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Publication Ethics

Publication ethics of Greater Mekong Subregion Medical Journal (GMSMJ)

Greater Mekong Subregion Medical Journal presents articles in the field of basic and advanced clinical research in medicine and related health sciences, medical education as well as community medicine in Thailand and international, especially in countries of Greater Mekong Subregion.

The journal publishes 3 issues a year: Issue 1 (January - April), Issue 2 (May - August) and Issue 3 (September -December). All submitted research articles and review articles will be evaluated by a single blinded peer-review process and reviewed by 2 experts who have knowledge, expertise, and experience in the field of medicine and related health sciences prior to publication. The journal encloses the information of authors and reviewers. In case of a difference of evaluation, the article evaluation will be considered and given a final decision.

Greater Mekong Subregion Medical Journal establishes the roles and duties for three different groups in the process of article publication: author (s), editor, and reviewers. The following information is given to the three groups of people so that they will strictly abide by its benefits of those concerns, including readers and others in academia.

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Original Article

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Greater Mekong Subregion Medical Journal

Epidemiology and Antimicrobial Susceptibility of Bloodstream Infections among Febrile Neutropenic Patients at a Thai University Hospital

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Abstract:

Background: Febrile neutropenia is a frequent and serious complication in patients undergoing chemotherapy for malignancies, often resulting in significant morbidity and mortality. Understanding local epidemiology and antimicrobial resistance patterns is critical to optimizing empirical therapy for bloodstream infections in this population.

Objective: This study aimed to evaluate the current microbiological profile and antimicrobial susceptibility patterns of bloodstream infections (BSIs) in neutropenic patients at Phramongkutklao Hospital. Additionally, the study sought to identify factors associated with drug-resistant infections and determine their impact on patient mortality.

Materials and Method: This retrospective analytical study reviewed the medical records of febrile neutropenic patients with bloodstream infections at Phramongkutklao Hospital between January 1, 2017, and December 31, 2021. The study aimed to describe the epidemiological trends and antimicrobial susceptibility patterns in febrile neutropenic patients with bacteremia.

Results: A total of 151 febrile neutropenic patients with bloodstream infections were included, with a mean age of 43.42 years. Males constituted 57.6% of the cohort. The most common underlying conditions were acute myeloid leukemia (60.9%), acute lymphoblastic leukemia (25.2%), and multiple myeloma (10.6%). Gram-negative bacteria were the most frequently isolated pathogens (73.5%), primarily *Escherichia coli* (33.7%), *Klebsiella pneumoniae* (19.9%), and *Pseudomonas aeruginosa* (10.6%). Gram-positive bacteria accounted for 19.9% of cases, and fungi 6.6%. Significant associations were observed between urinary catheter use and infections caused by carbapenem-resistant Enterobacterales (CRE) and vancomycin-resistant Enterococci (VRE) ($p < 0.05$), while parenteral nutrition use was linked to methicillin-resistant *Staphylococcus aureus* (MRSA) infections ($p = 0.02$). The overall 14-day mortality rate was 16.6%, with CRE and VRE infections contributing

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significantly to mortality ($p < 0.01$). A Pitt bacteremia score greater than 3 was also identified as a significant predictor of mortality.

Conclusion: Gram-negative bacteria remain the predominant pathogens in febrile neutropenic patients with bloodstream infections. Their prevalence and evolving antimicrobial resistance patterns are critical considerations in guiding empirical therapy at Phramongkutklao Hospital.

Keyword: Febrile neutropenia, Bloodstream infections, Antimicrobial resistance

Introduction

Chemotherapy-induced neutropenia is associated with fever in approximately 10-50% of patients with solid tumors and more than 80% of patients with hematologic malignancies. This fever is primarily attributed to underlying infections.[1] Given the critical nature of this condition, the administration of broad-spectrum antimicrobial therapy, aligned with established clinical guidelines, is imperative.[1-3] Delayed or insufficient initial antimicrobial therapy, especially in septic or septic shock conditions, significantly increases mortality risk.[4] Prompt and appropriate antimicrobial intervention has been shown to reduce morbidity and mortality rates among these patients.

The epithelial barrier serves as the body's primary defense mechanism against microbial invasion. However, chemotherapy disrupts not only cancer cells but also the epithelial lining of organs such as the digestive tract and oral cavity, enabling normal microbial flora to translocate into tissues and the bloodstream.[2,5] Additionally, chemotherapy-induced neutropenia diminishes both the quantity and functionality of neutrophils, the immune system's first responders to infections. As a result, patients undergoing chemotherapy face a heightened susceptibility to infections.[6] Notably, infection-related symptoms in neutropenic patients are often subtle or even asymptomatic, with fever being one of the few common indicators.[7] Thus, managing fever in these patients can be particularly challenging.

Severe complications arising from febrile neutropenia, including septic shock, acute renal failure, respiratory failure, and heart failure, occur in 25-30% of cases, with an associated mortality rate of approximately 11%. In cases involving severe infections, this rate can increase by up to 50%.[3] Mortality is influenced by factors such as comorbidities, the severity and duration of neutropenia, and the presence of bacteremia.[8] Mortality rates differ based on the causative pathogen, standing at 18% for gram-negative bacterial infections and 5% for gram-positive infections. Previous research suggests that gram-negative infections are more prevalent, accounting for 60–70% of cases.[9-13]

Antibiotic resistance poses a significant challenge to treatment outcomes in both the general population and patients with febrile neutropenia.[14,15] Therefore, maintaining up-to-date epidemiological data on pathogens within individual healthcare institutions is essential for optimizing the selection of broad-spectrum antimicrobials. Such data can contribute to lowering the rates of severe complications, morbidity, and mortality. This study aims to examine the epidemiology and antimicrobial susceptibility patterns of bloodstream

infections in febrile neutropenic patients at Phramongkutklao Hospital. Additionally, it seeks to identify factors associated with antimicrobial resistance and mortality in this patient population.

Materials and Method

Ethical considerations

The study received ethical approval from the Institutional Review Board of the Royal Thai Army Medical Department, Bangkok, Thailand (Approval number: IRBRTA 0805/2023). All procedures adhered to the Declaration of Helsinki and complied with the International Conference on Harmonisation Guidelines for Good Clinical Practice.

Study design and participants

This retrospective cohort study was conducted at Phramongkutklao Hospital in Bangkok, Thailand, over a five-year period from January 2017 to December 2021. The study population included patients admitted to the Department of Medicine with febrile neutropenia and confirmed bloodstream infections, based on laboratory findings.

Febrile neutropenia was defined as either a single axillary temperature of ≥ 38.3°C or a sustained temperature of ≥ 38°C for over one hour. Neutropenia was characterized as an absolute neutrophil count (ANC) of < 1,000 cells/mm^3 or < 1,500 cells/mm^3 with a predicted decline to < 500 cells/mm^3 within 48 hours, following the Infectious Disease Society of America (IDSA) guidelines.[16] Patients were excluded if they had known HIV infection, pathogen contamination, infections occurring after the diagnosis of febrile neutropenia, or infections with identifiable causes, such as pneumonia or urinary tract infections.

Procedures

Data were retrospectively extracted from inpatient medical records, including demographic information such as age, gender, comorbidities, underlying malignancy, chemotherapy regimen, and catheter use. Data on causative pathogens and antimicrobial susceptibility in bloodstream infections among neutropenic patients were also reviewed. The clinical presentation at the time of febrile neutropenia diagnosis and subsequent outcomes were documented using standardized case report forms.

Standard laboratory procedures were followed for blood cultures as part of routine clinical practice. Pathogen identification was performed using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was conducted through automated broth microdilution (Sensititre, Thermo Fisher, Cleveland, OH, USA), with minimum inhibitory concentration (MIC) values determined according to Clinical and Laboratory Standards Institute (CLSI) guidelines.[17]

Outcomes

The primary outcomes included microbiological isolates and antimicrobial susceptibility patterns. Clinical outcomes were evaluated based on the use of mechanical ventilation, ICU length of stay, incidence of organ failure, and mortality rates. Additionally, the Pitt bacteremia score was analyzed as a prognostic indicator for mortality.[18, 19]

Statistical analysis

The sample size was calculated using data from C. Gudio, et al.[9], which reported a 49% prevalence of bloodstream infections

with isolated cultures. To achieve statistical significance ($p < 0.05$) with 90% power, a minimum of 145 participants was required. Descriptive statistics were reported as means or medians for continuous variables and as frequencies and percentages for categorical variables. Comparisons of categorical variables were performed using the Chi-square test, while continuous variables were analyzed using the independent T-test or Mann-Whitney U test, as appropriate. Statistical analyses were conducted using STATA software, Version 14.0 (StataCorp, College Station, TX, USA). A p-value of < 0.05 was considered statistically significant

Results

Participants were recruited for the study between January 2017 and December 2021. Initially, 253 individuals diagnosed with febrile neutropenia were screened. Of these, 39 participants were excluded due to negative blood cultures, resulting in 214 eligible participants with positive blood cultures. Among these, 58 participants met exclusion criteria, and an additional 5 were excluded due to receiving palliative care or missing data. Consequently, 151 participants were included in the final analysis (Figure 1).

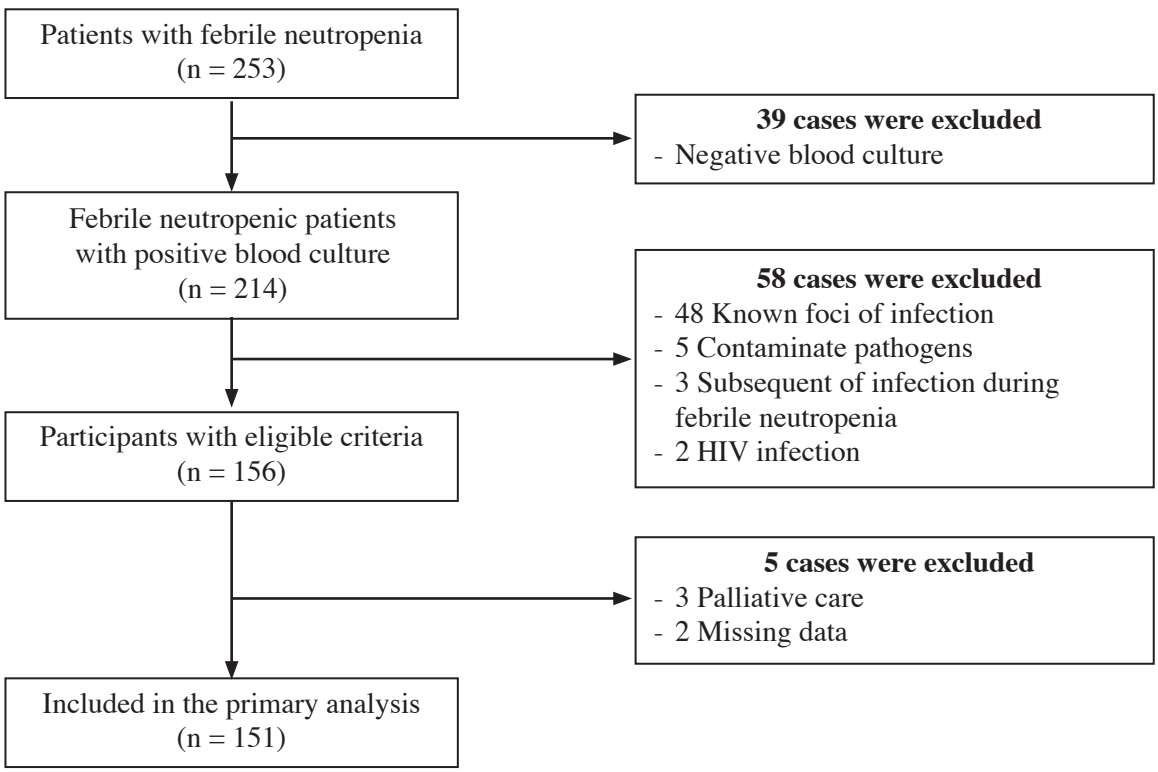

Figure 1 Patient enrollment and the flow of study

Baseline demographic and clinical characteristics are detailed in Table 1. The cohort comprised predominantly male participants (87/151; 57.6%), with a mean age of 43.42 ± 17.04 years. Hypertension was the most prevalent comorbidity, followed by chronic kidney disease and diabetes mellitus.

Hematologic malignancies were the most common underlying conditions (99.3%), with only 0.7% of cases attributed to solid malignancies. Acute myeloid

leukemia (AML) was the most frequent primary diagnosis (60.9%), followed by acute lymphoblastic leukemia (ALL) (25.2%) and multiple myeloma (10.6%). Chemotherapy regimens included FLAG-IDA (20.5%), Hyper-CAVD (17.9%), Int-DAC (13.2%), 7+3 (7.9%), and Hi-DAC (7.9%) (Table 1).

Table 1 Baseline demographic data of 151 participants with febrile neutropenia

Patient characteristics	**Number (%)**
Age, years (mean ± SD)	43.42 ± 17.04
Male sex	87 (57.6%)
Underlying disease	
Hypertension	36 (23.8)
Chronic kidney disease	14 (9.3)
Diabetes mellitus	11 (7.3)
Ischemic heart disease	10 (6.6)
Chronic lung disease	7 (4.6)
Cirrhosis	4 (2.6)
Underlying malignancy	
Acute myeloid leukemia	92 (60.9)
Acute lymphocytic leukemia	38 (25.2)
Multiple myeloma	16 (10.6)
Non-Hodgkin lymphoma	2 (1.3)
Chronic myeloid leukemia	2 (1.3)
Colonic cancer	1 (0.7)
Chemotherapy regimen	
FLAG-IDA regimen	31 (20.5)
Hyper-CVAD regimen	27 (17.9)
Int-DAC regimen	20 (13.2)
7+3 (cytarabie + anthracycline) regimen	12 (7.9)
Hi-DAC regimen	12 (7.9)
Concurrent corticosteroid therapy	50 (33.1)
Previous hospitalization (within 3 months)	139 (92.1)
Previous antimicrobial therapy (within 3 months)	126 (83.4)
Central venous catheter or PICC insertion	132 (87.4)
Urinary catheter insertion	40 (26.5)
Parenteral nutrition	7 (4.6)

Primary outcome

Among the 151 blood culture isolates, Gram-negative bacteria accounted for the majority (73.5%), followed by Gram-positive bacteria (19.9%) and fungi (6.6%). The most common Gram-negative pathogens were *Escherichia coli* (33.7%), *Klebsiella pneumoniae* (19.9%), *Pseudomonas aeruginosa* (10.6%), and *Acinetobacter baumannii* (3.9%). For Gram-positive isolates, *Corynebacterium* species (4.6%), *Enterococcus faecium* (4.6%), and *Staphylococcus aureus* (4.1%) were prevalent. Fungal isolates primarily included *Candida* species (5.2%) (Table 2).

Table 2 Causative organisms of 151 episodes of febrile neutropenia with positive blood culture

Isolated organisms	Number (%)	Isolated organisms	Number (%)
Gram-negative bacteria	**111 (73.5)**	**Gram-positive bacteria**	**30 (19.9)**
Escherichia coli	51 (33.7)	*Corynebacterium* spp.	7 (4.6)
Klebsiella pneumoniae	30 (19.9)	*Enterococcus faecium*	7 (4.6)
Pseudomonas aeruginosa	16 (10.6)	*Staphylococcus aureus*	6 (4.1)
Acinetobacter baumannii	6 (3.9)	Viridans group streptococci	5 (3.3)
Acinetobacter spp.	3 (2.0)	Coagulase-negative staphylococci	5 (3.3)
Aeromonas spp.	3 (2.0)	**Fungi**	**10 (6.6)**
Salmonella spp.	1 (0.7)	*Candida* spp.	8 (5.2)
Stenotrophomonas maltophilia	1 (0.7)	*Trichosporon asahii*	1 (0.7)
		Fusarium spp.	1 (0.7)

Antimicrobial susceptibility analyses revealed that 47.1% of *E. coli* and 43.3% of *Klebsiella pneumoniae* isolates were susceptible to ceftriaxone. Imipenem susceptibility varied across pathogens, with rates of 94.1% for *Escherichia coli*, 73.3% for *Klebsiella pneumoniae*, 81.3% for *Pseudomonas aeruginosa*, and 16.7% for *Acinetobacter baumannii*. Colistin susceptibility was high for *Pseudomonas aeruginosa* (100%) and *Escherichia coli* (90.2%) but lower for *Klebsiella pneumoniae* (56.7%) and *Acinetobacter baumannii* (83.3%). Amikacin exhibited strong activity against *Escherichia coli* (100%), *Klebsiella pneumoniae* (96.7%), and *Pseudomonas aeruginosa* (87.5%), though activity against *Acinetobacter baumannii* was limited (33.3%).

Among Gram-positive bacteremia cases (19.9%), methicillin-resistant *Staphylococcus aureus* (MRSA) was detected in 50%, while coagulase-negative staphylococci (CoNS) were entirely resistant to methicillin (MRCoNS). All strains of MRSA, MSSA, and CoNS were susceptible to vancomycin. Conversely, *Enterococcus faecium* exhibited 100% resistance to vancomycin, qualifying as vancomycin-resistant enterococci (VRE). Viridans group streptococci remained susceptible to ceftriaxone and vancomycin. Detailed susceptibility patterns are presented in Figures 2 and 3.

