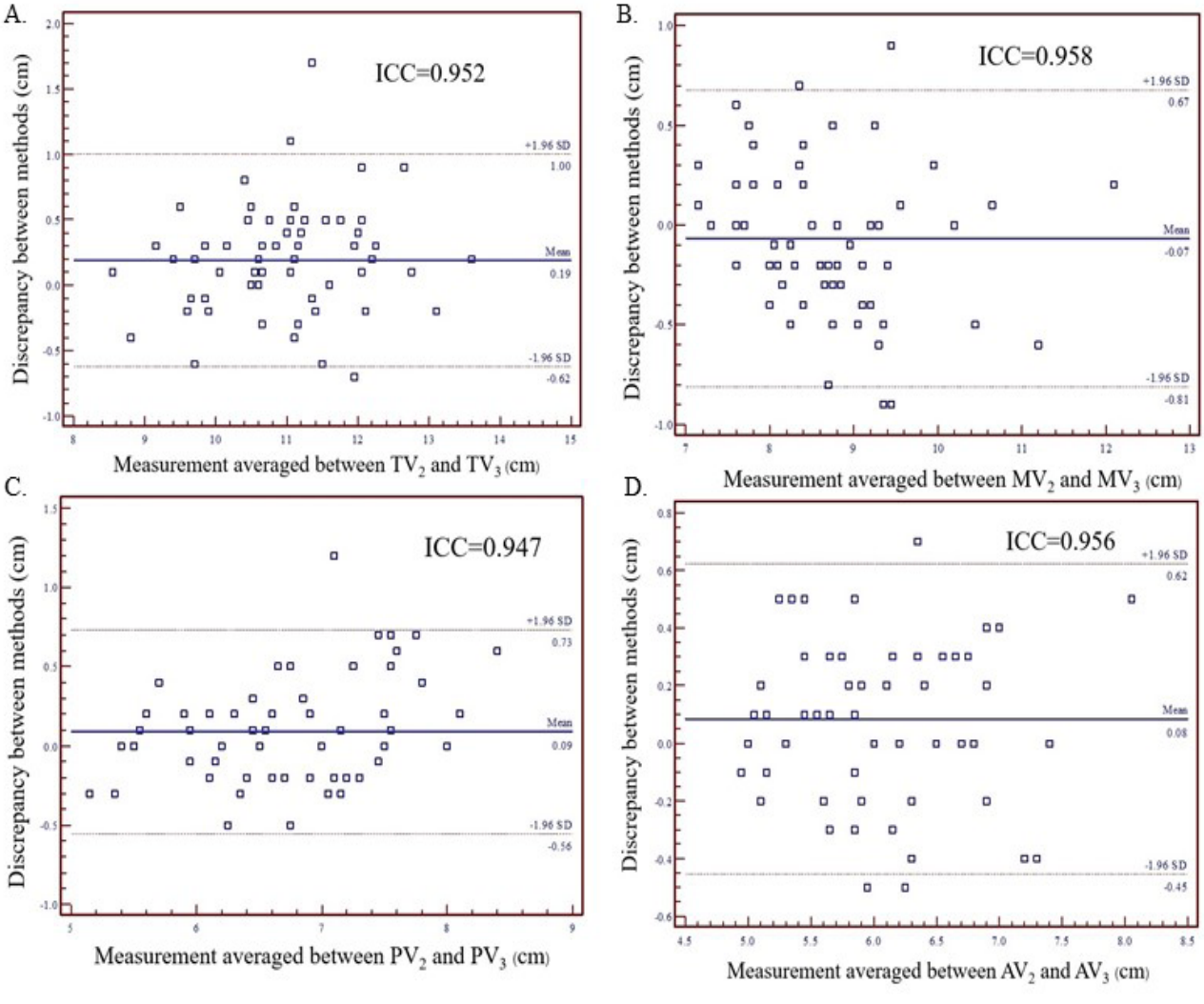

Fig 3. Differences between Methods 2 and 3: (A) Tricuspid valve; (B) Mitral valve; (C) Pulmonic valve; (D) Aortic valve; ICC: Intra-class correlation coefficient.