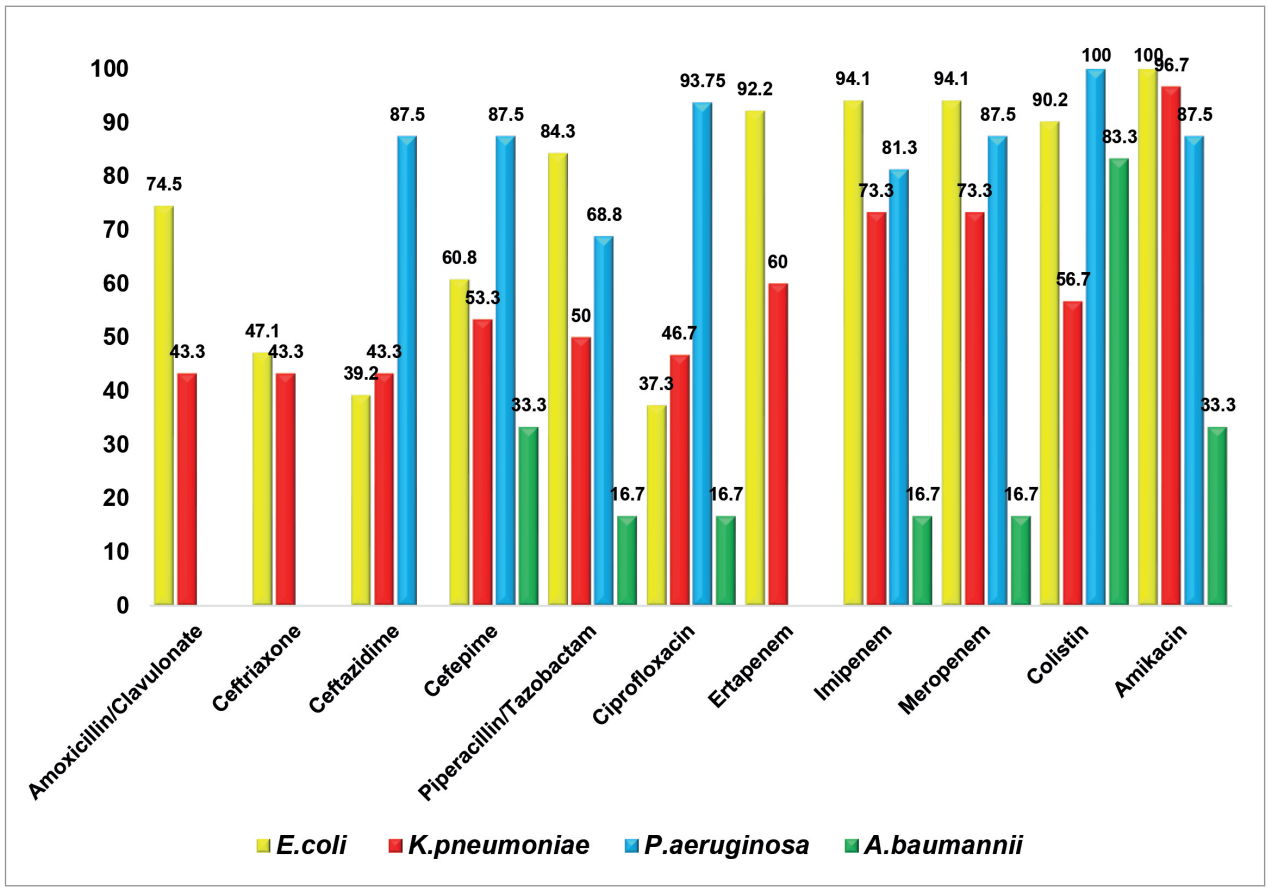

Figure 2 Antimicrobial susceptibility pattern of Gram-negative bacteria

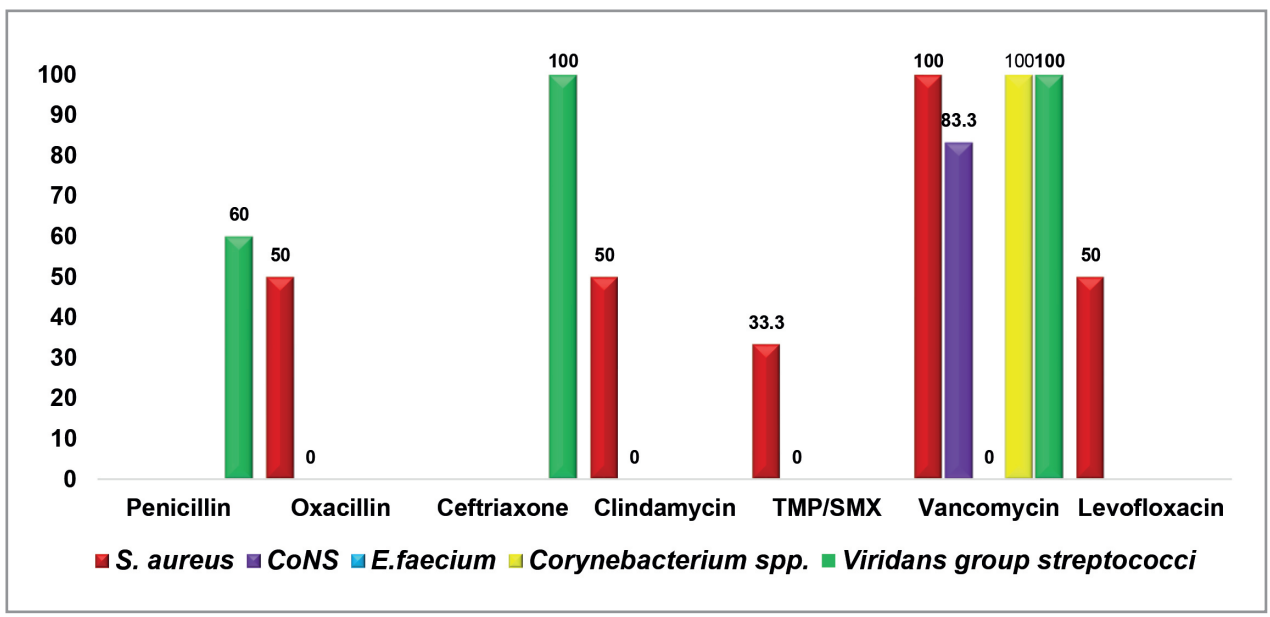

Figure 3 Antimicrobial susceptibility pattern of Gram-positive bacteria

Secondary outcomes

At diagnosis, 61.6% of patients presented with fever without localized symptoms, while 13.9% reported perianal pain, and 12.6% showed signs of thrombophlebitis. Laboratory findings revealed a mean absolute neutrophil count (ANC) of 172.48 ± 394.28 cells/mm^3, with an average neutropenia duration of 17.05 ± 11.92 days.

Complications included respiratory failure requiring mechanical ventilation in 14.6% of patients, critical illness necessitating ICU transfer in 13.9%, and a 14-day mortality rate of 16.6% (Table 3).

Table 3 Clinical presentation and outcomes in patients with febrile neutropenia

Clinical presentation and outcome	**Total episode (n = 151)**
Clinical presentation at diagnosis, n (%)	
Fever	93 (61.6)
Perianal pain	21 (13.9)
Thrombophlebitis	19 (12.6)
Gastrointestinal tract symptoms	6 (4.0)
Respiratory tract symptom	6 (4.0)
Pitt bacteremic score at diagnosis (mean ± SD)	1.78 ± 2.11
Absolute neutrophil count at diagnosis (cell/mm^3; mean ± SD)	172.48 ± 394.28
Duration of neutropenia (days; mean ± SD)	17.05 ± 11.92
Duration of hospitalization (days; mean ± SD)	34.02 ± 16.79
Clinical complication, n (%)	
Mechanical ventilation use	22 (14.6)
Intensive care unit transfer	21 (13.9)
Organ failure*	49 (32.5)
14-day mortality	25 (16.6)

*Organ failure: acute respiratory failure that needs O_2 therapy, acute heart failure, acute kidney injury defined by AKIN criteria, acute hepatocellular injury that AST/ALT elevated > 3UNL.

Subgroup analysis showed that patients with multidrug-resistant (MDR) organism infections had a higher 14-day mortality rate compared to those without MDR infections (50% vs 28.67%, p = 0.06). Specifically, bloodstream infections caused by carbapenem-resistant Enterobacterales (CRE) significantly increased the risk of 14-day mortality (HR 6.1; 95% CI 1.5–24.95; p = 0.01). (Table 4)

Table 4 Fourteen-day mortality rate in subgroup analysis of patients with multidrug resistance organisms infection

Mortality rate	With drug resistance, frequency (%)	Without drug resistance, frequency (%)	HR (95% CI)	p-value
Any MDROs	10 (50)	43 (28.67)	2.49 (0.97-6.40)	0.06
3GCephRE	2 (33)	27 (44.26)	0.63 (0.11-3.70)	0.61
CRE	5 (50)	10 (14.08)	6.1 (1.50-24.95)	0.01
MDR *P. aeruginosa*	0 (0)	5 (33.33)	0.64 (0.02-18.37)	0.79
CRAB	3 (100)	1 (33.33)	11.67 (0.32-422.17)	0.18

Abbreviations: MDROs, Multidrug resistant organisms; 3GCephRE, 3rd generation cephalosporin resistant Enterobacterales; CRE, Carbapenems resistant Enterobacterales; MDR, Multidrug resistant; CRAB, Carbapenems resistant *A. baumannii*

Risk factors for drug resistance included Foley catheter use and mechanical ventilation. Foley catheter insertion was associated with an elevated risk of CRE infection (HR 3.64; 95% CI 1.16-11.44; p = 0.03) and VRE infection (HR 7.79; 95% CI 1.45-41.92; p = 0.02). Similarly, mechanical ventilation significantly increased the likelihood of CRE infection (HR 5.45; 95% CI 1.35-22.04; p = 0.02) (Table 5).

Table 5 Factor associated drug resistance organisms

Factors associated with 3GCephRE	**HR (95% CI)**	**p-value**
• Present of Foley's catheter	0.39 (0.10-1.39)	0.15
• Mechanical ventilator use	0.49 (0.09-2.72)	0.41
• Previous antibiotic use within 3 months	0.58 (0.16-2.13)	0.41
• Previous hospitalization within 3 months	1.59 (0.27-9.33)	0.61
Factors associated with CRE	**HR (95% CI)**	**p-value**
• Present of Foley's catheter	3.64 (1.16-11.44)	0.03
• Mechanical ventilator use	5.45 (1.35-22.04)	0.02
• Previous antibiotic use within 3 months	2.80 (0.33-23.55)	0.34
• Previous hospitalization within 3 months	3.33 (0.18-62.38)	0.42

ROC analysis identified a Pitt bacteremia score of >3 as the optimal cut-off for predicting mortality in bloodstream infections. This score demonstrated a sensitivity of 88%, specificity of 91.3%, a positive predictive value of 66.7%, and a negative predictive value of 97.5% (Figure 4).

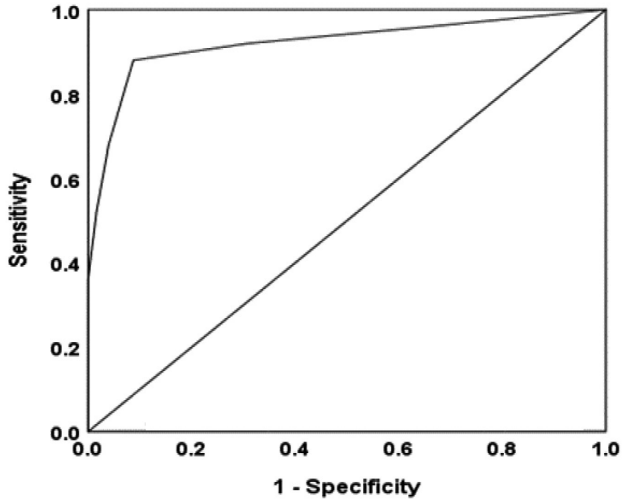

Figure 4 AUROC curve analysis Pitt bacteremic score to predictors of mortality in BSI

Discussion

Febrile neutropenia is a frequent and serious complication among patients with hematologic malignancies undergoing chemotherapy. Consistent with findings from previous studies[1], hematologic malignancies were identified as the predominant underlying cause of febrile neutropenia at Phramongkutklao Hospital. Acute myeloid leukemia, acute lymphoblastic leukemia, and multiple myeloma were the most commonly observed conditions, consistent with findings reported in similar research.[13]

This study reaffirms that Gram-negative bacteria (73.5%) remain the predominant causative agents in febrile neutropenic patients, followed by Gram-positive bacteria (19.9%) and fungi (6.6%). The variability in the incidence of Gram-positive infections compared to previous studies may reflect differences in geographic regions, hospital protocols, and time periods, with reported rates of 29.9%[11], 62%[20], and 67%.[9] While prior institutional data highlighted a higher prevalence of Gram-positive infections, our findings align with international reports identifying Enterobacteriaceae, specifically *Escherichia coli* (33.7%) and *Klebsiella pneumoniae* (19.9%), as the most isolated Gram-negative pathogens.[9, 11, 12, 20] The detection of *Pseudomonas aeruginosa* in 10.6% of cases reinforces its importance as a pathogen requiring coverage in empirical antibiotic regimens for febrile neutropenic patients, as evidenced by incidence rates of 7%[9], 14%.[12] Notably, this study identified an increased incidence of candidemia (5.2%), compared to previous studies reporting 1.5%.[9] which may be linked to the rising use of central venous catheters, peripherally inserted central catheters (PICC), and parenteral nutrition. Clinicians should consider fungal infections, such as candidemia, in patients who do not respond to antibiotic therapy, even though these infections occur less frequently than bacterial ones. Delayed recognition of fungal infections can lead to worse outcomes, so early consideration is crucial, especially in immunocompromised individuals or those with prolonged hospital stays.

The escalating prevalence of antimicrobial resistance remains a major global challenge in managing bloodstream infections. According to the World Health Organization (WHO) 2024 Bacterial Priority Pathogens List (BPPL), Gram-negative bacterial pathogens continue to be classified as critical threats, particularly carbapenem-resistant *Acinetobacter baumannii* (CRAB), carbapenem-resistant *Enterobacterales* (CRE), and third-generation cephalosporin-

resistant *Enterobacterales* (3GCRE).[21, 22] This study identified a higher resistance rate of Enterobacterales to ceftazidime (58.75%) and fluoroquinolones (58%) compared to previous reports.[23] Additionally, this study found an increased resistance to fluoroquinolones, with a resistance rate of 58%, compared to 38.2%[12] and 26%.[24] However, susceptibility to piperacillin-tazobactam (67.2%) compare with previous studies have reported varying levels of sensitivity, ranging from 53.2%[12], 87.5%[23], to 95%.[24] This trend aligns with the increasing burden of CRE and 3GCRE, which are ranked among the highest-priority pathogens due to their widespread prevalence, resistance mechanisms, and limited treatment options. The carbapenems remains relatively high, underscoring their role in the empirical treatment of resistant Gram-negative infections, According to the current IDSA guidance[25] for the treatment of third-generation cephalosporin resistance, carbapenems are recommended for treating infections caused by these organisms. Nevertheless, the development of carbapenem resistance, as observed in 7.8% of Enterobacterales isolates, similar to previous studies that reported 7.1%.[23] The biofilm-associated resistance increases the persistence of *Klebsiella pneumoniae* in hospital environments, emphasizing the necessity of stringent infection control measures, particularly in immunocompromised populations like febrile neutropenic patients especially those with indwelling catheters.[26] Furthermore, resistance to tigecycline among CRKP strains has been reported in Thailand indicating the emergence of multidrug-resistant (MDR) and extensively drug-resistant (XDR) Klebsiella pneumoniae strains. The present further treatment limitations, necessitating the careful selection of combination therapies to optimize patient outcomes.[27]

Given the rising prevalence of drug-resistant Gram-negative infections, healthcare providers must continuously adapt treatment strategies based on local epidemiology. Strengthening antimicrobial stewardship, optimizing infection control, and developing novel therapies are crucial to mitigating multidrug-resistant infections. The persistence of CRAB, CRE, and 3GCRE as WHO critical-priority pathogens highlights the need for a global AMR response. Effective catheter care, environmental disinfection, and targeted antibiotic strategies can prevent recurrent bloodstream infections, particularly those caused by *Pseudomonas aeruginosa*, while enhanced surveillance and antibiotic restrictions help reduce resistance and improve outcomes.

Bloodstream infections are a significant cause of morbidity and mortality in cancer patients with febrile neutropenia.[20] These infections can be reduced by prescribing appropriate empirical antibiotics based on the local antibiotic resistance patterns in hospitals.[4] The 14-day mortality rate of 16.6% is consistent with earlier studies from Thailand, with similar patient characteristics contributing to comparable outcomes 19.2%[11], 19%[28], and 19.7%[23], respectively. The similar mortality rates may be attributed to the comparable age and underlying comorbidities of the patient groups. Drug-resistant infections, particularly CRE infections, emerged as significant contributors to mortality, alongside elevated Pitt bacteremia scores. These findings emphasize the importance of identifying high-risk patients and tailoring treatment strategies accordingly. This finding aligns with studies on drug-resistant infections in the general patient population, not just those with neutropenia.[15]

This study highlights the evolving epidemiology of pathogens and resistance patterns in febrile neutropenic patients.

Ongoing surveillance of local pathogens and their susceptibility is essential to inform empirical antibiotic policies. Additionally, the increasing incidence of candidemia necessitates greater awareness and consideration in treatment protocols.

This study has several limitations. As a single-center study, the findings may not represent the broader population across Thailand. The retrospective design relied on medical records, leading to potential missing data and confounding variables that may affect the analysis of drug resistance. Future research should expand to multicenter studies across hospitals of varying levels—primary, secondary, and tertiary care to identify additional risk factors for drug-resistant infections in febrile neutropenic patients. Such studies will enhance the generalizability of findings and provide a more comprehensive understanding of this critical issue.

Conclusion

Gram-negative bacteria continue to pose significant challenges in managing febrile neutropenic patients with bloodstream infections. Infections caused by multidrug-resistant Gram-negative organisms are associated with increased morbidity and mortality, highlighting the need for appropriate empirical antimicrobial therapy guided by local resistance patterns. Continuous surveillance of antimicrobial resistance trends is imperative to optimize treatment strategies, prevent complications, and improve outcomes in this vulnerable population.

Conflict of Interest

Role of the funding source

The authors have no financial disclosures. The researchers had full and unrestricted access to all study data and maintained sole responsibility for the decision to submit this manuscript for publication.

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ICMJE Statement

Chutchawan Ungthammakhun contributed to the conception and design of the work. Nalinporn Deechat was responsible for the acquisition of data. Both Chutchawan Ungthammakhun and Nalinporn Deechat contributed to the analysis and interpretation of the data. Nalinporn Deechat drafted the manuscript, and the manuscript was revised collaboratively by the authors. Both authors reviewed and approved the final version of the manuscript and agree to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Sensors Based on the Internet of Things for Semi-Outdoor Air Cleaners: An Alternative Method for Controlling Haze and COVID-19

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Abstract:

Background: Particulate matter has been connected to COVID-19 occurrences, whereas Internet of Things (IoT) technology can be utilized to address the demand for ambient air quality monitoring.

Objective: The purpose of this research is to create a low-cost IoT platform that can be integrated with multi-air sensors and a ventilating fan controller for public semi-outdoor air conditions, and to protect us from the haze and the COVID-19 crisis.

Materials and Method: The IoT platform was designed and built, with two-way data communication, between a dual ventilating fan, air quality sensors, and a chatbot platform.

Results: The results of a preliminary test were collected and interpreted, including the operation of the ventilation system, aerosol reports, and IoT interfacing. When PM2.5 levels reached 50 $\mu g/m^3$, a set of ventilating fans was activated and ran indefinitely until the aerosol level dropped below 50 $\mu g/m^3$. The chatbot system was divided into two LINE groups, the first of which was used to send a machine code. This chatbot could respond within 1-5 seconds, depending on Wi-Fi stability, to monitor the status of the ventilation system as well as any types of aerosol levels (CO_2, formaldehyde, TVOC, PM2.5, PM10, temperature, and humidity). Another report system was used to only report machine status and aerosol levels every 1 hour or as users desired, which was designed for people who want to know the air quality levels in their location.

Conclusion: Multi-sensors and the LINE chatbot can both detect aerosols as well as control ventilation fans. With the exception of a SIM card included with the Wi-Fi 3G system, which is suitable for developing countries during the haze and COVID-19 crisis, all platforms are free for potential users.

Keywords: COVID-19, Internet of things, Particulate matter, Haze, Semi-outdoor air quality

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Introduction

Since the end of 2019, the COVID-19 pandemic has had an impact on many global sectors, particularly following declarations of lockdown policies in many countries. Haze pollution levels in some countries have changed as a result of the COVID-19 situation. A machine learning approach was used in Malta to predict the effect of COVID-19 on NO_2 and O_3 concentrations. The results revealed statistically significant reductions in NO_2 concentrations related to traffic, while O_3 levels increased.[1] Similarly in Mexico City, air quality improved during the lockdown due to lower NO_2 and PM2.5 levels, caused by lower motor vehicle emissions, despite higher O_3 levels.[2] Reduced activity in France as a result of lockdown policies resulted in lower NO_2, PM10, and PM2.5 concentration levels.[3] In China, 10 Chinese mega cities, indicators of traffic pollution, NO_2 which is one of the primary traffic pollution indicators, were significantly lower during the pandemic, whereas particulate matter pollution varied.[4] On a global scale, data from 34 countries and five continents revealed a 34.0 percent reduction in NO_2 concentration and a 15.0 percent reduction in PM2.5 concentration during strict lockdown periods, while O_3 concentration increased by 86.0 percent.[5]

Several studies have found that certain air contaminants, such as PM2.5, PM10, CO, NO_2, SO_2, and O_3, increase COVID-19 mortality rates. However, some research indicates that COVID-19's indirect effect may aid in the reduction of air pollution. The presence of particulate matter has been linked to an increase in COVID-19 incidents.[6] After controlling for many area-level confounders, a study in the United States found that higher historical PM2.5 exposures are associated with higher county-level COVID-19 mortality rates.[7] A rapid systematic review conducted between December 2019 and September 2020 discovered that pollutants independently associated with COVID-19 incidence and mortality were firstly PM2.5, followed by PM10, NO_2, and O_3 in the acute phase, while PM2.5 and NO_2 had similar relationships in the chronic phase. As a result, both acute and chronic air pollution exposure can have an impact on COVID-19 epidemiology.[8] Population density is strongly correlated with COVID-19 infection and mortality, as well as with PM2.5 concentration, this according to a global exploratory study of the relationship between population density, PM2.5 concentration, and confirmed COVID-19 ases.[9] Furthermore, according to satellite data, global air pollution is an important cofactor increasing the risk of COVID-19 mortality.[10]

To evaluate and monitor various parameters of air quality, Internet of Things (IoT) technologies and sensor networks such as comprehensive network communication, information tracking, Cloud-based decision making, and online management, have been developed in response to the need for ambient air quality monitoring.[11] For indoor air measurement, one of the existing infrastructures that can be developed into a model for marketing purposes is sensor-based home IoT, on the theme of air quality improvement.[12] Indoor aerosol concentrations, such as volatile organic compounds (VOC), CO, CO_2, and temperature-humidity, are typically reported and further analyzed using Cloud computing and a web server or application.[13] During the COVID-19 pandemic, a solution for indoor air quality monitoring and prediction, based on IoT and machine learning, was developed. GSM/Wi-Fi technology is used to transmit real-time air conditions to a web portal and a mobile application. The Long and Short Term Memory (LSTM) model is used to forecast aerosol concentrations.[14] For outdoor air quality monitoring, an air quality measurement device called "Smart-Air" was created that can detect

PM10 in South Korean subway tunnels and present it to an IoT gateway.[15]

A previous study found that a low-cost prototype of a water-based air purifier that can be used in semi-outdoor conditions could also be operated manually. It has out-of-the-box IoT connectivity.[16] Some details of ventilating fans combined with IoT systems are required to develop this machine. To realize an IoT-type electric fan, a Pulse Width Modulation (PWM) signal generation module was integrated with an embedded system with a Field-Programmable Gate Array (FPGA). The use of Wi-Fi and Long Term Evolution (LTE) technology is now a promising technology.[17] Another paper described an IoT system that was linked to the Cloud and a building control unit. The proposed system can balance Indoor Air Quality (IAQ) and outdoor air pollution before adjusting the proper airflow rate of the indoor ventilating fan.[18]

As previously stated, the goal of this study is to design and fabricate a low-cost IoT platform that can be integrated with multi-air parameter sensors and a ventilating fan controller for public semi-outdoor air conditions, as well as to provide protection from haze and the ongoing COVID-19 crisis.

Materials and Method

The method for using aerosol sensors and a ventilating system with an IoT platform is shown below;

Design Architecture

Figure 1 depicts two-way data communication between a ventilating fan and air quality sensors and a chatbot platform. According to the Air Quality Index (AQI), which was developed by the United States Environmental Protection Agency (EPA), an AQI of 0 to 50 is good and presents little or no threat to health.[19] Therefore, when the PM2.5 levels in the semi-outdoor area reached 50 μg/m^3, an air quality module reported the data to a microcontroller unit (MCU), using Transistor–Transistor Logic (TTL), and a dual ventilating fan was activated. After PM2.5 levels fell below 50 μg/m^3, the dual ventilating fan was turned off. The data was transmitted via a Local Area Network (LAN) that was linked to a 3G Wi-Fi module. The Cloud storage and IoT system then sent an air quality report to a chatbot every 1 hour or whenever the user specified. In this study, a twin duct ventilation fan was employed to evacuate air at a total rate of 1800 m^3/h.[20]

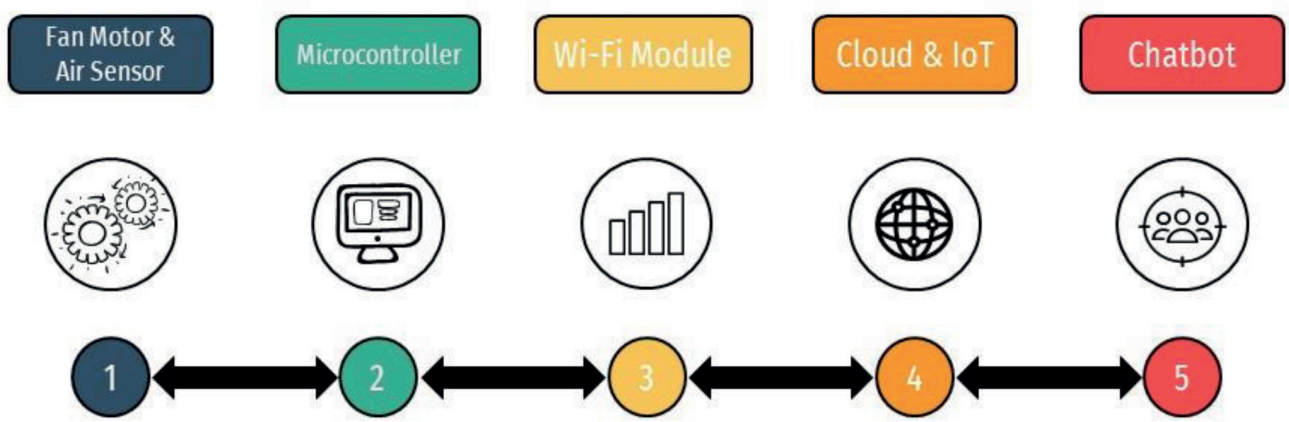

Figure 1 IoT system planning for semi-outdoor air cleaner.

Air Quality Module and Microcontroller

For environmental air quality monitoring, a seven-in-one air quality module (Model: AQM-RK14V), operating temperature 0-50°C, working humidity ≤ 95%RH, dimensions 61*48*13 mm (L×W×H) was used. CO_2 (400-5,000 ppm, resolution 1 ppm ± 25%), formaldehyde (HCHO 0-2,000 μg/m^3, resolution 1 μg/m^3 ± 25%), TVOC (0-50,000 μg/m^3, resolution 1 μg/m^3 ± 25%), PM2.5 (0-999 μg/m^3, resolution 1 μg/m^3 ± 10%), PM10 (0-1,000 μg/m^3, resolution 1 μg/m^3 ± 10%), temperature (-40-125°C, resolution 0.01°C ± 0.03°C), and also humidity (0-100%, resolution 0.04% ± 3%RH) were all measured and interpreted. The device was calibrated during its manufacture. After installation, the sensor calibration procedure comprises a quarterly comparison with reference-grade air monitors, taking into account ambient variables. This information would confirm the sensors' accuracy and data reliability. Every second, all seven groups of monitoring data were automatically sent to the MCU via a UART TTL interface. The sensor is positioned at the top of the air purifier to avoid interference and misinterpretation from the air inlet and outlets.

The MCU also included the Arduino Mega 2560 Rev3 board, the I-Autoc KSIM series single phase AC output solid state relay, and the XH-M609 12-36V battery low voltage disconnect protection module. A microcontroller was connected to a dual ventilation exhaust fan model HF-200, 130W, 220V, air flow rate 900 m^3/h. An electrical signal was delivered between the MCU and the ventilation system using the PWM signal. After receiving data from an air quality module, a feedback loop between MCU, air sensors, and a ventilating fan was introduced.

Wi-Fi Module and IoT Platform Development

A Wi-Fi 3G module also included an Ethernet shield (Model: W5500), and a D-LINK (DWR-920) Wireless N300 4G Router, allowing an Arduino board to connect to the internet via LAN cables. Using the Serial Peripheral Interface (SPI), the MCU could connect to an Ethernet shield.

The data was then sent to the NETPIE 2020 IoT platform via Message Queuing Telemetry Transport (MQTT), which can report real-time sensor monitoring as well as Cloud platform control. An Application Programming Interface (API) code was written in PHP language for chatbot development. This code was saved on the internet hosting service "GitHub", and the URL was then sent to a Cloud platform called "Heroku". Using the Heroku platform, all data was then transferred between the MCU and the LINE chatbot. Finally, the MCU transmitted PM2.5 levels to an outdoor P5 SMD LED screen module, 64 x 32 dot, 320 x 160 mm installed on top of an air purifier unit.

To test the system, the air purifier and accessories were used constantly for one month. The early tests of overall systems, which comprised the operation of the ven tilation system, aerosol reports, and IoT interfacing, were gathered and analyzed.

Results

Response of Ventilation System

When PM2.5 levels reached 50 μg/m^3, a set of ventilating fans was activated and worked continuously until the aerosol level dropped below 50 μg/m^3. Figure 2 depicts the operation of a real-time LED screen module seen at the air purifying station.

Figure 2 A semi-outdoor air cleaner with an onsite LED screening. PM2.5 levels were displayed on screen.

Ordering via LINE Application

The chatbot system was divided into two LINE groups, the first being the operation system, also known as the "BoonG" group. This LINE chatbot was used to send a machine code that included the words "on", "off", and "report" in both Thai and English. Within 1-5 seconds, depending on Wi-Fi stability, the chatbot was able to respond with the status of the ventilation system as well as any types of aerosol levels (CO_2, formaldehyde (HCHO), TVOC, PM2.5, PM10, temperature (0C), and percent humidity), as shown in Figure 3.

Figure 3 LINE messenger was used to send direct messages for air cleaner control.

Secondly, the reporting system, also known as the "BOONG 2021_Notify" group, was used to only report machine status and aerosol levels every 1 hour or as desired by users in the "BoonG" group. This chatbot was created for people who want to know the air quality levels in specific locations, as shown in Figure 4.

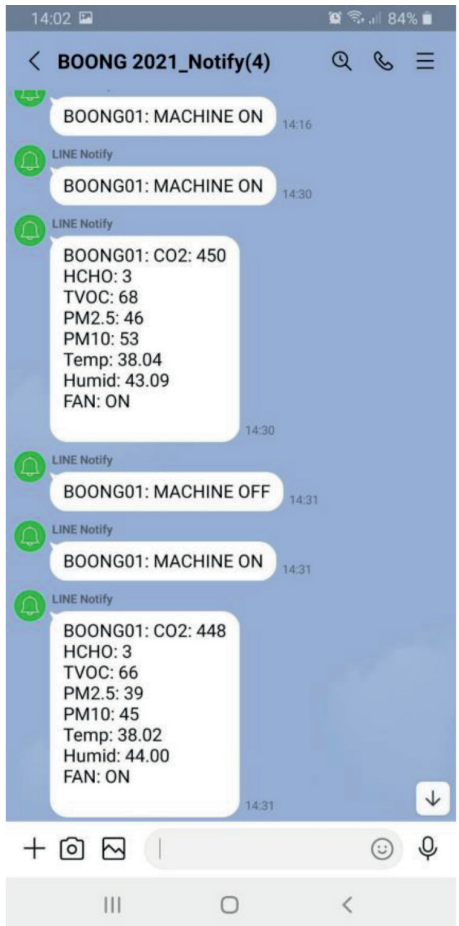

Figure 4 For multi-users, air pollution reports and machine status were available.

Discussion

This air quality IoT platform can be used to demonstrate a low-cost semi-outdoor air purifying control model. The advantages of this apparatus also include real-time air monitoring and hourly reporting via LINE chatbots. All platforms are free and suitable for many developing countries. In addition, an LED screen module is available for sharing real-time PM2.5 levels at the air ventilation site. Using a feedback loop between a microcontroller, PM2.5 levels, and a ventilation exhaust fan, the machine can limit the workload of a dual fan motor to extend its useful life. However, some issues that should be investigated further include, first and foremost, maintenance costs for a SIM card that was included with the Wi-Fi 3G system remain. Before implementing this instrument in those areas, infrastructures such as electricity and telecommunications networks must be completed. This machine should ideally be used for multi-air purifying nodes, using a grid system to collect air quality in each area in the future. Second, the IoT system should be capable of detecting or forecasting certain air conditions, such as conflagration, storm, rain, and earthquake. Third, the clear analysis of error margins, measurement accuracy, and probable causes of error in PM2.5 measurements should be

done. Typical error analysis would include comparing the sensors' results to those from a reference-grade monitor under various environmental conditions. Fourth, dependability parameters such as reading consistency over time, sensor drift, and performance under various temperature and humidity conditions should be evaluated. Fifth, comparing error and reliability statistics to data from similar air quality monitoring systems is currently challenging. Finally, any information should be collected and analyzed as part of big data.

Conclusion

The semi-outdoor air cleaner integrates IoT-based air quality monitoring and fan control to manage PM2.5 and other pollutants in public spaces. The system uses an air quality sensor module (AQM-RK14V) connected to an Arduino Mega 2560 microcontroller (MCU) to measure pollutants, including CO_2, formaldehyde, TVOCs, and particulate matter (PM2.5 and PM10). Positioned at the top of the air cleaner to avoid airflow interference, the sensor continuously sends data to the MCU, which activates dual ventilating fans via a relay if PM2.5 levels exceed 50 $\mu g/m^3$. The fans automatically turn off once the air quality improves, with their speed controlled by the MCU using PWM signals. Data transmission occurs via a D-LINK 4G router and Ethernet shield, which connects the MCU to the NETPIE 2020 IoT platform through the MQTT protocol for real-time data reporting. An LED display provides PM2.5 readings onsite, while users receive updates and control options through a LINE chatbot. This chatbot, divided into two groups, allows users to control the cleaner or receive periodic air quality reports. After sensor calibration and system testing, the device is deployed in a semi-outdoor area, offering a low-cost air quality management solution. The system's automation and ease of use make it ideal for public areas affected by haze and air pollution, providing accessible real-time air quality data and automatic pollutant management to enhance public health during events like the COVID-19 pandemic. This low-cost IoT platform was created to enable prediction and protection from haze and the COVID-19 crisis. Aerosol detection and ventilation fan control are made available via multi-sensors and the LINE chatbot. All platforms are free, with the exception of a SIM card included with the Wi-Fi 3G system, which is suitable for developing countries during the haze and COVID-19 crisis.

Conflict of Interest

The author has no conflict of interest to declare.

Author Contributions

Arnon Jumlongkul created, evaluated, and drafted the text; Watchara Jamnuch designed and tested the machinery system; while Pitchayapa Jumlongkul reviewed and proofread the article.

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สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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Prevalence and Factors Associated with Workplace Stress among Worker Population in Thimphu, Bhutan: A Cross-sectional Study

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Abstract:

Background: Workplace stress is a major public health problem worldwide. The impact of workplace stressors contributed to risk behaviors, poor family relationship, severe diseases. Several factors are associated with workplace stress i.e. health condition and job satisfaction.

Objective: This study aims to estimate the prevalence of workplace stress and to determine factors associated with workplace stress among the working population in Thimphu, Bhutan.

Method: A cross-sectional study was conducted to collect the information using a validate questionnaire and the Workplace Stress Scale (WSS) to assess stress levels from the worker who lived in Thimphu district, Bhutan. Multiple logistic regression was analyzed to assess the association between variables at a significance level of $\alpha = 0.05$.

Results: A total of 415 workers were recruited into the study. The most participant were 56.4% males with a majority (43.9%) ranging between 19 to 30 years. There were 54.0% completed their high school education, 32.3% hold a bachelor's degree, 10.6%, have received primary level education, and 3.1%, have no formal education. 92.8% of the participants were Buddhist followed by Hindu (5.3%), and Christianity (1.9%) respectively. The overall prevalence of workplace stress was 48.2% of which 39.3% experienced moderate stress, 8.4% experienced severe stress and 0.5% experienced potentially dangerous level of stress. Eight factors were found to be associated with workplace stress, including cannabis use, COVID-19 infection, kidney disease, diabetes, chronic diseases in the family, severe mental health problems among family members, job position, and job satisfaction. The specialists, professional, and managers experienced higher levels of stress compared to operational level.

Conclusion: Workplace stress associated with cannabis use, COVID-19 infection, kidney disease, diabetes, chronic family illnesses, severe mental health problems among family members, job position, and job satisfaction. Individuals with moderate to high stress levels should be referred for further evaluation and treatment. These findings emphasize the

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importance of implementing targeted interventions in the workplace, with a focus on training workers in stress management skills to mitigate these stressors effectively.

Keywords: Workplace; Stress; Factors; Job satisfaction; Mental health; Prevalence

Introduction

Workplace stress is one of a significant problem in public health issues worldwide and it has become a major mental health problem in both developed and developing countries.[1] Stress is a part of mental health definition defined by WHO 2022 stated that "mental health is a condition or a state of well-being in which an individual realizes his or her own capacities, can manage normal stresses of life, can work productively, and is suitable to make a contribution to his or her community".[2] This is a significant global public health challenge contributing to financial hardship on individuals and households and elevated rates of deaths and disability.[3] The prevalence of workplace stress varies widely across occupations and regions, with studies reporting rates ranging from 12.6% to 50.6% globally.[4] Bhutanese adults with hypertension demonstrate a high stress levels.[5]

Workplace stressors influence a wide-ranging effect on many aspects of an individual's life, including work productivity[6], psychological health[7], family relationships[8], and job satisfaction.[9] Several factors contributed the workplace stress, including high job demands, poor work-life balance, lack of control over job tasks, and poor interpersonal relationships.[1] Moreover, the COVID-19 pandemic has introduced additional stressors, such as job insecurity and health concerns.[10] Studies have shown that workplace stress can lead to reduce the efficiency of work, this may affect to the worker and organization performance. The persistent stress can lead to other mental health symptoms such as anxiety, depressive syndrome, and job burnout. Stress can disrupt a family issue in term of serious relationship and the emotional exhaustion. Moreover, job dissatisfaction can lead to lower motivation, reduced commitment, and higher turnover rates.

However, there needs necessary for further studies on stress burden on working population to better understanding the causes and consequences of workplace stress. These are crucial for developing effective interventions aimed at improving employee well-being and enhancing organizational performance. There were limited studies focused on workplace stressor in Bhutan, and it required more better understanding a prevalence and impact on worker well-being. This study was to estimate the prevalence of workplace stressor, and identify the key factors associated with workplace stress among Bhutanese workers. By addressing this gap, the findings would be contributed to a better clarify of workplace stress in Bhutan and inform the development of targeted interventions and policies to improve worker health, productivity, and job satisfaction.

Methodology

Study design

A cross-sectional study was conducted to collect the information from the worker who lived in Thimphu district, Bhutan.

Study setting

This study was carried out in Thimphu district, the capital city of Bhutan, situated in the western region of the country. It was chosen because Thimphu district is considered as one of the developed districts

in the country having a significant presence of both private and government sector organization.

Study population and eligible population

The study population comprised individuals who were employed, aged between 18 and 60 years, and actively working in both government and private sectors within the Thimphu district, Bhutan. The participants were required to have a minimum of 1 year of work experience in their respective sectors and express their willingness to take part in the study. The study excluded employee's individuals who did not provide their consent to take part in the study were excluded from the research.