DISCUSSION

It is well known that tissues shrink after formalin fixation. With reference to previous studies, however, a variable reduction in the percentage of tissue shrinkage was observed.[16-20] Most of these works used different soft tissues as their samples and they reported different results. Pritt *et al*. reported that there was no significant change in 96% of the breast tumor size between the fresh and fixed states.[17] Also, Jonmarker *et al*. found no significant decrease in prostate tissue diameter after formalin fixation, but observed a significant weight loss.[18] In contrast, a different result was found by Hsu *et al.* in their study, whereby they found that the measured dimensions of the oral cavity mucosa and tongue muscle shrank by 30.7% after formalin fixation.[19] It was also reported that the mean diameter of breast cancer tissues decreased by 4.5% after formalin fixation.[20] Lastly, the average shrinkage of the maximum diameter of head and neck cancer samples was 4.4% in the study by Chen *et al.*[16] In our study, the average heart valve circumference after formalin fixation was 15-20% smaller than that before formalin fixation. Regarding the variable shrinkage reported in the aforementioned works, it is worth mentioning that possibly the constitution and type of tissue (e.g., amount of elastic tissue or fat) may have had an effect on the overall degree of shrinkage.[16]

Many studies have been carried out in formalin-fixed hearts rather than fresh human hearts.[6,9-11,13,15] Formalin is widely used to preserve tissues for routine histological examination. The most common formula is 10% formalin, consisting of 3.7% formaldehyde in water with 1% methanol.[16] When using to preserve tissues, the process includes a two-phase fixation. The first phase is the alcohol fixation phase, described as alcohol-induced dehydration and hardening of the tissue. Shrinkage of the tissue occurs during this phase. Subsequently, this is followed by the cross-linking phase formed by a cross-linking of formaldehyde and peptide. There are two factors that influence formaldehyde fixation: penetration and fixation.[21] The former is the potential of the solution to penetrate the tissue, while the latter is the ability of the formaldehyde to form cross-linking. The completion of formalin fixation is dependent on many factors, such as the temperature, pH, time, type of tissue, and the concentration of formaldehyde.[21-22] Therefore, it can be assumed that the average heart valve circumference varies among different studies using different fixative methods, as we found in this study.

Various techniques have been conducted using a variety of methods, such as the thread and ruler method, the direct measurement, echocardiography, and computer software. Currently, there is no standard method to measure heart valve circumference. With reference to previous studies[5-8,10,12,13], the thread and ruler technique was the most popular practice to evaluate the heart valve circumference. Because none of the above-mentioned studies provided data on direct measurement, no comparisons can be made. In this study, we measured the heart valve circumferences by the direct measurement using a specially designed instrument and by the thread and ruler technique. The results showed that there was excellent reliability between the two types of measurement. Thus, we recommend the direct measurement method for assessing the heart valve circumference because of its easy manipulation.

The next issue we considered was that of the variation in heart valve circumference. Westaby *et al.*[11] reported a wide individual variation in heart valve circumference and identified that the size of the heart valve circumference was unrelated to the body habitus. Many studies have performed detailed analyses of the heart valve circumferences in various populations (Table 3). However, the data in the previous studies showed some different results compared with this study. Before formalin fixation, the average heart valve circumference in this study was slightly higher than in almost all the previous studies[5-14], but lower than that reported by Tei *et al.*[15] and coincided with those reported by Jatene *et al.*[8] and Alison *et al.*[23] Nevertheless, the average heart valve circumference after formalin fixation was slightly lower or similar to the above-mentioned studies. Why there was such a difference between our study and previous studies might be explained by the different socio-economic status and population groups from which the hearts were obtained as well as by the factors affecting formalin fixation, as mentioned above.

To the best of our knowledge, this study is the first study to investigate the effects of formalin fixation on heart valve samples. Previous studies were conducted using formalin-fixed hearts and these may be different from the values from fresh hearts. In this study, an effort was made to describe this finding, which might be used to compare the valve orifice size before and after formalin fixation. Thus the data from this study can be taken as a useful guide in medical practice, especially being a reference in the diagnosis and treatment of valvular heart disease. This study should help in the choice of prosthetic replacement as well. It is realistic to use a mean circumference of each heart valve as a guide for valve surgery. The measurements that we have taken will help a surgeon to estimate the correct size of prosthesis to fit accurately in the valve orifice in a person. Further

TABLE 3. Comparison with the previous studies' heart valve measurements.