Study sample and sample size

The sample size was calculated according to the following standard formula of a cross- sectional study[11]: $n = [Z^2\alpha/2*P*(1-P)]/e^2$, wherein Z is the value from the standard normal distribution corresponding to the desired confidence level (Z = 1.96 for 95% CI), P = the prevalence of workplace stress scale from a previous study, and e = the accepted deviation to the predicted prevalence of the study, which was set at 4.2%. A previous study in Bhutan reported the prevalence of psychological stress in Bhutan was 75.2%.[5] So, 415 participants were included in this study, with adding 10.0% error throughout the study.

Research instruments

Data on the socio-demographic characteristics, behavioral factors, physical health information, and work-related factors of the participants were collected using a validated self-administered questionnaire. This study applied the Person-Environment (P-E) Fit Theory as a conceptual framework.[12] The workplace stress questionnaire was utilized to assess the prevalence and association of workplace stress scale. The questionnaire includes questions into the following section:

Part 1: The participants completed a validated self-administered questionnaire, which was divided into four sections. Section One: Socio-demographic information for each subject was collected using a researcher-designed questionnaire such as age, gender, educational attainment, religion, marital status, a number of children, the total number of family members, conflicts with the partner, the frequency of conflicts with the partner, conflicts with other family members (including children), and debt information including total debt. Section two (behavioral factors): The information regarding the participants' drinking behaviors was collected using four questions, while an additional four questions were used to gather information on tobacco and drug use. Section three (physical health information): The participants were asked seven questions to gather information about their physical health, including whether they had tested positive for COVID-19, had kidney disease, hypertension, diabetes, any chronic diseases in their family, severe mental health problems among their family members or any disabled family members. Section four (work-related factors): Fourteen questions were used to collect information related to work including the working sector, occupational sector, employment type, position at work, monthly salary, work experience, working hours per week, the total number of workers in the department, satisfaction with the current job, supervisors understanding towards job problems and needs, help and support from the supervisor, supervisor willingness to listen to the work-related problem, relationships with the supervisors and finally relationships with the co-workers.

Part 2: Workplace stress scale (WSS), the Marlin Company, based in North Haven, CT, USA, in collaboration with the

American Institute of Stress in Yonkers, NY, USA, developed the workplace stress scale (WSS) in 2001. The workplace stress scale consists of eight items, and it aims to assess how frequently a respondent experiences emotion towards their job. Scoring assigns, a score to each item on a five-point Likert scale, ranging from 1 (indicating never) to 5 (indicating very often). The scores for item numbers 6, 7, and 8 are reversed. Increased scores are indicative of elevated levels of job-related stress. Interpreting the scores of the respondents: The scoring system categorizes individuals with scores of 15 and below as relatively calm, scores of 16-20 as fairly low, scores of 21-25 as moderate levels of work stress, scores of 26-30 as severe levels of work stress, and scores of 31-40 as potentially dangerous levels of work stress (The Marlin Company, 2001).[13] In our study, we employed a cut-off point of > 20 to ascertain the prevalence of workplace stress among the participants. Scores workplace stress level ≤ 15 is chilled out and relatively calm, 16-20 is fairly low, 21-25 is Moderate stress, 26-30 is Severe, and 31-40 is stress level is potentially dangerous. In our study, we reported a Cronbach's alpha reliability coefficient of 0.601 for the entire scale WSS. The scale's internal consistency, assessed using Cronbach's alpha, was 0.762 (Xhakollari et al., 2020).[14] Soltan et al. (2020)[15] conducted a study and found that the entire scale of WSS had a Cronbach's α coefficient values of 0.80. A study conducted by Mekonen et al., 2021[16], among bank workers in Gondar city, Northwest Ethiopia, determined a Cronbach's α reliability coefficient of 75.7%.

Data collection

The researcher employed a stratified random sampling method to select employees from various agencies in both the private and government sectors. The government offices were 51 health sectors, 45 finance sectors, 79 education sectors, and 35 engineering sectors, and the private sectors were 69 construction sectors, 46 tourism sectors, 80 hoteliers, and 10 others. The data collection took place from August to September 2023. After receiving ethical approval from the Human Research Ethical Committee at Mae Fah Luang University and the heads of different sectors, the researcher personally approached the participants and obtained their informed consent. A total of three data collectors were chosen, with two employed in the government sector and one in the private sector. They underwent a two-day training session that covered various topics, including participant engagement, research methodology, questionnaire contents, and key concepts and definitions. The data collectors received comprehensive training on the utilization of the questionnaire to accurately record responses and transmit them to the principal researchers. Data collectors underwent a simulated interview and training session to familiarize themselves with the sequence of the questions. We carefully evaluated and deliberated upon the feedback and opinions from the mock interview to ensure consistency during the actual data collection process.

The research procedure commences with data collection from employees employed in various government and private sectors in Thimphu, conducted by data collectors. The survey questionnaires were printed, and the participants were instructed to complete all the questions. After obtaining the necessary authorization and agreement from the relevant establishment, we individually approached and conducted direct, face-to-face interviews with each eligible participant. We requested the participants to complete the survey questionnaire while ensuring the

confidentiality of their personal information. All participants met the inclusion criteria and consented to take part in the study. After completing the questionnaire, the researchers carefully examined all the completed questionnaires to verify the completeness of the data. The data collectors then forwarded all the hard copies of the questionnaires to the principal researcher. The data were inputted into Excel, encoded, and analyzed using SPSS.

Statistical analysis

The data collected was encoded, inputted, and processed using SPSS software Version 20.0. Descriptive statistics were analyzed using frequency (mean, maximum, minimum, and standard deviation) and percentages. Inferential statistics were chi-square tests and fisher exact tests (more than 20% of cells have expected cell counts less than 5) are employed to ascertain if there is a statistically significant relationship between independent variables and outcome variables, with a significance level of $\alpha = 0.05$. A logistic regression analysis was conducted to identify the risk factors associated with workplace stress.

Results

General characteristic

A total of four hundred fifteen (415) workers working in different sectors in Thimphu participated in the study. The most participant were 56.4% males with a majority (43.9%) ranging between 19 to 30 years. The majority of participants (54.0%) have completed their high school education, 32.3% hold a bachelor's degree, 10.6%, have received primary level education, and 3.1%, have no formal education. 92.8% of the participants were Buddhist followed by Hindu (5.3%), and Christianity (1.9%) respectively. The most participants were 70.4% married, 22.4% single, 7.0% divorced, and 0.2% widowed. The most participants were having three or more family members and having two children. There were 30.6% experiencing conflicts (disagreements) with their partners, reported encountering conflicts on a weekly basis, and 33.5% indicated that they did not experience any conflicts with their family members, including their children. Approximately 16.4% of the study participants were discovered to have accumulated debt, with 89.2% of these individuals had debt amounts below 50,000 BTN, which is roughly equivalent to 600 USD. The percentage of participants who reported never using cannabis was 90.8%, whereas 5.3% reported using it on a daily basis, and 3.9% reported using it sometimes. (Table 1)

The majority of the participants (78.1%) had tested negative for COVID-19 test, while 3.9% were uncertain about their COVID-19 test results, while 18.1% tested positive for the virus. The findings of the study indicate that a significant proportion of the participants (84.8%) do not exhibit history of kidney disease, whereas 6.5% have been diagnosed with kidney disease. Approximately 8.7% of individuals lack knowledge regarding their kidney disease status. A majority of the individuals (75.7%) did not have hypertension, but a smaller proportion (7.7%) expressed uncertainty regarding their hypertensive status. Approximately 16.6% of the population have been diagnosed with hypertension. Similarly, it was found that 85.1% of the participants said that they did not have diabetes, whilst 7.7% of the participants were unaware of their diabetic status, and 7.2% of the participants reported suffering from diabetes. 84.3% of the respondents reported that none of their family members are diagnosed with such conditions. In contrast, a total of 9.2% of respondents indicated that their family members have chronic diseases, and 6.5% of respondents

claimed being unaware of any chronic diseases among their family members. Approximately 6.0% of respondents reported the presence of severe mental health issues among their family members, whereas 85.5%, claimed the absence of any family member experiencing severe mental health problems. A total of 8.2% of respondents indicated a lack of knowledge regarding the subject matter. The majority (94.5%) of the respondents indicated that they do not have any family members with disabilities, whereas 5.5% reported having impaired family members.

The majority of participants were employed in the government sector (50.6%), while 49.5% worked in the private sector. The most occupation sectors were hotels (19.3%), education (19.0%), construction (16.6%), healthcare (12.3%), and tourism (11.1%). In terms of job positions, 52.8% were operational-level positions, 26.3% were in supervisory or support positions, 15.2% occupied professional positions, and 5.8% were executives or specialists. Participants had a wide range of work experience, from 1 to 35 years, with a mean of 19.27 years (SD = 9.30). Nearly half (49.2%) had worked for 20–29 years, followed by 22.7% (10–19 years), 20.2% (1–9 years), and 8.0% (≥ 30 years). Most participants (63.2%) had regular employment, while 34.7% were contract workers, and 2.2% were temporary employees. The majority of the participant's monthly income was between 12,000 to 20,000 BTN (37.1%) which is approximately equal to 144-240 USD.

The majority of participants (59.8%) reported that they worked between 60 and 69 hours per week, whereas 10.1% reported working between 70 and 79 hours per week. The participants provided information regarding the number of workers in their respective departments. They reported total number of workers ranged from one to one hundred fifty, with an average of 16.48 workers (SD = 16.02). Approximately 81.0% of participants reported having between less than or equal to twenty-nine workers in their department, while 17.6% reported having between thirty to fifty-nine workers. The study found that 44.1% of participants were satisfied with their job, with 5.5% being extremely satisfied, while 10.9% reported dissatisfaction. Regarding supervisory support, 76.1% perceived their supervisors as somewhat understanding of their job-related problems and needs, and 6.0% believed their supervisors lacked understanding entirely. Supervisor support was reported as sometimes (58.3%), while 32.5% always support the job issues. Additionally, 51.1% stated that their supervisors occasionally listened to work-related concerns, whereas 39.0% always to listen the job problems. Most participants were good relationships with their supervisors (59.8%) and coworkers (60.0%).

The overall prevalence of work-related stress among the working population in Thimphu, Bhutan was 48.2% of which 39.3% experienced moderate stress, 8.4% experienced severe stress and 0.5% experienced potentially dangerous level of stress. (Table 1)

Table 1 The general characteristics of the participants

Characteristics	n	%
Total	415	100.0
Sex		
Male	234	56.4
Female	181	43.6
Age (years)		
19-30	182	43.9
31-40	179	43.1
41-50	51	12.3
> 50	3	0.7
Mean = 32.4, Median = 31.0, Minimum = 19, Maximum = 57, SD = 6.6		
Education level		
No education	13	3.1
Primary school	44	10.6
High school	224	54.0
Bachelor degree and higher	134	32.3
Religion		
Buddhism	385	92.8
Hinduism	22	5.3
Christianity	8	1.9
Marital Status		
Single	93	22.4
Married	292	70.4
Divorced	29	7.0
Widowed	1	0.2
Number of Children		
None	123	29.6
One	101	24.3
Two	124	29.9
Three or more	67	16.1
Total number of family members		
1	40	9.6
2	107	25.8
3 and more	268	64.6

Table 1 The general characteristics of the participants (con.)

Characteristics	n	%
Conflicts with the partner		
Yes	127	30.6
No	288	69.4
Frequency of conflict with the partner (n = 128)		
Every day	16	12.5
Once a week	62	48.4
Twice a week	32	25.0
More than twice a week	18	14.1
Conflicts with family members including children		
Never	139	33.5
Rarely	123	29.6
Sometimes	138	33.3
Often	15	3.6
Debt		
Yes	68	16.4
No	347	83.6
If yes, how much debt do you have? (n=68)		
< Nu. 50,000	23	33.8
Nu. 50,000-150,000	29	42.6
> Nu. 150,000	16	23.6
Mean = 148,782, Min = 10,000, Max = 800,000, SD = 178,043.38		
Cannabis (marijuana) use		
Yes	22	5.3
Sometimes	16	3.9
No	377	90.8
COVID-19 positive		
Yes	75	18.1
Do not know	16	3.9
No	324	78.1
Kidney disease		
Yes	27	6.5
Do not know	36	8.7
No	352	84.8

Table 1 The general characteristics of the participants (con.)

Characteristics	n	%
Hypertension		
Yes	69	16.6
Do not know	32	7.7
No	314	75.7
Diabetes		
Yes	30	7.2
Do not know	32	7.7
No	353	85.1
Chronic disease among family members		
Yes	38	9.2
Do not know	27	6.5
No	350	84.3
Severe mental health problems among family members		
Yes	25	6.0
Do not know	34	8.2
No	356	85.8
Disabled family members		
Yes	23	5.5
No	392	94.5
Working Sector		
Government sector	210	50.6
Private sector	205	49.4
Occupational Sector		
Education sector (Teachers)	79	19.0
Engineer	35	8.4
Finance sector	45	10.8
Health sector (Nurses/doctors)	51	12.3
Construction sectors	69	16.6
Tourism sector	46	11.1
Hoteliers	80	19.3
Others (call center)	10	2.4

Table 1 The general characteristics of the participants (con.)

Characteristics	n	%
Position at work		
Executives and Specialist	24	5.8
Professional and management	63	15.2
Supervisory and support	109	26.3
Operational	219	52.8
Employment type		
Regular	262	63.1
Contract	144	34.7
Others (temporary)	9	2.2
Monthly salary (Ngultrum)		
Less than Nu.12,000	64	15.4
Nu.12,000-Nu. 20,000	154	37.1
Nu. 20,000-Nu. 30,000	92	22.2
More than Nu. 30,000	105	25.3
Duration in the current job (work experience) (years)		
1-9	84	20.2
10-19	94	22.7
20-29	204	49.2
> 30	33	8.0
Mean = 19.27, SD = 9.30, Min = 1, Max = 35		
Working hours per week		
Less than 30 hrs/week	29	7.0
30-39 hrs/week	31	7.5
40-49 hrs/week	28	6.7
50-59 hrs/week	29	7.0
60-69 hrs/week	248	59.8
70-79 hrs/week	42	10.1
More than 80 hrs/week	8	1.9
Total numbers of workers in the department		
≤ 29	336	81.0
30-59	73	17.6
60-89	5	1.2
> 90	1	0.2
Mean = 16.48, SD = 16.02, Min = 1, Max = 150		

Table 1 The general characteristics of the participants (con.)

Characteristics	n	%
Satisfaction with the current job		
Very dissatisfied	26	6.3
Dissatisfied	19	4.6
Neutral	164	39.5
Satisfied	183	44.1
Extremely satisfied	23	5.5
Supervisor understands jobs problem and needs		
Not at all	25	6.0
Somewhat	316	76.1
To a great extent	74	17.8
Supervisors help and support		
Never	38	9.2
Sometimes	242	58.3
Always	135	32.5
Supervisor willingness to listen to work-related problems		
Never	41	9.9
Sometimes	212	51.1
Always	162	39.0
Relation with supervisor		
Poor	15	3.6
Fair	152	36.6
Good	248	59.8
Relation with co-workers		
Poor	17	4.1
Fair	149	35.9
Good	249	60.0
Level of workplace stress		
Relatively calm and relaxed	67	16.1
Fairly low	148	35.7
Moderate	163	39.3
Severe	35	8.4
Dangerous	2.0	0.5

Nu. = Ngultrum, the official currency of Bhutan. (1 USD ≈ 83 NU)

Factors associated with workplace stress

For the purpose of analyzing associated factors, workplace stress levels were classified into two categories: relative calm and relaxed to fairly low stress (51.8%) and moderate to dangerous stress (48.2%). Five variables were found to be associated with workplace stress in the multiple regression analysis. Those individuals who use cannabis had 2.32 times (95% CI = 1.09-4.95) greater odds of getting workplace stress than those who did not use. Those individuals who had history of COVID-19 positive had 2.15 (95% CI = 1.18-3.91) times greater odds of getting workplace stress than those who did not. Those individuals who had not know about severe mental health problems in their family had 6.53 (95% CI = 2.47-17.29) times greater odds of getting workplace stress than those who did not have. Those individuals who being executives and specialists and professional and manager had 2.70 (95% CI = 1.03-7.03), and 4.31 (95% CI = 2.21-8.43) times greater odds of getting workplace stress than those individuals who were operational positions, respectively. (Table 2)

Table 2 Factor associated with workplace stress among the workers

Factors	Workplace stress scale				OR	95%CI	p-value	ORadj	95%CI	p-value
	No N (%)	Yes N (%)	Chi-square	p-value						
Total	215(51.8)	200 (48.2)								
Cannabis (marijuana) use			5.19	0.023*						
Yes/Sometimes	13 (34.2)	25 (65.8)			2.22	1.10-4.47	0.026*	2.32	1.09-4.95	0.030*
No	202(53.6)	175 (46.4)			1.00			1.00		
COVID-19 positive			8.32	0.016*						
Yes	29 (38.7)	46 (61.3)			1.98	1.17-3.31	0.009*	2.15	1.18-3.91	0.012*
Do not know	6 (37.5)	10 (62.5)			2.08	0.74-5.87	0.165	0.87	0.25-2.97	0.822
No	180(55.6)	144 (44.4)			1.00			1.00		
Kidney disease			7.47	0.024*						
Yes	16 (59.3)	11 (40.7)			0.79	0.36-1.75	0.558			
Do not know	11 (30.6)	25 (69.4)			2.61	1.24-5.46	0.011*			
No	188(53.4)	164 (46.6)			1.00					
Diabetes			6.03	0.049*						
Yes	15 (50.0)	15 (50.0)			1.17	0.55-2.46	0.687			
Do not know	10 (31.3)	22 (68.8)			2.56	1.18-5.57	0.017*			
No	190(53.8)	163 (46.2)			1.00					
Chronic disease in family			6.29	0.043*						
Yes	18 (47.4)	20 (52.6)			1.30	0.67-2.55	0.437			
Do not know	8 (29.6)	19 (70.4)			2.79	1.19-6.54	0.018*			
No	189(54.0)	161 (46.0)			1.00					

Table 2 Factor associated with workplace stress among the workers (con.)

Factors	Workplace stress scale		Chi-square	p-value	OR	95%CI	p-value	ORadj	95%CI	p-value
	No N (%)	Yes N (%)								
Severe mental health problems among family members			14.77	<0.001*						
Yes	15 (60.0)	10 (40.0)			0.79	0.35-1.81	0.580	0.51	0.20-1.28	0.152
Do not know	7 (20.6)	27 (79.4)			4.57	1.94-10.76	<0.001*	6.53	2.47-17.29	<0.001*
No	193(54.2)	163 (45.8)			1.00	1.00		1.00		
Position at work			23.81	<0.001*						
Executives and Specialist	9 (37.5)	15 (62.5)			2.53	1.06-6.03	0.037*	2.70	1.03-7.03	0.043*
Professional and Management	17 (27.0)	46 (73.0)			4.11	2.21-7.62	<0.001*	4.31	2.21-8.43	<0.001*
Supervisory and support	57 (52.3)	52 (47.7)			1.38	0.87-2.20	0.169	1.32	0.81-2.15	0.259
Operational	132(60.3)	87 (39.7)			1.00			1.00		
Satisfaction with the current job			11.26	0.010*						
Very dissatisfied	8 (30.8)	18 (69.2)			3.27	1.36-7.86	0.008*			
Dissatisfied	9 (47.4)	10 (52.6)			1.61	0.63-4.14	0.320			
Neutral	76(46.3)	88 (53.7)			1.68	1.11-2.54	0.014*			
Satisfied/Extremely Satisfied	122(59.2)	84 (40.8)			1.00					
The supervisor understands the job problem and needs			1.000	0.606						
Not at all	11 (44.0)	14 (56.0)			1.58	0.63-3.94	0.325	2.37	0.84-6.74	0.105
Somewhat	163(51.6)	153 (48.4)			1.17	0.70-1.94	0.554	2.42	1.30-4.52	0.006*
To a great extent	41 (55.4)	33 (44.6)			1.00			1.00		

Discussion

The nearly half of working populations in Thimphu, Bhutan were experienced workplace stress. One-third were neutral of satisfaction with the current job. Two-third were had supervision understand job problems and needs and supports. More than half had good relationship with supervisor and co-workers. Cannabis use, COVID-19 positive, kidney disease, diabetes, chronic disease in family, severe mental health problems among family members, position at work, satisfaction with the current job were associated with workplace stress.