Study	Nationality	Heart status	Measurement Method	Heart valve	Circumference (cm)	
					Mean	S.D.
Ilankathir[4]	India	Fresh	Thread	TV	10.37	-
				PV	6.82	-
				MV	8.29	-
				AV	7.54	-
Deopujari *et al*.[6]	India	Fresh	Thread	MV	8.27	1.25
Jatene *et al*.[7]	Brazil	Fresh	Thread	AV	7.38	1.01
Udhayakumar and Yasawardene[11]	Sri Lanka	Fresh	Thread	AV	6.47	0.70
Alison *et al*.[22]	U.S.	Living	Echocardiograph	MV	10.70	1.46
Tei *et al*.[14]	U.S.	Fresh	Ruler	TV	13.50	0.80
				MV	11.40	0.70
		Formalin-fixed	Ruler	TV	12.20	0.80
				MV	10.70	0.50
Gupta *et al*.[8]	India	Formalin-fixed	Image analysis	MV	9.11	0.44
Nayak *et al*.[9]	India	Formalin-fixed	Thread	MV	7.92	0.50
Westaby *et al*.[10]	U.S.	Formalin-fixed	Ruler	TV	11.63	1.39
				PV	7.63	0.93
				MV	9.79	1.23
				AV	7.28	0.92
Garg *et al*.[5]	India	Formalin-fixed	Thread	PV	6.50	0.59
Lama *et al*.[12]	Nepal	Formalin-fixed	Thread	TV	11.22	0.20
				MV	9.22	1.49
The present study	Thailand	Fresh	Equipment	TV	13.33	0.92
				PV	8.42	1.01
				MV	10.62	1.13
				AV	7.12	0.79
		Formalin-fixed	Equipment	TV	11.02	1.10
				PV	6.75	0.80
				MV	8.71	0.99
				AV	6.09	0.70
			Thread	TV	10.81	1.02
				PV	6.66	0.70
				MV	8.65	0.91
				AV	6.01	0.69

studies concerning a survey of a larger sample size and comparisons with radiological and echocardiographic examinations should be conducted.

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The Influence of Electromagnetic Field Pollution on Human Health: A Systematic Review

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ABSTRACT

Objective: Recent technological advances have exponentially expanded glob with harbouring upon Electromagnetic fields (EMF). The utilization of Electromagnetic field has become universal from everyday usage of electronic appliances such as micro wave ovens, tablets and portable computers to telecommunication systems like mobile phone towers, radio- television broadcast systems and electronic power transmission systems resulting in electromagnetic field and associated radiations. EMF can have biological effects on cell at microlevel and have the potential ability to cause cell dysfunction manifesting in various biological effects. This review tried to gather evidence from the existing literature about the biological effects of EMF on human health.

Materials and Methods: We did extensive literature search using PubMed and Cochrane database using key words, "electromagnetic fields", "Extremely low frequency electromagnetic fields (ELF-EMFs)", "biological effects", "health effects", "public health". We included 20 studies conducted from Dec 2009 to Dec 2019 in our systematic review. Data from each study was extracted by two independent researchers and discrepancies were resolved by consensus.

Results: Significant biological effects of EMF exposure were reported on human health ranging from anxiety, depression, sleep disturbance, increased risk of Alzheimer's disease and ALS (Amyotrophic Lateral Sclerosis), hypersensitivity to infertility and increased risk of multiple carcinomas.

Conclusion: Application of preventive measures in order to minimize the exposure becomes the need of the hour especially so in occupational settings.

Keywords: Electromagnetic fields; health effects; biological effects; carcinogen (Siriraj Med J 2021; 73: 485-492)

INTRODUCTION

Since the arrival of 20th century everyone is exposed to a complex mix of weak electric and magnetic fields, at home as well as at work places, from the generation and transmission of electricity, domestic appliances and industrial equipment, to telecommunications and broadcasting resulting in electromagnetic field and associated radiations. With ubiquitous expansion of current technology system globally in the last few decades, EMF has crept up as a new type of pollution in the physical environment due to resulting electromagnetic radiations. This anthropogenic pollution is much stronger than the known natural sources of electromagnetic fields or radiation. One of the first reports of their potentially harmful effects on living organisms report in epidemiological research report published in 1979 by Wertheimer and Leeper.[1] They studied the health status of children from Denver (Colorado, USA), who lived in homes exposed to magnetic fields of high intensities and concluded that the children exposed to higher intensity magnetic fields had slightly higher risks of developing leukaemia.[1]

Anthropogenic electromagnetic fields can be classified by their physical parameters such as frequency, and intensity. They can range from extremely low frequency (associated

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with electricity supplies and electrical appliances) to low, medium, high, and extremely high frequency (mostly associated with wireless communication). Electronic devices such as smartphones, tablets, microwave ovens, radio, and television sets emit low intensity electromagnetic radiation at frequencies from 300 MHz to 300 GHz that can be associated with microwaves. On the other hand, power transmission lines and electric devices are strong sources of electromagnetic fields and radiation of much lower frequencies but much higher intensities. Electromagnetic fields and/or electromagnetic radiation, as electromagnetic pollution, affect various elements of the environment and living organism. EMF pollution in public health literature refers to the hazard bestowed by non-ionising radiations with a frequency towards the lower half of the electromagnetic spectrum. Tiny electrical circuits exist in the human body that occur as part of the normal bodily functions like transmission of electric impulses for brain activities, heart beating and even due to chemical reactions for digestion of food. Low-frequency electric fields influence the human body which is made up of charged particles, so influence the distribution of electric charges and causes small currents inside. Similarly, Low-frequency magnetic fields may also induce circulating currents within the body depending on the intensity of the magnetic field. Both electric and magnetic fields induce voltages and currents in the body that are usually very small. However, if sufficiently large, these currents could cause stimulation of nerves and muscles or affect other biological processes. Commonly, the effects of EMF radiations can be broadly classified as thermal and non-thermal effects. Though thermal effects are well documented in public health literature the non-thermal effect poses greater challenge for the upcoming research as there are conflicting results of different epidemiological studies done on this matter.

We are currently living under this large gamut of EMF with a limited knowledge of its biological impact. Although in-vitro studies have proven negative impact of EMF at cellular levels, lacunae exist in providing evidence towards possible adverse outcomes in terms of health. Hence, it becomes very important to appropriately determine the nature and related side effects of electromagnetic pollution and its impact on human health. The International Agency of Research on Cancers (IARC) has declared EMF as "Possibly carcinogenic to human health (category 2B)".[2] The effect of these radiation on environment is of course research-worthy yet practically difficult to conduct.

Objective: We conducted this systematic review with the objective to identify and map the available evidences regarding the possible biological effect of EMF pollution on human health so that its public health effects could be addressed.

MATERIALS AND METHODS

Literature search

We conducted a systematic search of Medline database and the Cochrane Library in January 2020 to identify all relevant peer-reviewed papers published using key words, "electromagnetic fields", "Extremely low frequency electromagnetic fields (ELF-EMFs)", "biological effects", "health effects". The key words were arranged in different Boolean combinations with different search phrases. The search was further refined using filters/ mesh terms, "free full text", "10 years", "English", "MEDLINE", "Humans".

Inclusion and exclusion criteria: We included human laboratory trials and epidemiological studies published in English in last 10 years from Dec 2009 to Dec 2019. The health effects due to EMF were then rearranged in line with different human systems affected. However, we excluded in-vitro studies, studies in animals and studies discussing therapeutic effects of EMF.

Data extraction: The data from each study were extracted independently by two researchers and recorded. The form extracted information about study design study sample, sampling procedure, exposure, results and health effects. Differences concerning data extraction were resolved by consensus.

Selection of studies: In total, 2611 potentially relevant abstracts were identified; from where 445 full text articles were considered; based on our inclusion and exclusion criteria 20 studies were finally included in the review (Fig 1). Of the 20 articles included in the analyses, 8 were original studies and 12 were review articles.

RESULTS

Multiple adverse effects of EMF on different human organ systems have been reported by different studies. Different varieties of biological effects were observed in presence of different type of electromagnetic radiations. Findings from various epidemiological studies and their major gaps have been listed in Table 1. Seven original studies out of the eight, included in the review, suggested possible association of presence of various physical symptoms and cell morphology alteration with exposure to EMF.[3-8] One accepted mechanism of action of EMF to exert their non thermal biological effect is via breaking DNA strands in cell type dependent manner.[9]

Ten out of 12 review studies included in the review suggested possibility of linkage of EMF with cellular pathways like apoptosis and other cellular regulatory