This study found that 48.2% of worker populations experienced moderate to severe workplace stress. These findings align with systematic review study on teacher stress in China, Brazil, the United States, India, and Spain, where the prevalence of workplace stress ranged from 12.6% to 50.6%.[4] The variation in stress prevalence across studies may be influenced by differences in occupational demands, work environments, and socio-cultural factors. Compared to a study on healthcare workers in Singapore,[17] where 33% reported workplace stress, our findings suggest a higher prevalence. The high stress levels observed in this study emphasize the need for targeted workplace interventions, such as stress management programs, mental health support, and organizational policy improvements, to mitigate the impact of workplace stress on worker well-being and productivity.

Our study showed a job satisfaction in medium level, this was similar to the systematic review study reported a neutral level of satisfaction from China, South Korea, Egypt, and the United States.[9] The study on job satisfaction among Austrian pediatricians showed that gender and working hours were found to be associated with job satisfaction.[18] One key factor influencing job satisfaction is supervisory support[19] and leadership style.[20] Our findings revealed that while worker received consistent support from their supervisors and understand the job issues. These would suggest that workers feel heard and supported from the supervisor, their job satisfaction tends to increase, whereas a lack of understanding and responsiveness may contribute to workplace dissatisfaction and stress reported by Radulović AH, et al.[21]

This study highlighted the association between COVID-19 positive, chronic diseases (such as kidney disease and diabetes), family history of chronic illness, and workplace stress. The findings suggest that individuals with health conditions and a family history of chronic diseases may be more vulnerable to higher stress levels, which could affect both physical and mental health outcomes. A case-control study in Iran reported that workplace stress is high in the outbreak of COVID-19 situation[22] and aligned with the study in Japan focusing on job stress and loneliness among desk workers, with a focus on the impact of remote working in a world pandemic of COVID-19.[23] The study of the association between metabolic syndrome and job stress in Iran revealed that an association between job-related stress in the presence of metabolic syndrome among the medical university staff.[24] Several studies have also reported a link between metabolic syndrome and job stress. For instance, Chandola et al.[25] identified a prolong exposure to workplace stress over a 14-year period and an increased risk of metabolic syndrome. Several studies reported that the impact of kidney diseases associated with stress,[26] low health conditions[27, 28] and poor quality of life.[29, 30] Individuals with kidney disease often experience declined physical function, which can negatively impact their work performance and job-related activities. Additionally, patients with have family member's kidney disease are more susceptible to workplace stress.

Our study found that cannabis use associated with workplace stress. A study of cannabis uses and stress response[31] and another study in the United State[32] showed that cannabis use associated with stress response. The individuals who use cannabis may reduce their workplace stress level. The odds of getting stress more likely to be those who had experienced a positive COVID-19 that align with a study in Corrente M, et al,[33] and the study in Brazil.[10] The COVID-19 pandemic has contributed to increased workplace stress globally. Individuals who were unaware of severe mental illness in their family were more likely to experience workplace stress. This may be due to a lack of mental health awareness, making it difficult for them to manage their own stress. A study from Hong Kong emphasized the importance of comprehensive assessments of family members' psychosocial needs to guide appropriate interventions and strengthen coping skills.[34]

This study found that specialists, professional, and managers experienced higher levels of stress compared to operational level. This could be attributed to greater job responsibilities, decision-making pressures, and high-performance expectations associated with these roles reported by a study in Japan that job stress of managers was significantly higher than that for general workers.[35] Therefore, targeted workplace interventions and stress management strategies should be implemented to mitigate the impact of workplace stress among specialists, professionals, and managers.

A few limitations were found in this study. The study was conducted within the working population of Thimphu, the capital city of Bhutan. However, it is important to note that the findings are applicable solely within this specific setting. Furthermore, it is worth noting that the sample of occupational categories chosen for this study is limited, perhaps resulting in a lack of representativeness for the broader working population inside the country.

Conclusion

The working population in Thimphu, Bhutan, experienced workplace stress, while one-third expressed neutral job satisfaction. Two-thirds reported that their supervisors understood job-related issues and provided support, and more than half had good relationships with their supervisors and co-workers. Eight factors were found to be associated with workplace stress, including cannabis use, COVID-19 infection, kidney disease, diabetes, chronic diseases in the family, severe mental health problems among family members, job position, and job satisfaction. These findings highlight the need for targeted workplace interventions to provide the training of stress management skills, improve job satisfaction, promote the health campaign in prevention of chronic diseases, enhance supervisory support in high positions.

Competing Interests

The authors declare that there is no conflict of interest associated with this study.

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Author contributions

TT and PS designed the study, reviewed the literature, collected data, analyzed data.

PS drafted final version of the manuscript. PS and PW conceived and designed this study, analyzed and interpreted data, drafted and approved the final version of the manuscript. All authors contributed to the writing and approved the final manuscript.

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สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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Preparation of Hydroxyapatite Scaffolds from Cockle Shell for Bone repair

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Abstract:

Background: There is a growing demand for effective bone repair materials to replace and reduce the import of materials from abroad, utilizing waste materials from natural resources in Thailand. The goal is to create medical devices and develop inventions into innovations that can be commercialized.

Objectives: This study aimed to prepare hydroxyapatite (HA) scaffolds using a solid-state reaction with raw materials of $CaCO_3$ and $NH_4H_2PO_4$.

Materials and Method: Scaffolds were synthesized under two conditions with sintering temperatures ranging from 1100 to 1300°C for 2 hours. The phase contents, physical, and mechanical properties were investigated. Additionally, to evaluate the maximum HA phase content, each condition was assessed in the subcutaneous soft tissue of laboratory rats over 7, 30, and 90 days. Characterization techniques included XRD, SEM, porosity analysis, bending strength testing, hardness measurement, and histological studies using optical microscopy.

Results: The findings indicated that the scaffolds sintered at 1300°C for 2 hours (Condition 2) had the highest HA content. After 90 days, the scaffold's reaction with soft tissues showed mild inflammation and good tissue compatibility due to the high HA content.

Conclusion: Our results conclude that HA scaffolds prepared from cockle shells have potential for use as bone grafts, as the samples were found to be nontoxic and biocompatible with soft tissues.

Keywords: Bone graft, Cockle shells, Scaffolds, Soft tissue, Histological

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Introduction

Calcium phosphate bioinorganics are compounds that are widely studied for orthopedic applications. The most widely used of these compounds include hydroxyapatite (HA), β-tricalcium phosphate (β-TCP), and biphasic calcium phosphate (BCP).[1] HA is one of the most effective biocompatible materials and is found to be the major component of bone. A quantitative XRD analysis should indicate a minimum HA content of 95% in relative peak intensities for surgical implants.[2] HA has been widely used as a bone graft for bone replacement, substitution, or proliferation. The need for bone grafts depends on the complexity of the bone defects. For instance, if the defect is minor, bone has its own potential to self-remodel within a few weeks. Therefore, surgery is not required. However, in the case of severe defects and loss of bone volume, the bone cannot heal on its own. In such cases, grafting is necessary to restore function without damaging living tissues.[3,4] The preliminary study in this work focused only on the soft tissue reaction to HA scaffolds, evaluating their biocompatibility and safety for soft tissues. The hard tissue reaction to the implanted sample has not yet been studied, as this will be the focus of future research. Furthermore, studying the reaction of the sample to soft tissue is crucial when developing bone grafts, as the HA scaffold not only interacts with bone but also with the surrounding soft tissue. Therefore, the results of this study may be applied in the future for human bone grafts. Scaffolds are made from HA powders, which are synthesized using several methods such as the wet method[5], sol-gel[6], hydrothermal[7], aqueous precipitation[8], and solid-state reaction.[9-11] Solid-state reaction is a decomposition reaction of two reactants: a solid mixture of $CaCO_3$ and phosphate, and it provides appropriate mechanical and thermal energy to the mixture to synthesize HA ceramics. However, there is limited research on the solid-state reaction method because the reaction tends to produce adhesion forces between fine particles, which readily agglomerate. Nevertheless, this method is very simple, low-cost, and highly productive. The advantages of using calcium carbonate ($CaCO_3$) from cockle shells, instead of synthetic sources, include a very low raw material cost. In addition, the product yield from this method is very high. Therefore, HA produced from cockle shells via the solid-state reaction method can be a viable alternative.

Cockle shells *(Tegillarca granosa)* are natural materials from biological sources, consisting of micro-laminated composites of minerals (95-99% $CaCO_3$) and 1-5% organic macromolecules, which are primarily located within the inter-crystalline boundaries. In addition, the unique composition and composite microstructure of cockle shells result in an enhancement in toughness by three orders of magnitude compared to non-biogenic $CaCO_3$.[12] Therefore, the aim of this work is to study HA scaffolds derived from cockle shells, investigate their properties, and examine the reaction of optimized samples to subcutaneous soft tissue.

Materials and Methods

Materials

Cockle shells were purchased from a commercial seafood market in Chiang Rai, Thailand. Hydrogen peroxide (H_2O_2) (50%), stearic acid, and ethanol (95%) were purchased from World Chemical Group Co. Ltd., Thailand. Tiletamine, zolazepam, xylazine HCl, povidone iodine, penicillin G, and streptomycin were obtained from Sigma-Aldrich, Thailand. Analytical-grade $NH_4H_2PO_4$ was purchased from Merck, Germany.

Samples preparation

Cockle shells were treated with H_2O_2 solution, rinsed with distilled water, oven-dried at 80°C for 24 hours, and finally calcined in air at 550°C for 5 hours. The $CaCO_3$ powder derived from cockle shells was ground and screened through a 325 mesh.[13] The starting powders were combined in precise quantities: 0.58 mole of $CaCO_3$ powder and 0.36 mole of $NH_4H_2PO_4$ powder for Condition 1, and 0.59 mole of $CaCO_3$ powder and 0.35 mole of $NH_4H_2PO_4$ powder for Condition 2. The composition of the preparation is shown in Table 1.

Table 1 The composition for preparation of HA

Condition	Reactant compositions	
	$CaCO_3$ (Mole)	$NH_4H_2PO_4$ (Mole)
1	0.58	0.36
2	0.59	0.35

In the first step, $CaCO_3$ powder was mixed with $NH_4H_2PO_4$ powder in a nylon polyamide milling jar with yttrium-stabilized zirconia grinding media. The jar was then placed in a planetary ball mill for 45 minutes. In the second step, the obtained powder was pressed using an uniaxial pressing machine into a disc shape with dimensions of 4 mm in thickness and 16.5 mm in diameter, under a stress of approximately 3 MPa in a cylindrical stainless steel die. The die wall was lubricated with a liquid solution of 5 wt% stearic acid in ethanol. Finally, the green bodies of the samples were sintered at temperatures of 1100, 1150, 1200, 1250, and 1300°C for 2 hours in an electric furnace (Eurotherm controller 3504, U.S.A.). Sintering was carried out with a heating ramp rate of 60°C/h up to 400°C, followed by a soaking time of 30 minutes. The temperature was then increased at a ramp rate of 120°C/h up to 650°C, with a soaking time of 1 hour. Next, the heating rate was increased to 300°C/h, and the sample was isothermally sintered at different temperatures with soaking times ranging from 2 to 5 hours. Afterward, the temperature was decreased with a ramp rate of 120°C/h to 850°C, where it was held for 1 hour. Finally, the furnace was turned off, allowing the samples to cool to room temperature with a ramp rate of 240°C/h. The scaffold sample is shown in figure 1.

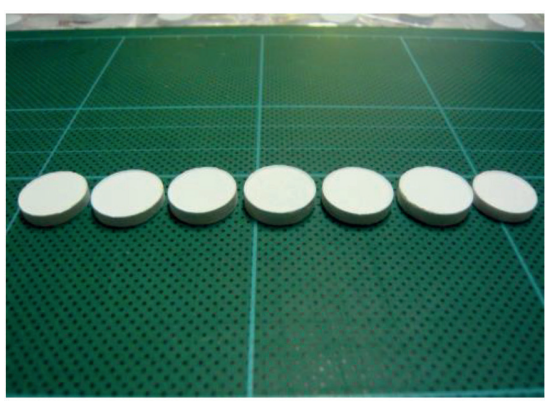

Figure 1 The photographs of scaffolds

Animals testing

This study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University. We adhered to the principles of the 3Rs concerning the use of laboratory animals. Twelve healthy adult Wistar rats (1 to 2 months of age, non-specific gender), weighing approximately 300 to 400 g, were used in this study. These rats were obtained from the Laboratory Animal House, Faculty of Medicine, Chiang Mai University, and we followed the guidelines for the care and use of laboratory animals.[14] Surgery was performed under anesthesia using a mixture of Tiletamine (50 mg/1 c.c.) and Zolazepam (50 mg/1 c.c.) at a dose of 40 mg/kg, along with Xylazine HCl at 5 mg/kg. The dorsal neck area (approximately 1x1 square inch) was shaved, washed, and disinfected with 70% alcohol and povidone iodine solution. The wound was then carefully closed with an absorbable suture to prevent movement of the samples. After surgery, the laboratory rats were allowed to recover from anesthesia. Simultaneously, penicillin G and streptomycin were administered to prevent bacterial contamination, with antibiotic doses of 20,000 IU/kg post-operation. Finally, the individually marked rats were housed in standard cages ($266 \times 425 \times 185$ mm^3 in size, with an area of 820 cm^2).[15]

For the rat trials, the disc scaffolds were cut and lathed into a cylindrical shape, with diameters ranging from 1 to 6 mm and lengths ranging from 10 to 20 mm. The samples used in the animal trials were those prepared under the optimized conditions of Condition 1 and Condition 2. In each rat, two samples were implanted: one under Condition 1 (right side, Group 1) and one under Condition 2 (left side, Group 2). At least four rats, each with two implanted samples, were required for each implantation period. The implantation periods were 7, 30, and 90 days.[16,17] The surgical procedure was as follows: The sample from Condition 1 was implanted on the right side (Group 1) and the sample from Condition 2 was implanted on the left side (Group 2) of the subcutaneous tunnel on the back of the rat's neck. A small incision, approximately 1 cm long, was made on each side of the rat's neck to facilitate implantation.

Characterization

1. The multiple phases of all scaffolds were analyzed using X-ray diffraction (XRD) (Bruker, D8 Advance, England), operated at 40 kV and 30 mA, with Cu Kα radiation (λ = 1.54056 Å). The scanning speed was set to 0.04°/min, and the 2θ range was from 10° to 80°.

2. The microstructure of the HA scaffolds was examined using scanning electron microscopy (SEM) (JEOL, JSM-6335F, Japan). The fracture morphology of the scaffolds was studied using a field emission SEM. The sintered samples were coated with a thin layer of gold (Au) for imaging purposes.

3. The density and porosity of the scaffolds (six samples) were measured using the Archimedes method, with distilled water as the fluid medium.[18] The average grain size was determined using the linear intercept method.[19]

4. Bending strength was investigated using a ball-on-ring test[20] with a universal testing machine (Shimadzu, AGS-500A, Japan). The span length was 20 mm, the crosshead speed was 5 mm/min, and the load cell capacity was 2500 N. Six samples were tested for each condition, and the average result for each condition was calculated. The bending strength (σ) of the samples was determined using Equation (1).

$$\sigma = \frac{3F(1+\nu)}{4\pi t^2}\left[\frac{(1-\nu)}{(1+\nu)} \times \frac{2a^2 - b^2}{2R^2} + 2\ln\left(\frac{a}{b}\right) + 1\right] \quad (1)$$

where σ is the strength in MPa; F is the breaking load in Newtons; ν is Poisson's ratio; a is the radius of the support; R is the radius of the sample; t is the thickness of the sample; and b is t/3.

5. Microhardness measurements were conducted using a Vickers hardness (HV) tester with a pyramidal diamond indenter, applying a 50 g load for 15 seconds. The lengths of the diagonals of the hardness indents were measured using an optical microscope. Five indentations were made for each sample, and the average value was calculated using Equation (2).[21]

$$HV = 0.1891 \frac{F}{d^2} \quad (2)$$

where F is the indentation load in Newtons (N) and d is the mean diagonal length of the indentation in micrometers.

6. Histological analysis was performed following euthanasia. During necropsy, any gross abnormalities in color or consistency were recorded in the tissue surrounding the samples. Each sample, along with the surrounding tissue (at least 4 mm in thickness), was removed. The tissue was then collected in a 10% formalin solution for fixation. Histological examination involved the description of inflammatory cells and the evaluation of the soft tissue response. The results from each histological test were evaluated using optical microscopy.

Statistical analysis

All quantitative data were analyzed with origin 8.0 (OriginLab Corporation, USA) and presented as the mean ± standard deviation. Statistical comparisons were carried out using analysis of variance (ANOVA, Origin 8.0). A value of $p < 0.05$ was considered to be statistically significant.

Results and Discussion

The XRD patterns of the scaffolds obtained from conditions 1 and 2 were recorded after sintering at temperatures ranging from 1100 to 1300°C for 2 hours. The sintered scaffolds were composite ceramics consisting of mixtures of HA, β-TCP, and CaO phases. All patterns were compared with the standard pattern from the JCPDS file no. 09-0432.[22] (Figures 2)

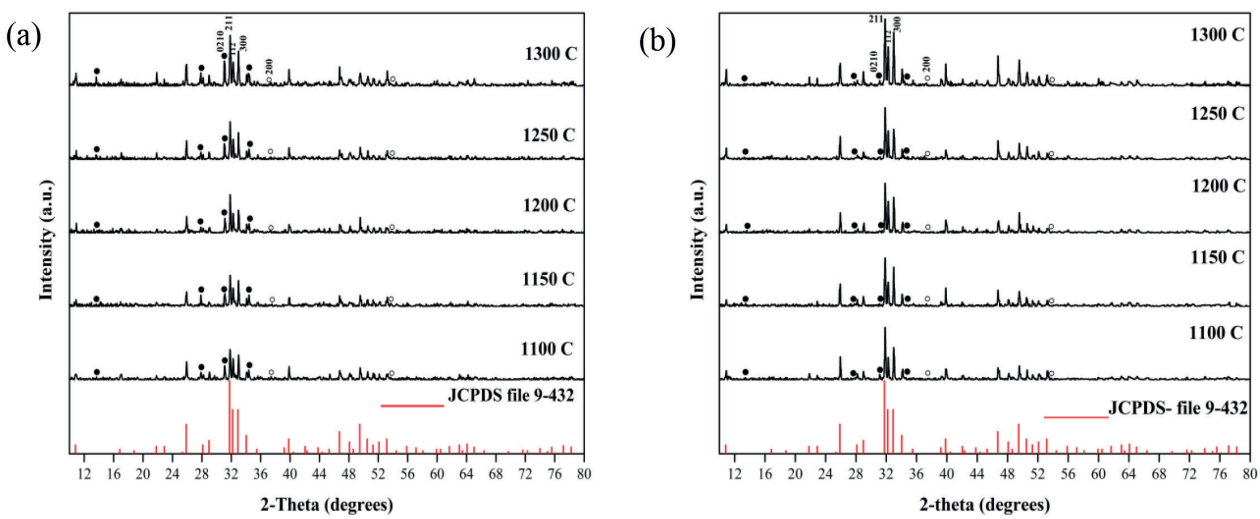

Figure 2 The XRD patterns of the sample powders from condition 1 (a) and condition 2 (b) at various temperatures with the JCPDS file no. 09-0432 of pure HA. The symbols represent β-TCP (•) and CaO (o).