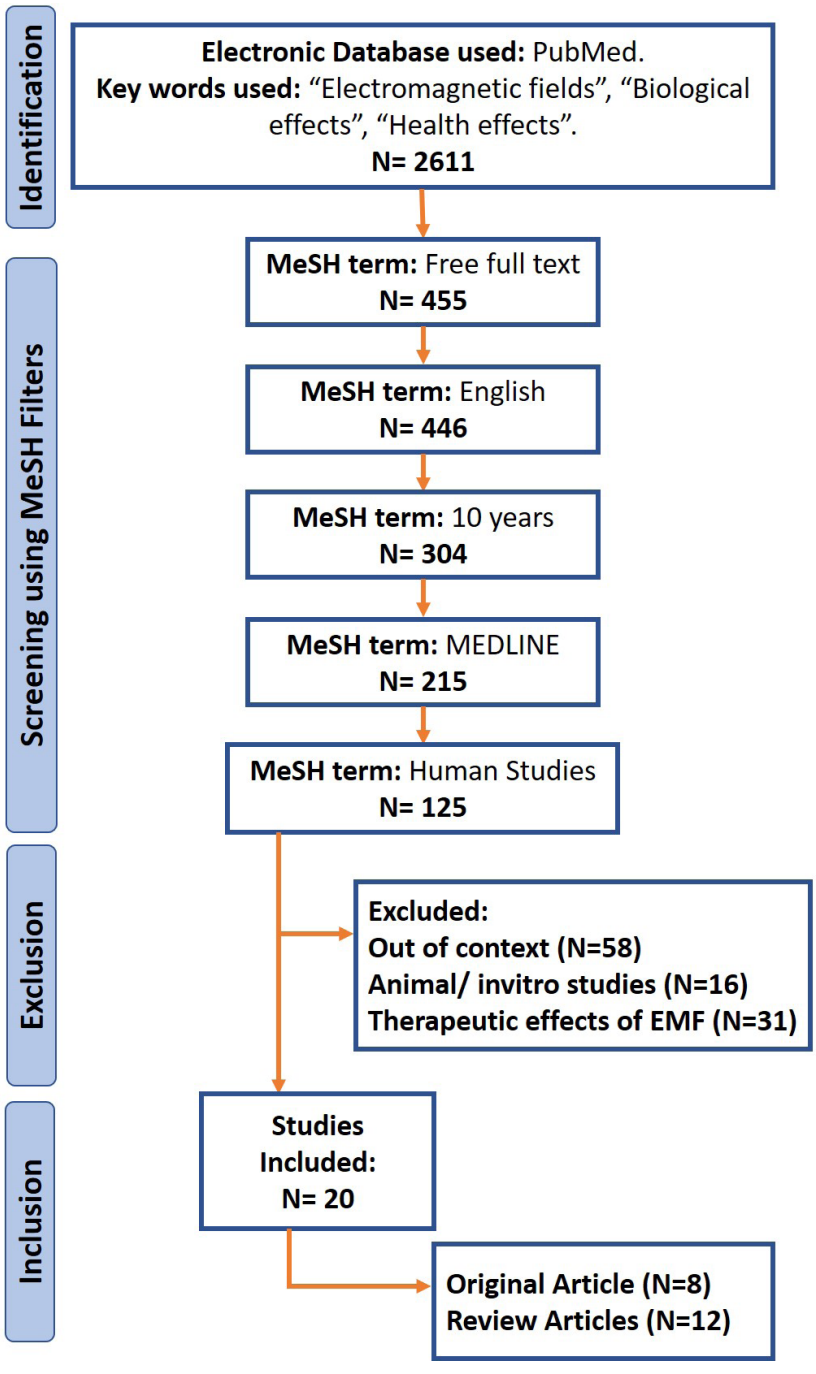

Fig 1. Flowchart showing the identification and selection of studies on the health effects of exposure to electromagnetic fields (EMF)

mechanism which may lead to carcinogenesis.[10-19] One study also reported increased reactive oxygen species on exposure to EMF in human cell lines leading to cellular damage.[11] However, two review articles reported unlikely or inconsistent findings of any effect of EMF exposure on cognitive functions and brain tumours.[20,21]

We identified multiple studies conducted at different places and time that have provided evidence regarding effects of EMF on multiple organ system. Based on the studies included in the review we summarised the potential effects of EMF on various organ systems. (Fig 2)

Effects on nervous system

Studies have also reported a positive correlation between EMF exposure and neurodegenerative diseases and autism.[18,23] Gunnarsson LG et al in their study on occupational exposure of EMF reported 10% increase

TABLE 1. Summary of various epidemiological studies included in the systematic review.

Authors Article Types Publication Year	Findings	Challenges in understanding the biological effects of EMF radiations
Bogers RP et al.[6] (Original Article), 2018	• Possible association of Nonspecific Physical symptoms (Positive or negative) with EMF exposure	• Small sample size (n=7) due complex study design including use of explosimeters to assess the exposure.
Lasalvia M et al.[5] (Original Article), 2018	• Possible alterations of morphology of lympho-monocytes of exposure to microwave radiation.	• Larger samples required to assess the biological consequences of findings.
Jazi SD et al.[27] (Original Article), 2017	• No effect of EMF on physiological tremor and EEG	• Generalization of the study findings was limited to small sample size and nature of the study
Kapri-Pardes E et al.[7] (Original Article), 2017	• Evidence of potential of ELF-EMF towards cellular proliferation and oncogenic transformation.	• No sufficient rise in ERK1/1 phosphorylation on EMF exposure sufficient to justify oncogenic potential.
Fang Q et al.[3] (Original Article), 2016	• Change in RR interval on short term exposure to EMF. No change in rest of the ECG intervals on EMF exposure.	• In view of overlapping ECG frequency and ELF-EMF operating frequency, it is difficult to conclude definitive effect of EMF on ECG.
Luo Q et al.[2] (Original Article), 2013	• EMF exposure adversely affects placental functions and foetal development among pregnant mothers.	• Altered protein expression on foetus cannot be verified due to ethical concerns.
Balamuralikrishnan B et al.[4] (Original Article), 2012	• Genotoxic potential of ELF-EMF in peripheral lymphocytes among workers exposed to prolonged low level non ionizing radiation	• It was a case control study, however only 20 controls for 50 exposed were taken. Control group could have been increased.
Augner C Et al.[8] (Original Article), 2010	• Higher incident of psychological strain and anxiety among people living 100 meters or less, from the tele-communication base stations.	• The findings were generated with the help of participant's subjective outlook regarding the EMF exposure.
Naarala J, et al.[10] (Review Article), 2019	• Linkage of Radiofrequency MF with pathways like apoptosis, cellular regulation and cytoskeleton maintenance. • Effects of EMF on circadian rhythm and sleep cycle.	• Lack of consistency regarding effects of EMF exposure by different studies. • Inhomogeneous study designs.
Singh R et al.[14] (Review Article), 2018	• Possible mechanism of action of non-thermal effects can be production of reactive oxygen species. • Effect of EMF on reproductive system by causing decreased sperm motility, viability as well as altered sperm morphology.	• Different exposure parameters, variations in body structures and environment.

TABLE 1. Summary of various epidemiological studies included in the systematic review. (Continue)

Authors Article Types Publication Year	Findings	Challenges in understanding the biological effects of EMF radiations
Santini SJ et al. [18] (Review Article), 2018	• Cellular effects like altered molecular pathways, apoptosis and dysregulated cell cycle. • Raised reactive oxygen species. Possible role of EMF as co-carcinogen. • Increased risk of neurodegenerative diseases, autism. • Possible effect of both male and female reproductive systems.	• Different biological models used in different settings, diverse exposure. • Controversial findings among various studies.
Kesari KK et al. [13] (Review Article), 2018	• Detrimental effect of EMF on quality of sperms including count, morphology and motility.	• Even in presence of significant evidence, the true mechanism behind effect of EMF on reproductive system inaccessible.
Wang H et al. [11] (Review Article), 2017	• Increased levels of reactive oxygen species on exposure to EMF in majority of the reviewed research.	• Disparities among various studies which could be due to magnetic field type/ intensity/frequency.
Carlberg M et al. [19] (Review Article), 2017	• Potential association of gliomas and EMF exposure on the basis of nine Bradford Hill viewpoints.	• Findings are based on Hills viewpoint of causality and analyses secondary data
Medeiros LN et al. [12] (Review Article), 2015	• Association between EMF exposure and tinnitus, especially in persons with electromagnetic hypersensitivity.	• Prospective cohort studies are further required for providing definitive evidence of the findings.
Teepen JC, et al. [16] (Review Article), 2012	• Increased potential risk of Childhood Leukaemia for EMF exposure although its causal association cannot be confirmed.	• Limited epidemiological studies on impact of EMF with inbuilt biases in the present studies.
Pall ML et al. [15] (Review Article), 2015	• Non thermal biological effects of EMF need to be emphasized, esp. the genotoxic potential in presence of vast array of literature with conflicting results.	• Emphasis of selection of only consistent studies while addressing the research question was preformed
Vijaylaxmi et al. [17] (Review Article), 2014	• Even in presence of contrasting findings from different group of experts about the biological effects of EMF, a preventive approach towards the same remains the key.	• It was compilation of the various guidelines and conclusions of studies on the biological effects of RF exposures, from various national and international expert groups.
Swerdlow AJ et al. [21] (Commentary), 2011	• Unlikely evidence of increased brain tumours among adults.	• Presence of recall misclassification in the case control studies, limited time duration.
Regel SJ et al. [20] (Review Article), 2011	• Inconsistent findings of any effect of EMF exposure and cognitive functions.	• Reason behind the inconsistent findings could be lack of validated tools, study designs and different sample sizes.