The XRD patterns of the sample powders from condition 1, sintered at various temperatures, were compared with the JCPDS file no. 09-0432 for pure HA. The four dominant HA peaks were observed at $2\theta = 25.9°$ (002), $2\theta = 31.8°$ (211), $2\theta = 32.2°$ (112), and $2\theta = 32.9°$ (300). The peak for the (0210) plane of β-TCP was observed at $2\theta = 31°$ (JCPDS file no. 09-169), while the (200) plane of CaO appeared at $2\theta = 37.35°$ (JCPDS file no. 37-1497). The XRD analysis of the scaffolds obtained from condition 1 is presented in Figure 2(a). The XRD results revealed that the scaffolds contained multiple phases. The crystalline phase content of HA, β-TCP, and CaO in the scaffolds was determined using the reference intensity ratio (RIR) method.[23-25] For the scaffolds from condition 1, the percentage of crystalline phases was found to be: HA (67-69%), β-TCP (25-27%), and CaO (5-7%). The scaffolds from condition 2, presented in Figure 2(b), had the following phase percentages: HA (85-91%), β-TCP (4-8%), and CaO (3-6%). These results suggest that the scaffolds obtained from condition 2 are more likely to form a single-phase HA than those from condition 1 when sintered under the same temperature and time conditions. At various sintering temperatures, the amount of HA increased with the sintering temperature. When sintered at 1300°C for 2 hours, condition 1 reached a maximum of 69% HA, while condition 2 reached 91% HA. The highest HA content (91%) obtained from condition 2 was close to the standard of pure HA (≥ 95%). The effect of composition and sintering temperature on the formation of the HA phase can be explained by the reactions between the starting materials, which resulted in good densification of the mixed powders according to the firing schedule. The microstructure of the scaffolds sintered at different temperatures showed significant variations in crystalline phases. This variation could be influenced by factors such as particle size, isothermal temperature, heating rate, and densification, which serve as driving forces for the synthesis of the HA phase.[26] This is consistent with previous studies, which demonstrated that the formation of β-TCP is faster than that of HA. This is due to the continuous reaction of CaO with the phosphate group to form β-TCP. However, the reaction was carefully controlled in this study by adjusting the time and temperatures in the firing schedule.[27] Additionally, previous studies have reported that the thermal decomposition of synthetic HA, processed between 1160 and 1300°C, leads to its dehydration in two phases. This results in the formation of oxyhydroxyapatite ($Ca_{10}(PO_4)(OH)_{2-2x}O_xV_x$) and oxyapatite ($Ca_{10}(PO_4)6O_x$), where V represents a lattice vacancy in the OH position. Oxyapatite may decompose into a mixture of β-TCP and CaO. In this study, the rehydroxylation of oxyapatite was reversed to HA by the cooling and annealing steps in the firing schedule, although the reversed phase may not have undergone a complete reaction, as HA, β-TCP, and CaO phases were still observed in the XRD analysis.[28]

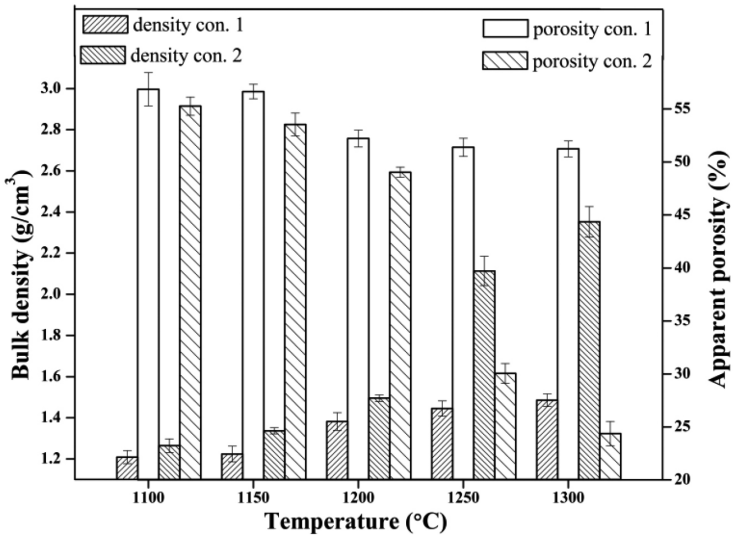

Figure 3 Bulk density and apparent porosity with con. 1 and 2 at different temperatures

Figure 3 displays the physical properties of scaffolds obtained from condition 1 (con.1) and condition 2 (con.2) as a function of temperature. It was found that, for scaffolds from con. 1, as the sintering temperature increased, the bulk density increased, while the apparent porosity decreased. A similar trend was observed for scaffolds from con. 2. The composition and temperature have an effect on the synthesis of HA because the heat causes the calcium and phosphorus particles to bond. As a result, the sample under condition 2 tends to synthesize into HA. The bulk density of con. 2 was 2.375 ± 0.074 g/cm^3, which is lower than the standard value for pure HA (3.16 g/cm^3). However, this value is higher than the density of natural cortical bone (1.8-2.0 g/cm^3) and natural cancellous bone (0.1-1.0 g/cm^3). This can be explained by the fact that the bulk density of the scaffold includes the volume of the solid phase, as well as both open and closed pores, while the density of pure HA represents only the solid phase. Nevertheless, the bulk density of the scaffold falls within the density range of natural bone (both cortical and cancellous bone), which also consists of solid mass with open and closed pores. Both conditions yielded porous materials with open and closed pores. It was observed that the densification of scaffolds from con. 1 resulted in slight shrinkage of the bodies when sintered at 1100-1300 °C, which corresponded to minimal changes in bulk density and apparent porosity. In contrast, scaffolds from con. 2 exhibited slight shrinkage at 1100-1200°C, but showed more significant shrinkage at 1250°C and 1300°C.

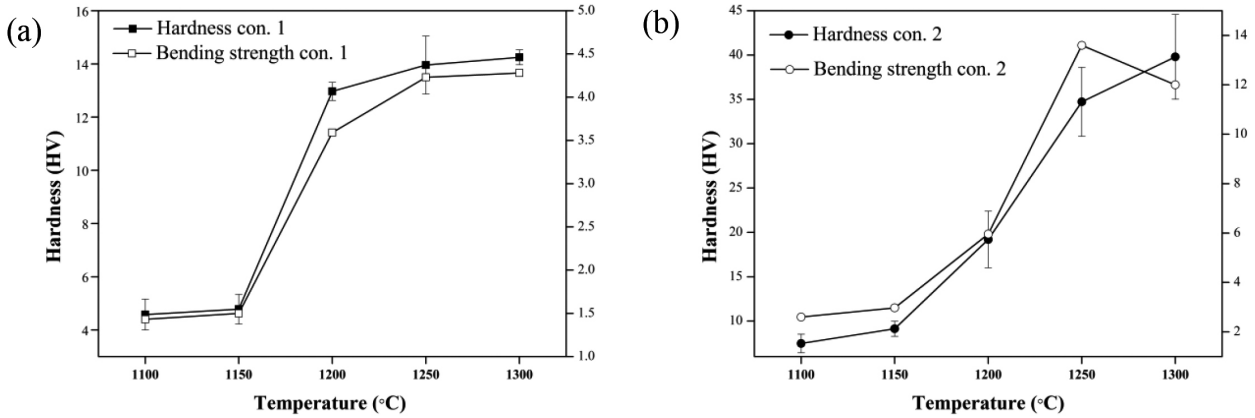

Figure 4 Vickers hardness and bending strength with con. 1 (a) and con. 2 (b) as a function of temperature

Figure 4 (a) shows the variation in the Vickers hardness and bending strength of the samples from con. 1 was a function of temperature. An increase in both characteristics was observed with rising temperatures. The Vickers hardness increased from 4.58 ± 1.15 HV at 1100°C to 14.25 ± 0.55 HV at 1300°C for con. 1. The bending strength of the scaffolds increased from 1.43 ± 0.39 MPa at 1100°C to 4.28 ± 0.40 MPa at 1300°C. Figure 4(b) shows the evolution of the Vickers hardness and bending strength of the samples from con. 2 was a function of temperature which similar to con. 1, and increase in both characteristics was observed with increasing temperature. The Vickers hardness increased from 7.48 ± 2.10 HV at 1100°C to 39.80 ± 9.56 HV at 1300 °C for con. 2. Meanwhile, the bending strength of the scaffolds increased from 2.60 ± 0.59 MPa at 1100°C to 13.60 ± 1.55 MPa at 1250°C, before decreasing slightly to 12.00 ± 1.21 MPa at 1300°C. This decrease in bending strength at 1300°C may be attributed to the reduction in closed pores and the increase in finer grain sizes at higher temperatures. Additionally, the formation of pores after the dehydroxylation of HA, leading to the formation of oxyapatite, could also contribute to this phenomenon. The maximum average Vickers hardness was 39.80 ± 9.56 HV at 1300°C for con. 2, which is lower than the theoretical value of 600 HV for pure HA. However, this value is close to the hardness of natural cortical bone (40.4 HV) and higher than that of natural cancellous bone (35.2 HV). The bending strength was 13.60 ± 1.55 MPa at 1250°C for con. 2, which is lower than the theoretical range of 115-200 MPa for pure HA.

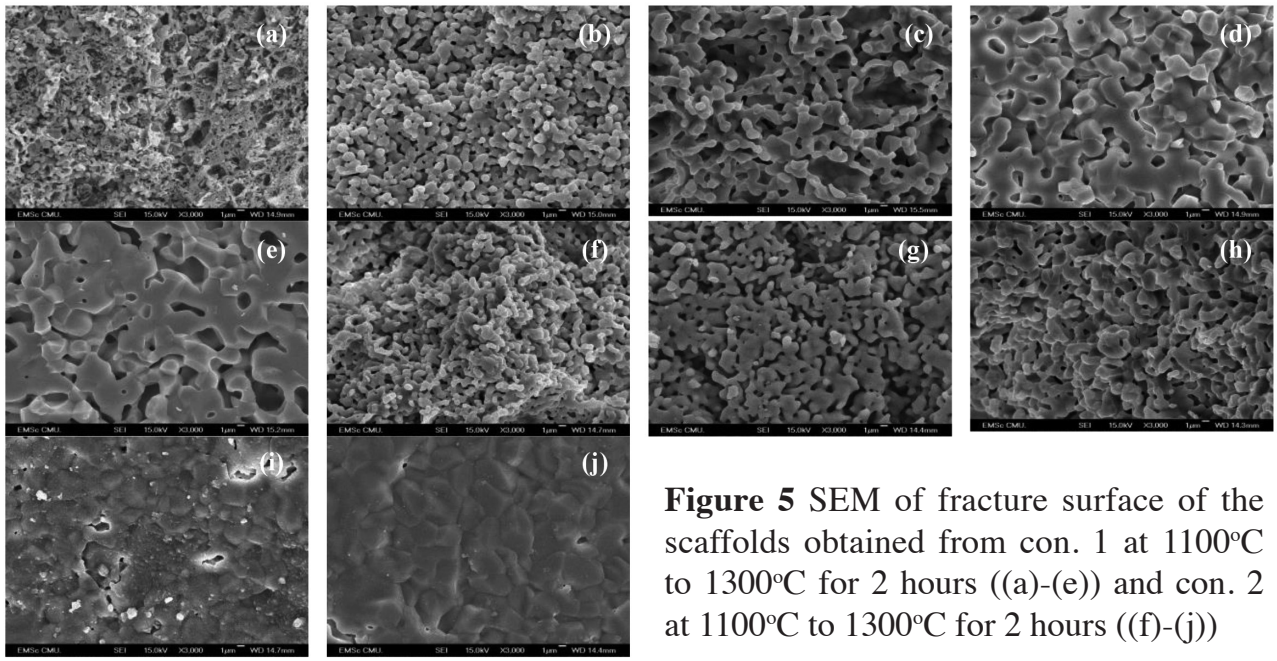

Figure 5 SEM of fracture surface of the scaffolds obtained from con. 1 at 1100°C to 1300°C for 2 hours ((a)-(e)) and con. 2 at 1100°C to 1300°C for 2 hours ((f)-(j))

The SEM images of the scaffolds obtained from con. 1 and con. 2 are illustrated in Figure 5. The micrographs show that the average grain size of the scaffold obtained with con. 1 was 0.3 μm, and the average pore size ranged from 0.4 - 6 μm. The porosity and pore size of the obtained scaffold were measured by image analysis and the Archimedes principle. The porosity of the sample was approximately 57% at 1100°C (Figure 5(a)). For the scaffold obtained from con. 2, the average grain size was 0.6 μm, the average pore size ranged from 1 to 10 μm, and the porosity was approximately 53% (Figure 5(f)). The fracture surfaces of the scaffolds obtained from con. 1, sintered at 1150°C, showed an increase in grain size, with an average range of 0.3 - 0.8 μm. The average pore size was 6 μm, and the porosity was approximately 56% (Figure 5(b)). For the scaffolds obtained from con. 2, the average grain size was 0.9 μm, the average pore size was 7 μm, and the porosity was approximately 51% (Figure 5(g)). At 1200°C, the fracture surface of the scaffolds obtained from con. 1 showed an increase in grain size, ranging from 0.8 to 13 μm. The average pore size was 7.2 μm, and the porosity was approximately 52% (Figure 5(c)). For con. 2, the average grain size was 1.5 μm, the average pore size was 2.2 μm, and the porosity was approximately 50% (Figure 5(h)). This temperature led to the formation of smaller pores, ranging in size from 1 to 2 μm, alongside the development of larger pores ranging from 3 to 8 μm. In addition, the formation of closed pores began at this temperature. At 1250°C, the fracture surface of the scaffolds obtained from con. 1 showed an average grain size of 3.5 μm, an average pore size of 4.2 μm, and a porosity of approximately 51% (Figure 5(d)). For the scaffolds obtained from con. 2, the average grain size was 2.7 μm, the average pore size was 1.5 μm, and the porosity was approximately 33% (Figure 5(i)). At 1300°C, the fracture surface of the scaffolds from con. 1 showed an average grain size of 5.9 μm, an average pore size of 5.2 μm, and a porosity of approximately 50% (Figure 5(e)). For con. 2, the average grain size was 7.9 μm, the average pore size was 0.5 μm, and the porosity was approximately 26% (Figure 5(j)). Grain growth was clearly observed at this temperature.

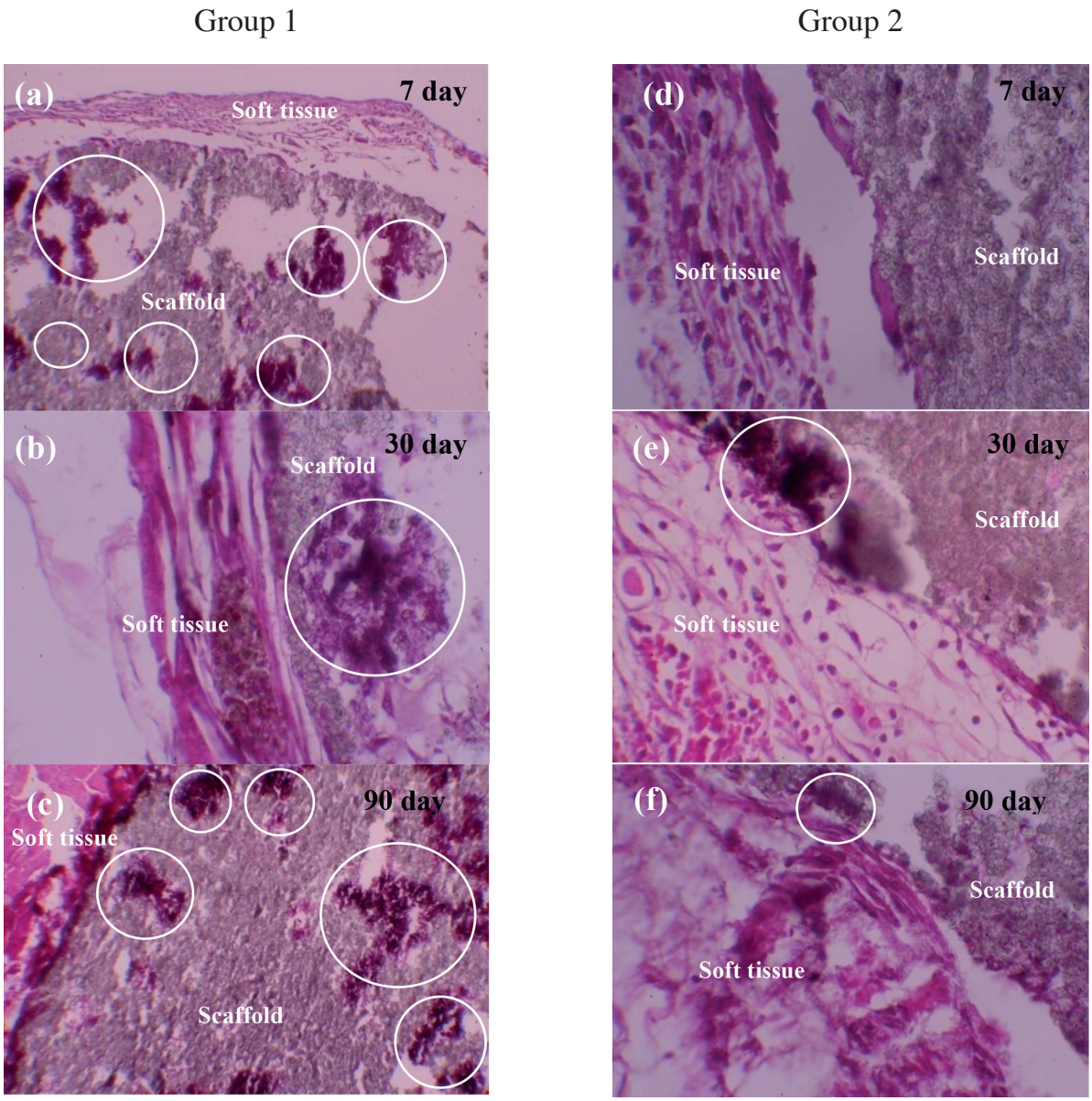

Figure 6 Optical microscope images of scaffolds obtained from Group 1 ((a)-(c)) and Group 2 ((d)-(f)) with magnification of X40

The results of animal testing show the tissue response to the scaffolds after implantation for 7, 30, and 90 days (Figure 6). Microscopic examination of the sections revealed fragments of the scaffolds from Group 1 and Group 2, surrounded by fibro-adipose tissue.