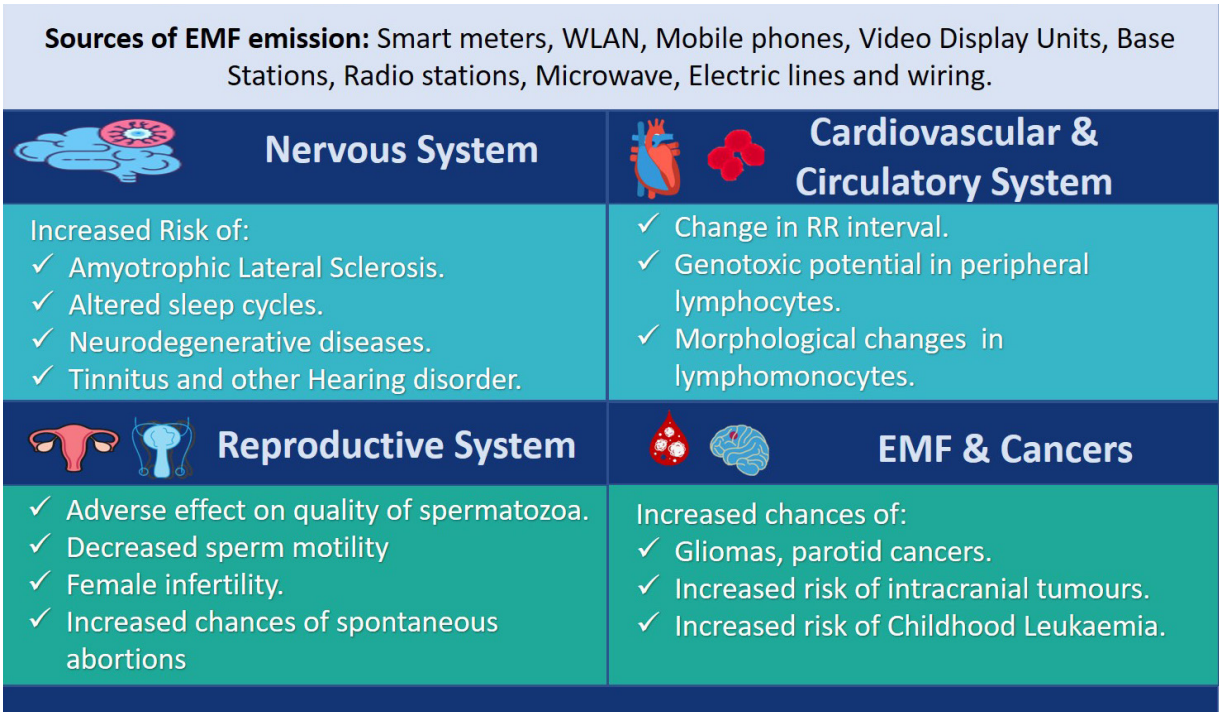

Fig 2. Figure showing possible adverse effects of EMF on different organ system.

in the risk of ALS (Amyotrophic Lateral Sclerosis) and Alzheimer's disease due to exposure.[24] In an Indian study amongst school going students, "Ringxiety" or phantom ringing is seen in the students frequently using mobile phones in classroom and library.[25] Augner C et al, also reported higher psychological stress and anxiety among those living near base stations.[8] Medeiros LN et.al, in a review, reported association of long-term mobile phone use and tinnitus and other hearing disorders.[12] A study by Reale et.al, also provide data on potential effect of ELF- EMF on neurodegenerative processes which further needs to be established with the help of experimental models.[20] EMFs have also been reported to alter pineal melatonin concentration, affect sleep cycle, lower mood, reduced concentration, and depression. Decrease in release of melatonin hampers maintaining the molecular structure of DNA strands, entitling EMFs as teratogenic and mutagenic.[26] A study by Jazi AD et al., however reported no effect of EL-EMF on physiological tremors and EEG.[27]

Effects on reproductive system

Kesari et al. have reported negative effect of EMF on male fertility due to adverse effect on quality of spermatozoa.[13] A decrease in the normal sperm morphology, motility and count on exposure to EMF including mobile phones has also been reported in literature.[14,18] Another case control study done in Iran reported 4 times higher risk of infertility among women living within 500 mts proximity of high voltage power lines in comparison to women living at more than 1,000 mts distance.[28] Clinical studies on pregnant women exposed to ELF RF like Video display terminal (VDT) have indicated a significant increase in spontaneous abortions.[29] Exposure of EMF in early embryo stage may also have an adverse effect on nervous system development and cellular proliferation.[30]

Effect of cardiovascular and circulatory system

Fang et al in their study reported a significant change in RR interval on ECG on short term exposure to ELF-EMF, with no significant change on other intervals.[3] However, physiological implication to the above findings requires further research.[3] Evidence also supports genotoxic potential of ELF-EMF in peripheral lymphocytes among workers exposed to prolonged low level non-ionizing radiation.[4] Possible effect of EMF exposure on the lymphomonocyte morphology was also reported but was limited to further research in terms of EMF type/ intensity/ frequency, etc.[5]

Electromagnetic Hypersensitivity Syndromes (EHS)

Some individuals reported dermatological symptoms like redness, itching relating to exposure to EMFs termed as Electromagnetic Hypersensitivity syndromes (EHS). It was sometimes associated with or without vegetative or neurasthenic symptoms like nausea, general weakness,

dizziness, nystagmus, sometimes even haemoptysis and paralysis.[2] Bogers et.al, also reported association of non-specific physical symptoms (including, headache, fatigue and dizziness that cannot be explained by other medical condition), with radio frequency electromagnetic fields.[6] However, after conducting several double-blinded cohort studies, the causal association of EHS with EMF could not be established. EHS was then regarded as the consequence of predicament of assuming harmfulness of EMF rather than EMF itself by many leading health professional and scientific bodies.[31]

EMF and cancers

A study by Kapri-Pardes et al., yielded some evidence of potential of ELF-EMF towards cellular proliferation and oncogenic transformation.[7] Teepen JC, et al, also provided epidemiological evidence of higher population attributable risk of Childhood Leukaemia among children exposed to ELF-EMF levels above 0.3 μT.[16] The Interphone study published in 2010, reported more than double the risk of brain Glioma in the people using mobile phones for more than 10 years.[32] A meta-analysis done by Bortkiewicz A, et al, also provided evidence increased risk of increased risk of intracranial tumours with long term use of mobile phones.[33] However, many other studies proved inconclusive evidences of mobile phone usage and cancers.[15,21,34] A study relating occurrence of cancers (Glioma, Acoustic Neuroma and Parotid gland cancers) with respect to the residential distance from GSM or UMTS found no relationship between the occurrence of cancer and various distances..[35] Carlberg M et al., using Bradford Hill viewpoints also concluded a positive association of gliomas and EMF exposure.[19]

DISCUSSION

As, there are multiple sources of EMF in any particular residence or workplace, proper epidemiological evaluation of this matter is quite ambiguous. As the time of use of electronic appliances and telecommunication tools like mobile phones and other EMF devices will increase in coming years, we are exposed to EMF radiation from multiple sources simultaneously every day at work and home. So accurate data regarding EMF pollution from any epidemiological studies could not possibly made in real human population. Experimental studies indicate that short-term exposure at the levels present in the environment or in the home do not cause any apparent detrimental effects. Thus, till the time a definitive health effect has been proven, considering a high index of suspicion, a need arise for proper legislative measure that should be taken to reduce usage of materials that contributes to electromagnetic field pollution. Such as, limitation of numbers of radio stations in crowded area or base station in public place. Electric lines and wiring should be done as such that EMF emission should be least. IEC activities should be undertaken targeting young population to decrease mobile phone time in their daily life, which is increasing day-by-day. Awareness of young population regarding the EMF emission from video displaying units would markedly reduce screen time, thus, electromagnetic field pollution.

WHO established the International EMF Project in the year 1996 to provide an international platform for coordinated response towards EMF issues. However, International Commission on Non-Ionizing Radiation Protection (ICNIRP) aided by Institute of Electrical and Electronics Engineer (IEEE) puts up the guidelines regarding the exposure limits of EMF in residential and occupational fields. They do the research regarding the EMF and its importance in environment and present their analysed information to WHO for making guidelines and decisions regarding EMF. Though WHO and its auxiliary organisations have repeatedly told that the effect is not detrimental to health, but it didn't satisfy a large number of researchers, who have argued that only short term effects had been taken into consideration in the epidemiological studies and not the long term and non-thermal effects. WHO also issued a risk-assessment monograph EHC (Environmental Health Criteria) for EMF but a group of researchers from Karolinska University reported that the committee presiding over the EHC risk-assessment study mostly comprised of people who are from ICNIRP and IEEE itself and so can be biased.[22]

CONCLUSION

There is no denying that the existing research works are pointing towards greater risk of adverse health effects ranging from irritability and sleep disturbance to paralysis and cancers. There with the need of the hour is undertaking various preventive measures in order to minimize the exposure in the occupational as well as non-occupational settings. There should be Mass media effort to generate awareness about the possible health impacts of EMF, particularly focusing on young population and proper legislative measures should be taken to minimize EMF exposure at occupational settings. In long run for overall benefit and sustainable development we should start searching option to substitute the contemporary technologies with ones having favourable benefit-risk ratio.

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