At 7 days after implantation, Group 1 showed a mild foreign body reaction with mixed cellular inflammatory infiltration and the presence of fibroblasts. In contrast, Group 2 exhibited a moderate foreign body reaction, with minimal epithelioid cells and occasional small monocytoid foreign body giant cells. Additionally, cellular infiltration into the scaffold bodies was observed in Group 1, but was absent in Group 2 (Figure 6 (a) and (d)). This difference can be attributed to the lower porosity of the scaffolds in Group 2, as high porosity promotes cellular infiltration into the scaffold bodies. This phenomenon highlights the potential of Group 1 scaffolds as a suitable material for bone grafting, as

previously discussed in.[29] Furthermore, dystrophic calcification was observed in the bulk scaffolds of Group 1, but was absent in Group 2. This difference is due to the higher amount of the CaO phase (circled) in Group 1, as confirmed by XRD analysis. The circled areas contained calcium salts that were deposited among the injured tissues and other phases of the scaffold. These calcium salts were visible to the naked eye as purple, non-cellular lesions on routine histology. In addition, the degradation of scaffold components into the surrounding host tissues was observed in both groups through XRD analysis. However, Group 2 exhibited lower amounts of β-TCP phases compared to Group 1 at 1300°C for 2 hours. This phenomenon has been previously explained in.[30,31]

At 30 days post-implantation, the inflammatory response to the scaffolds in Group 1 was moderate, while in Group 2, it was mild. Group 2 showed a decrease in inflammatory cells, while Group 1 exhibited an increase in inflammatory cells within the scaffold bodies. Additionally, cellular infiltration was present in the bodies of Group 1 but absent in Group 2, which had lower porosity compared to Group 1. Furthermore, dystrophic calcification was observed in both Group 1 and Group 2, as the CaO phase (circled) remained present in both scaffold bodies. Finally, the degradation of scaffold components into the surrounding host tissues was evident in both groups, likely due to the high solubility of β-TCP in the scaffold bodies (Figure 6(b) and (e)).

At 90 days post-implantation, the inflammatory response to the scaffolds in Group 1 was severe, with the appearance of some epithelioid cells and large multinucleated foreign body giant cells. In contrast, Group 2 exhibited a mild inflammatory response. Group 2 showed a consistent level of inflammatory cells, while Group 1 exhibited an increase in inflammatory cells within the scaffold bodies (Figure 6(c) and (f)). This difference may be attributed to the higher amount of β-TCP phase in the structure of Group 1, which likely contributed to the higher solubility of the scaffolds. Additionally, cellular infiltration was present in Group 1 but absent in Group 2, likely due to the significant difference in porosity between the two groups. Dystrophic calcification was observed in the bulk of both Group 1 and Group 2 scaffolds, as the CaO phase (circled) remained present in both. However, small areas of calcification were found in the microstructure of Group 2, while large areas of calcification were observed in Group 1. Moreover, the degradation of scaffold components into the surrounding host tissues was observed in Group 1 but absent in Group 2. This difference may be due to Group 2 starting to stabilize in the soft tissues of the rats, whereas Group 1 remained unstable due to the continuous dissolution of ceramic components.

Table 2 Phenomenon of foreign body reaction and some changes of tissues

Tissue reaction	Time periods (days)			
	Group	7	30	90
Reaction grading	1	♦	♦♦	♦♦♦
	2	♦♦	♦	♦
Cellular infiltration into ceramic body	1	√	√	√
	2	⊗	⊗	⊗
Calcification in ceramic body	1	√	√	√
	2	⊗	√	√
Displacement of ceramic components into surrounding host tissue	1	√	√	√
	2	√	√	⊗

Note: Foreign body reaction grading; Symbol (♦) is mild reaction, (♦♦) is moderate reaction and (♦♦♦) is severe reaction.
Cellular infiltration, calcification and displacement of ceramic components; Symbol (√) is absent and (√) is present.

Table 2 presents the overall changes observed over the time period from 7 to 90 days. The scaffolds obtained from Group 2 exhibited lower levels of inflammation compared to those from Group 1. This result supports the finding that Group 2 had a higher HA content than Group 1, which could explain the greater stability of the scaffolds in Group 2. In contrast, Group 1 demonstrated resorbable behavior due to the higher amount of β-TCP phase compared to Group 2. These findings are consistent with previous studies, which reported that the solubility rate of pure HA sintered in subcutaneous tissue was 0.1 mg/year, whereas β-TCP is a resorbable material that dissolves much faster than pure HA 12.3 times faster in an acidic solution and 22.3 times faster in a basic medium.[30,31] The apparent porosity of Group 1 was 51.2%, indicating that it is a porous material, while Group 2 had an apparent porosity of 24.37%, making it a relatively dense material. Apparent porosity is a crucial parameter that supports cellular infiltration into the scaffold bodies. SEM analysis of Group 2 revealed pore sizes ranging from 1 to 10 μm, but no cellular infiltration was observed. According to[29], scaffolds made of HA with 52% porosity implanted in the bone of laboratory rats allowed bone cells to rapidly grow into the scaffold. Calcification was another factor that inhibited cellular infiltration into the scaffold bodies of Group 2, as the CaO phase remained present in the microstructure. However, we hypothesize that cells from surrounding tissues may have occupied the scaffold bodies, because after implantation, the high solubility of β-TCP in the body fluid of the rats likely caused the smaller pores to enlarge over time.

Conclusion

Cockle shells were converted into calcium phosphate scaffolds with various HA and β-TCP compositions via solid-state reaction. The aim of the research is to synthesize HA through a solid-state process, which requires HA to be more than 95%. However, the synthesis results from this process revealed the formation of secondary phases, namely TCP and CaO. The phase composition of the HA scaffolds depended

on the concentration of $CaCO_3$ and phosphorus precursors, as well as the sintering temperature. The optimized conditions were those that resulted in the highest quantity of HA, which in turn influenced the physical, mechanical, and biological properties of the scaffolds. A comparison of the phase content, physical, and mechanical properties of scaffolds obtained from conditions 1 and 2 revealed that the highest HA content was 69% for condition 1 and 91% for condition 2, both sintered at 1300°C for 2 hours. The impurities in the scaffolds from condition 1 included 25.10% β-TCP and 5.76% CaO, while in condition 2, the impurities were reduced to 5.18% β-TCP and 3.38% CaO. Furthermore, condition 2 demonstrated greater potential for synthesis and exhibited superior physical and mechanical properties, making it more suitable for human bone repair compared to condition 1. The scaffold obtained from Group 1 and Group 2 was non-toxic and biocompatible with soft tissues. Group 2 scaffolds exhibited fewer inflammatory cells than those from Group 1, were more stable, and showed greater potential for use as bone grafts. However, the scaffolds from Group 2 had smaller pore sizes (lower than the theoretical range of 100-500 μm) and a lower percentage of porosity compared to the ideal parameters for bone tissue engineering.

Acknowledgements

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Effects of Oral Supplement L-Theanine on Relaxation Indexed by Alpha Brainwave

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Abstract:

Background: This study used electroencephalography (EEG) to measure brain wave activity to investigate the physiological impact of L-theanine, an amino acid found naturally in green tea, on a state of relaxation.

Objective: The purpose was to assess whether L-Theanine supplementation enhances relaxation by modulating brain waves in healthy middle-aged adults. This study focused on how L-Theanine affected alpha wave activities in different brain regions.

Materials and Method: Thirty healthy middle-aged participants were randomly assigned to receive either 200 mg of oral L-Theanine or a placebo. EEG recordings were obtained at baseline and 90 minutes after intake.

Results: Even though the results weren't statistically significant, there was a clear pattern of higher alpha wave power in the L-Theanine group, especially in the left frontal and parietal cortex 90 minutes after consumption, which suggests that they were more relaxed.

Conclusion: Despite the lack of statistical significance, the findings demonstrated that L-Theanine might modulate alpha wave activity. However, this finding may require further investigation with longer usage of L-theanine supplementation and extended observation periods.

Keywords: L-Theanine, Brain, Electroencephalogram, Brain wave activity, Alpha wave, Relaxation

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Introduction

In our fast-paced modern society, preserving cognitive function is crucial for efficient learning and productivity, as distractions increase due to technology in daily life. The brain, as the principal organ of cognition, regulates essential functions for learning, such as attention, memory, perception, problem-solving, and self-control. As individuals age, cognitive skills inherently decline, producing difficulties in memory retention and cognitive functioning. To address these issues, previous studies have focused on L-theanine, an amino acid that is naturally present in green tea and edible mushrooms.[1,2] L-Theanine is an amino acid that dissolves in water and is not a protein. Its backbone is glutamine, and it can also be found as an ethylamine derivative of glutamate. Its chemical formula is C7H14N2O3. L-Theanine can be obtained through various methods, including extraction from tea leaves, chemical synthesis, or biosynthesis.[1]

L-Theanine has attracted interest for its neuroprotective attributes, which foster neural connections and improve cognitive functions, including learning and memory. Studies indicate that L-Theanine can modulate brain waves, specifically the alpha wave linked to relaxation and focused attention. This alteration in cerebral activity emphasizes the potential of L-Theanine to enhance cognitive ability.[3,4] Previous studies have demonstrated that L-Theanine administration can enhance memory and cognitive function. It has been noted to improve memory and the functioning of the hippocampus, a brain region essential for long-term memory. L-Theanine has been shown to enhance mood, motivation, cognition, and memory by regulating neurotransmitters including dopamine, serotonin, 5-hydroxytryptamine (5HT), glycine, and GABA, while also reducing cortisol levels.[5,6]

Theanine, a prevalent non-protein amino acid, was first identified in green tea leaves in the 1940s by Sakato. The International Union of Pure and Applied Chemistry (IUPAC) refers to theanine chemically as 2-amino-4-(ethyl carbamoyl) butyric acid.[7] It usually appears in its L-(S) enantiomeric form. Theanine is distinctive in nature and is predominantly located in the Camellia genus, especially in tea-producing species such as C. *sinensis* var. *sinensis* and C. *sinensis* var. assamica. Theanine substantially influences the unique flavor of tea, with teas that possess elevated levels of theanine frequently considered superior in quality.[8] An enzyme called theanine synthetase turns glutamic acid and ethylamine into theanine. This process mostly happens in the roots of tea plants before it is sent to the new shoots.[9] Theanine concentrations in tea leaves may fluctuate based on factors including cultivation circumstances, tea grade, variety, and harvest timing. Certain investigations indicate that regulated exposure to sunlight can elevate theanine concentrations in tea. Throughout post-harvest processing, theanine concentrations remain stable across various tea types.[10] Studies indicate that the use of theanine may enhance individual health and well-being, including stress reduction, enhanced cognitive function, and possible protective effects against specific diseases. Achieving doses linked to beneficial benefits exclusively through tea drinking may prove challenging due to the substantial volume required to attain them. The inclusion of caffeine in tea complicates the achievement of certain levels, potentially leading to side effects prior to realizing the desired benefits.[11]

L-Theanine demonstrates promising neuroprotective benefits and the capacity to boost cognitive function, especially memory, indicating its potential for regulating brain waves. The goal of this study is to learn more about how L-Theanine affects the

electroencephalogram in healthy middle-aged people by looking at how taking supplements with L-Theanine affects their cognitive functions. This study aims to investigate the effects of L-Theanine supplementation on brain activity through EEG measurements taken at baseline and 90 minutes after intake. Previous investigations have primarily concentrated on the effects of L-Theanine in younger, healthy individuals or those with elevated health metrics; however, there is a lack of studies investigating its impact on the healthy middle-aged group.[1,3,4,12] This study seeks to address the issue by evaluating the activity of alpha brain waves after L-Theanine ingestion in healthy middle-aged persons, an age group that commonly utilizes supplements for relaxation and cognitive enhancement, hence yielding more significant insights into its effects. Thus, the aim of the present study was to determine the effect of L-Theanine in different regions of the brain and each hemisphere. When participants were administered L-Theanine, the brain waves' power was measured across various brain regions at different time points, including baseline and 90 minutes post-ingestion.

Materials and Method

This study involved a randomized, double-blind, single-dose, placebo-controlled experiment. This study utilized a conceptual framework to investigate the impact of 200 mg L-Theanine oral supplementation on changes in alpha brain waves at various time intervals, namely at baseline and 90 minutes post-ingestion, in healthy middle-aged adults.

Participants

Participants consisted of male and female volunteers aged 40 to 60 years, with a BMI ranging from 18.5 to 25 kg/m^2 and no prior medical history. The trial was conducted at the MAS Neuroscience Center, School of Anti-Aging and Regenerative Medicine, Mae Fah Luang University, Bangkok, Thailand. All participants were screened for the following inclusion and exclusion criteria. Inclusion criteria included (a) healthy male and female participants aged between 40 and 60 years old, (b) BMI 18.5-25 kg/m^2, (c) healthy individuals with no underlying illness, and (d) non-shift workers with a regular sleep schedule, with bedtime being between 10 PM and 12 AM. (e) Not a regular alcohol drinker (an intake of > 14 U of alcohol per week for females and 21 U for males (a unit of alcohol in the United Kingdom is defined as 7.9 g or 10 ml of pure alcohol; an average beer has 10 ml of alcohol). A shot of whiskey comprises 18 ml of alcohol. (f) Participants who consented to refrain from all caffeine- and theanine-containing items 24 hours before the test day; (g) Volunteers who gave written consent and agreed to follow the instructions. Exclusion Women who were pregnant or breastfeeding, people who had a history of psychiatric or emotional problems, people whose systolic blood pressure was higher than 140 mm Hg or their diastolic blood pressure was higher than 90 mm Hg and whose resting heart rate was lower than 40 beats per minute, people who had a history of substance abuse, people who smoked more than 5 cigarettes or the equivalent every day or drank more than 2 caffeinated drinks every day, people who had a family history of a disorder similar to schizophrenia, and people who took supplements a week before the test were not allowed to take part. The Mae Fah Luang University Ethics Committee on Human Research approved the study protocol (COA No. 088/2022, Protocol No.: EC 22033-20, authorized on May 19, 2022). All subjects gave informed consent, and the investigation followed their regulations.

Materials

Each participant received 200 mg of encapsulated purified L-Theanine for this investigation. The dosage was established based on the optimal effect noted in the previous study, 7 which demonstrated that 200 mg of L-Theanine exerted substantial effects on the human brain.

Procedure

Participants were recruited based on specific inclusion and exclusion criteria and subsequently randomized into one of two groups: either the L-Theanine group or the placebo group. The lab assistant positioned the EEG electrode according to the 10-20 regulations. We performed the test twice: once at baseline and again 90 minutes after administering either L-Theanine or a placebo. Each session lasted for five minutes. We continuously recorded EEG during each five-minute test to monitor brain activity.

We recorded EEG activity at 90 minutes post-ingestion based on prior research and pharmacokinetic data. The brain detects levels of L-Theanine within approximately 30 minutes post-ingestion, indicating its relatively quick absorption.[13] Studies have observed alpha wave changes as early as 40 minutes after ingestion, with EEG monitoring conducted at various intervals, including 30-, 45-, and 60-minutes post-dose.13 But 90 minutes seemed like a good time to catch the full effect of a single 200 mg dose, since some research shows that the highest levels of L-Theanine in the brain happen one to two hours after consumption.[14]

This timeframe also helped minimize participant discomfort by reducing the need for repeated EEG recordings, which could lead to fatigue or fluctuations in alertness unrelated to the supplement itself. Previous research has also shown that L-Theanine effects last longer than this window. For example, one study found that frontal alpha power increased significantly about three hours after taking it during a stress challenge.[15] Given these considerations, 90 minutes provided an optimal balance between capturing L-Theanine's expected peak effects and maintaining participant comfort during the study session.

Electroencephalographic Recording

The experiment employed an EEG apparatus based on the 10-20 system for the display, analysis, and recording of EEG activity. EEG signal frequency analysis was performed with a Fast Fourier Transform (FFT) technique over a 2-second interval. The frequency bands examined were delta (0.1-3.0 Hz), theta (4.0-7.0 Hz), alpha (8.0-12.0 Hz), and beta (13.0-30.0 Hz). For each subject, we initially measured the alpha wave power across a five-minute interval while they were seated in a relaxed state. The recorded power, quantified in microvolts, was subsequently averaged to yield one value denoting the group frequency band for each electrode. The electrodes were subsequently arranged to denote distinct regions of the brain, as illustrated below.

Group the electrodes for each brain region: Frontal area (F): FP1, FPZ, FP2, F7, F3, FZ, F4, F8; Temporal region (T): FC5, T7, CP5, FC6, T8, CP6; Parietal region (P): CP1, CP2, P3, PZ, P4, P7, P8; Central region (C): FC1, FC2, C3, CZ, C4; Occipital region (O): O1, OZ, O2.

Group the electrodes for the left and right hemispheres. Right: Fp2, F8, P8, O2; Left: Fp1, F7, P7, O1.

Statistical analysis

We used SPSS version 23.0 to document the demographic data and outcome results in the current study. We averaged the power of the alpha waves across different conditions to measure the selective enhancement of

brain activity. To do so, a linear mixed-effects model predicted the power of frequency band (alpha), group (test, placebo), and time (baseline, 90 minutes). Baseline power was measured at 0 min and was used to control for initial differences in neural activity while accounting for variance between participants. The Mann-Whitney U test and linear mixed model were utilized for significance testing because of the data's non-normality. The comparison assessed the mean difference of each brain wave across several brain areas at baseline (before L-theanine administration) and 90 minutes post-administration among all subjects. A p-value less than 0.05, or 5%, qualifies as statistically significant.

Results

Thirty participants were recruited and randomized to receive either L-Theanine (n =15) or a placebo (n =15). All participants successfully completed the experiment without difficulties. The sample size for identifying variations in mean changes in relative alpha power was established based on the findings of Evans, et al,[15] who performed a comparable placebo-controlled crossover study examining the effects of a single 200 mg dose of AlphaWave® L-Theanine. We eliminated one outlier from each group during the analysis. The primary outcome measure of this study was the relative power of alpha brainwaves, recorded in microvolts, across various brain regions. The alpha power values presented in Table 1 represents the mean relative alpha activity recorded during each five-minute EEG session. These numbers are given as relative power ratios, which were found by comparing the activity in the alpha band to the overall EEG power spectrum. Higher relative alpha power indicates a greater predominance of alpha activity. Table 1 shows that the frontal region alpha power didn't change significantly in the placebo group (N = 14) at any time points after the drug was taken compared to the start ($p=0.250$). Similarly, no significant changes were observed in the temporal, parietal, central, occipital, left, or right regions at any of the post-ingestion time points. However, the frontal and temporal regions of the brain show a decreasing trend, whereas the parietal, central, occipital, left, and right regions show an initial increase in power and then return to baseline or slight decrease after 90 minutes. In the L-Theanine group (N =14), however, the frontal region alpha power showed an increasing trend at 90 minutes compared to baseline (mean = 0.33 ± 0.09, $p = 0.765$). Although this trend was not statistically significant, it indicated a potential increase in alpha power overtime. Similar increasing trends were observed in the temporal (90 minutes: Mean = 0.26 ± 0.06, $p = 0.662$), parietal regions (90 minutes: Mean = 0.25 ± 0.09, $p = 0.818$), left hemispheres (90 minutes: Mean = 0.22 ± 0.04, $p = 0.161$), and right hemispheres (90 minutes: Mean = 0.25 ± 0.06, $p = 0.565$), though these were also not statistically significant. Even though there were no changes that were statistically significant, the data show that the power of alpha waves increased in the frontal, temporal, and parietal areas, as well as in both the left and right hemispheres, after the L-Theanine group took it compared to the placebo group. These results suggest that the peak effect within the 90-minute test period occurs at the 90-minute mark and may potentially increase further if the experiment were extended.

Table 1 L-Theanine and placebo groups' alpha wave power in different brain areas at baseline and 90 minutes after consumption. The mean ± standard error of mean (SEM) values in μV is shown.

Alpha wave	Measurements				
	Baseline		90 minutes		
	Mean	SEM	Mean	SEM	*p*-value [α]
Placebo (N = 14)					
Frontal area	.31	.05	.23	.04	.25
Temporal area	.33	.09	.22	.05	.19
Parietal region	.17	.03	.14	.02	.50
Central area	.21	.04	.21	.06	.51
Occipital area	.15	.02	.15	.04	.42
Left hemisphere .	.29	.05	.22	.04	.16
Right hemisphere	.29	.06	.25	.06	.57
L-Theanine (N = 14)					
Frontal area	.22	.04	.33	.09	.77
Temporal area	.22	.05	.26	.06	.66
Parietal area	.18	.03	.25	.09	.82
Central area	.18	.03	.18	.04	.66
Occipital area	.17	.03	.18	.05	.84
Left hemisphere	.20	.04	.32	.09	.60
Right hemisphere	.23	.04	.29	.08	.89

[α] *p*-value was calculated using Mann-Whitney test.

Discussion

This study aimed to investigate the effects of L-Theanine on cognitive functions through changes in the activity of alpha brain waves measured using EEG. The study involved 30 healthy middle-aged participants, although one participant from each group was removed due to being an outlier, who were randomly assigned to either the L-Theanine group or the placebo group. The main results show that there were no statistically significant changes, but there was a trend toward higher alpha power after taking L-Theanine, especially in the left frontal and parietal regions.

After 90 minutes post-ingestion, alpha power increased from 0.22 ± 0.04 at baseline to 0.33 ± 0.09 in the frontal area and from 0.18 ± 0.03 to 0.25 ± 0.09 in the parietal area. These increases were more pronounced in the left hemisphere compared to the right one, where the alpha wave power rose from 0.23 ± 0.04 to 0.29 ± 0.08. Even though these differences were not statistically significant (*p*-values of 0.765 for the frontal area, 0.818 for the parietal area, and 0.596 for the left hemisphere), the direction of change is consistent with what other research has shown, which is that L-theanine may increase alpha activity in these areas.

The increase of alpha power is generally associated with relaxation and focused attention.[3,4] In our results, the most significant change is in the frontal left region. The frontal regions, particularly on the left side, are associated with emotional regulation and attentional control.[16] Increased alpha activity in these regions has been interpreted as an indicator of a relaxed but alert mental state.[17] This pattern suggests that L-Theanine may promote a relaxed focus, potentially supporting cognitive processes that require sustained attention without inducing drowsiness. The parietal cortex, which is involved in the integration of sensory information and the allocation of attentional resources, also shows increased alpha power. Elevated alpha activity within this region has been consistently associated with internally directed attention and a reduction in the processing of external sensory stimuli.[18] Additionally, researchers believe that alpha oscillations in the parietal regions function as a gating mechanism, blocking irrelevant sensory input and promoting selective attention.[19] This study suggests that L-Theanine may help shift toward an internally focused cognitive state by inhibiting certain sensory inputs, potentially enhancing tasks that require mental imagery, working memory, or meditative awareness. Taken together, these findings suggest that L-Theanine may help shift the mind toward a focused and more relaxed cognitive state by reducing external sensory interference.

Compared to the placebo group, which typically showed reductions in alpha power from baseline to 90 minutes post-ingestion, the L-Theanine group showed a trend toward increased alpha activity. This contrast suggests that L-Theanine may affect the decline in alpha power typically observed during prolonged wakefulness or cognitive load. By maintaining or increasing alpha activity over time, L-Theanine may contribute to prolonged states of relaxation and mental clarity, potentially counteracting the cognitive fatigue often experienced during extended periods of concentration. However, this hypothesis requires further investigation in future studies.

The earlier studies by Chu et al.[20] showed that taking 200 mg of L-Theanine dissolved in 100 ml of water caused alpha waves to appear mostly in the occipital and parietal regions of young female volunteers (aged 18 - 22), and the more recent randomized, triple-blind, placebo-controlled crossover study by Evans et al.[15] found that 200 mg of AlphaWave® L-Theanine significantly increased frontal region and whole-scalp alpha power three hours after consumption in moderately stressed adults (19 - 60 years old). Our investigation targeted healthy middle-aged adults (aged 40 - 60), providing insights into the effects of L-Theanine across a broader age range; we also have a different observation period of 90 minutes. We observed that the increase in alpha power was most pronounced in the left frontal and parietal regions, which are areas associated with emotional regulation, attentional control, and sensory integration. According to these results, L-Theanine may change neural activity in different ways depending on age and brain region. This could affect how it is used to help middle-aged people feel calm and focused. Furthermore, it is possible that middle-aged adults require a longer latency period before significant changes appear, or that a single dose may be insufficient to provide significant effects in this population. Further research is warranted to explore these age- and brain region-specific effects, as well as their relevance to cognitive performance and emotional well-being.

To place our findings in a practical context, we considered how the observed effects of L-Theanine supplementation might translate to everyday tea consumption. On average, a standard cup of tea contains approximately 20 to 25 mg of L-Theanine,

though the amount can vary depending on the tea variety and brewing conditions.[7,10] Based on this estimate, the 200 mg dose used in our study would be equivalent to consuming about eight to ten cups of tea. It is unlikely that an individual would consume such a large quantity for relaxation purposes. Previous research has indicated that relaxation effects typically become more pronounced at higher doses of L-Theanine than what is commonly present in a single cup of green tea.[13] However, lower doses, like 50 mg from about two cups of tea, have been linked to increases in alpha wave activity within about 40 minutes, with effects lasting for at least 90 minutes.[21] This suggests that moderate consumption may elicit a mild state of relaxation, albeit less than the effects observed with a concentrated 200 mg dose of L-Theanine. Additionally, it is important to note that tea contains caffeine, which was not present in the pure L-Theanine capsules administered in our study. Caffeine and L-Theanine in tea are known to work together to produce a stronger effect, which is often described as a balanced state of relaxation with sustained focus. This is because L-Theanine can lessen some of the stimulating effects of caffeine.[22,23] From a practical standpoint, moderate tea consumption may contribute to stress reduction and relaxation, although the impact on alpha brain waves is likely to be more subtle compared to a higher dose L-Theanine supplement. For individuals seeking more pronounced relaxation effects without excessive caffeine intake or fluid consumption, options such as decaffeinated tea or L-Theanine supplements may offer a more effective alternative. This context helps highlight the real-world relevance of our findings and offers practical guidance for those interested in using L-Theanine to promote relaxation.

We acknowledge that 40 - 60 is a somewhat broad range, spanning two decades; we feel that it was appropriate for a pilot exploratory study, as it captures the population of middle-aged adults who might use supplements for relaxation or cognitive health. However, all participants met strict inclusion criteria to ensure relative health status. This range represents middle age, a life stage where subtle cognitive and neurophysiological changes begin. Research suggests that certain cognitive functions start to decline as early as the mid-40s.[24] We were interested in whether L-Theanine's relaxation and potential cognitive benefits would be evident in this age group, as many prior studies using EEG have examined younger adults.[25,26]

Both male and female participants were included to enhance the generalizability of our findings. We did not consider the sex differences in response to L-Theanine; rather, inclusion criteria were based on health and age parameters. It should be noted that we did not control for the menstrual cycle phase in female participants, as some studies indicated that alpha waves could be influenced by the menstrual cycle and progesterone level.[27,28] However, we use a random assignment to treatment groups and ensure a balanced gender ratio, minimizing potential sex-related effects. We acknowledge this as a potential limitation in our design and recommend future studies account for menstrual phase to eliminate this variable.

Conclusion

This study shows some early signs that L-Theanine may change brain activity, mainly by increasing the power of alpha waves in the left frontal and parietal regions. These are known to play important roles in controlling emotions, paying attention, and processing sensory information. While

the results didn't reach statistical significance, there is a consistent trend of increased alpha activity that suggests L-Theanine could help promote a relaxed yet alert mental state in healthy middle-aged adults. However, there are a few important limitations to acknowledge. The sample size was relatively small, and the study only explored the effects of a single dose. We also used a broad age range (40 - 60 years), and we didn't control for menstrual cycle phases in female participants, both of which could have introduced variability in the results. While we ensured a balanced gender ratio and randomized assignment, future studies should take these factors into account to better isolate L-Theanine's effects. Future research should explore different doses and timing to better understand how L-Theanine works over overtime and at varying levels. Longer-term studies could also determine whether regular use has long-term benefits. Including cognitive testing, like the CDR computerized battery, along with mood assessments such as visual analog scales, would give a clearer picture of how L-Theanine impacts both mental performance and emotional well-being. Adding biochemical measures, such as neurotransmitter levels and salivary cortisol, could also compare how L-Theanine affects other stress markers as well. And finally, excluding female participants during their menstrual cycles may help improve the results by reducing hormonal fluctuations that might influence brainwave activity.

Contribution

Conceptualization: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Methodology: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Data Curation: Nipapan Sangmanee and Phakkharawat Sittiprapaporn; Resources: Phakkharawat Sittiprapaporn; Formal analysis: Chong Ie Yern and Phakkharawat Sittiprapaporn; Validation: Phakkharawat Sittiprapaporn; Investigation: Phakkharawat Sittiprapaporn; Writing-original draft preparation: Chong Ie Yern and Phakkharawat Sittiprapaporn; Writing-review and editing, Phakkharawat Sittiprapaporn; Project administration: Phakkharawat Sittiprapaporn; Funding Acquisition: Nipapan Sangmanee and Phakkharawat Sittiprapaporn. All authors have read and agreed to the published version of the manuscript.

Disclosure of interest

The authors report no conflict of interest.

Data Availability Statement

The data presented in this study are available within the article.

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สำนักวิชาแพทยศาสตร์ มหาวิทยาลัยแม่ฟ้าหลวง
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Exploring Medical Cannabis Knowledge and Attitudes among Health Care Providers in Northern Thailand After Legalization

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Abstract:

Background: In June 2022, the Ministry of Public Health of Thailand announced the legalization of cannabis for medical and recreational purposes under controlled conditions. However, despite this regulatory shift, there exists a paucity of evidence-based research regarding the therapeutic efficacy of cannabis. Moreover, within the Thai context, there persists a notable gap in understanding the comprehensive spectrum of health benefits and concerns associated with cannabis usage.

Objective: To investigate the knowledge and attitudes of healthcare providers regarding medical cannabis.

Materials and method: This cross-sectional study was conducted among healthcare providers at Mae Fah Luang University Medical Center Hospital (MFU-MCH) and the Thai-Chinese Medicine Institute (TCMI) in November 2022. The survey instrument comprised two components. Firstly, it evaluated the participants' foundational knowledge concerning medical cannabis. Secondly, it measured their attitudes toward medical cannabis utilizing a Likert Four-Point scaling system. Data were collected through online surveys administered via Google Form.

Results: 74 healthcare providers were recruited. The prevalence of low knowledge scores was found to be 13.51%. Attitudes toward medical cannabis use were evenly split, with 50% expressing agreement and 50% expressing disagreement.

Conclusion: Basic knowledge about cannabis appears to be limited among healthcare providers, while attitudes towards its medical use are evenly split. Given that healthcare providers play a crucial role in advising patients and the public on medical cannabis, there is a pressing need to enhance their knowledge in this area.

Keywords: Cannabis, Knowledge, Attitudes, Health, Provider

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Introduction

Presently, a global trend of relaxing regulations surrounding cannabis usage is evident. Countries such as Canada, France, Mexico, South Africa, and select states within the United States have embraced legalization, encompassing both medical and recreational applications. Similarly, Argentina, Australia, Brazil, Finland, Denmark, Germany, Greece, and Italy have legalized cannabis strictly for medicinal purposes.[1, 2]

In Thailand, the legalization journey commenced in 2019 with the implementation of The Narcotic Act (Version 7), sanctioning the therapeutic utilization of medical cannabis. Subsequently, three years later, the Ministry of Public Health of Thailand extended this authorization to encompass both medical and recreational domains, including provisions for home cultivation.[3] This legislative shift has triggered extensive discourse within societal realms, probing the merits and drawbacks of such legalization endeavors.

Several studies in Thailand have scrutinized the health literacy levels pertaining to cannabis among the general population. Findings indicate a notable deficiency, with approximately one-third of the population exhibiting low health literacy, particularly in aspects concerning communication, decision-making, and self-management.[4] Furthermore, prevailing sentiments endorse the restricted application of cannabis solely for medical purposes, underscored by a collective desire for research conducted with stringent safety protocols.[5]

Within the healthcare landscape, providers wield significant influence as conduits of accurate cannabis-related information to the public. Nonetheless, past investigations reveal a spectrum of beliefs among healthcare professionals, encompassing both skepticism and endorsement regarding the efficacy of medical cannabis in augmenting patient quality of life.[6] Notably, none of these studies have examined the knowledge and attitudes regarding cannabis use among healthcare providers in Thailand.

Objective

This study aims to investigate the knowledge and attitudes of healthcare providers regarding medical cannabis.

Materials and Method

Study design and participants

A cross-sectional analytic study was conducted in November 2022 at Mae Fah Luang University Medical Center Hospital (MFU-MCH) and the Thai-Chinese Medicine Institute (TCMI). The study received approval from the Mae Fah Luang University Ethics Committee on Human Research, Thailand (EC 22104-21). The sample size was determined using infinite population proportions based on the proportions of high knowledge of medical cannabis observed in public hospital pharmacists ($p = 0.53$).[7] The margin of error and alpha value were set at 0.1 and 0.05, respectively. A total of 74 healthcare providers were recruited and provided electronic consent to participate in the study. Subsequently, participants completed electronic questionnaires via Google Forms. The general information questionnaire included inquiries regarding gender, age, working organization, occupation, and education level. Additionally, 10 dichotomous questions assessing fundamental knowledge of medical cannabis (table 2) and 12 questions evaluating attitudes toward medical cannabis (table 3), each rated on a Likert scale ranging from 1 to 4 points (strongly agree, agree, disagree, and strongly disagree), were administered based on a previous study.[7]

Criteria assessment of knowledge and attitudes regarding cannabis use

The level of knowledge was categorized into binary endpoints using Bloom's criteria[8]: individuals achieving a correct answer rate of more than 80% were classified as having high knowledge, while those with a correct answer rate of less than 80% were classified as belonging to the low knowledge group. Similarly, the level of attitudes was categorized into binary endpoints as previous study[9]: participants with an attitude level higher than the mean were classified as favoring, while those with an attitude level lower than the mean were classified as not favoring.

Statistical analysis

Data analysis was performed using Stata Statistical Software, version 16.0 (StataCorp LLC, College Station, TX, USA). A p-value of less than 0.05 was considered statistically significant. No missing data were found, allowing for a complete case analysis. Categorical variables were described by frequency and percentage. Continuous variables were presented with mean and standard deviation or median and interquartile range (IQR), based on data distribution.

Results

Participant's profiles

Seventy-four participants were included in this study. Most participants were female, constituting 72.97%, with a mean age of 33.58 ± 10.85 years. Additionally, most participants were employed at MFU-MCH (85.14%), and the most common occupation among participants was registered nurse (51.35%). The predominant education level attained by participants was a bachelor's degree (70.27%). (Table 1)

Table 1 Participant's Profiles (N = 74)

	n (%)
Female	54 (72.97)
Age (years)*	33.58 ± 10.85
Working Organization	
MFU-MCH	63 (85.14)
TCMI	11 (14.86)
Occupation	
Physicians	26 (35.14)
Registered Nurses	38 (51.35)
Pharmacists	3 (4.05)
Thai Traditional Medicine Practitioners	4 (5.41)
Chinese Traditional Medicine Practitioners	3 (4.05)
Education Level	
Bachelor's Degree	52 (70.27)
Master's Degree or Equivalent	14 (18.92)
Doctorate Degree or Equivalent	8 (10.81)

Mean ± SD., MFU-MCH = Mae Fah Luang University Medical Center Hospital, TCMI = Thai-Chinese Medicine Institute

Question for evaluation of fundamental knowledge of medical cannabis

The proportion of low knowledge among healthcare providers was 13.51% (n = 10). The question with the highest proportion of correct answers was question number 3 (Marijuana contains a psychoactive compound known as delta-9-tetrahydrocannabinol (THC), which induces the sensation of "euphoria."). Similarly, question number 10 (Practitioners such as physicians, dentists, pharmacists, traditional Thai medicine practitioners, and local doctors must undergo training to obtain permission to use marijuana) received a high correct answer rate of approximately 94.59%. Conversely, the question with the lowest proportion of correct answers was question number 8 (Extracts from marijuana do not typically cause drug interactions when used concomitantly with other medications) with only approximately 22.97% of respondents answering correctly. (table 2)

Table 2 Question for evaluation of fundamental knowledge of medical cannabis

Question for evaluation of attitudes toward medical cannabis

Question (N = 74)	Correct answer n (%)
1. The flower buds of female marijuana plants have a stronger effect on the nervous system compared to other parts	69 (93.24)
2. Marijuana plants are capable of synthesizing and producing naturally occurring substances called cannabinoids	67 (90.75)
3. Marijuana contains a psychoactive compound known as delta-9-tetrahydrocannabinol (THC), which induces the sensation of "euphoria"	70 (94.59)
4. Medical authorities allow the use of marijuana for medical purposes in individuals aged 18 and above to mitigate potential health risks	52 (70.27)
5. It is not recommended to use marijuana products as first-line therapy for treating or managing symptoms in all cases	63 (85.14)
6. The use of marijuana with THC extracts in AIDS patients can increase appetite, potentially aiding in weight gain	51 (68.92)
7. Administering marijuana oil extracts orally can result in faster onset of effects compared to sublingual administration	22 (29.73)
8. Extracts from marijuana do not typically cause drug interactions when used concomitantly with other medications	17 (22.97)
9. The permitted amount of marijuana extract (THC) allowed for use is typically not exceeding 0.2% by weight	56 (75.68)
10. Practitioners such as physicians, dentists, pharmacists, traditional Thai medicine practitioners, and local doctors must undergo training to obtain permission to use marijuana	70 (94.59)

The proportion of favoring cannabis use was 50.00% (n = 37). The majority of participants expressed agreement with the statements advocating for "there should be a systematic monitoring and reporting system for undesirable outcomes from medical marijuana usage", as well as "there should be stringent controls on advertising or promoting the sale of marijuana extracts". However, a significant portion of participants disagreed with "marijuana should be used for recreational purposes or other benefits beyond medical treatment." (table 3)

Table 3 Question for evaluation of attitudes toward medical cannabis

N = 74	Strongly Agree N (%)	Agree N (%)	Disagree N (%)	Strongly disagree N (%)	Median (IQR)
1. There is permission granted to cultivate marijuana for medical purposes	10 (13.51)	35 (47.30)	20 (27.03)	9 (12.16)	3 (2-3)
2. Marijuana should be used for recreational purposes or other benefits beyond medical treatment	2 (2.70)	7 (9.46)	21 (28.38)	44 (59.46)	1 (1-2)
3. The indications for marijuana in certain diseases should undergo more research and gather more observational evidence before being applied for actual treatment	38 (51.35)	32 (43.24)	3 (4.05)	1 (1.35)	4 (3-4)
4. Marijuana can treat certain diseases	6 (8.11)	42 (56.76)	21 (28.38)	5 (6.76)	3 (2-3)
5. Advertisements about medical marijuana provide the public with accurate, comprehensive, and sufficiently effective information	8 (10.81)	13 (7.57)	31 (41.89)	22 (29.73)	2 (1-3)
6. There should be more promotion of knowledge regarding the benefits and drawbacks of marijuana to the public, such as offering courses on medical marijuana to increase public awareness	42 (56.76)	26 (35.14)	4 (5.41)	2 (2.70)	4 (3-4)
7. Administering inappropriate amounts of marijuana for treatment can lead to subsequent problems, such as excessive use or recreational use without consultation with a physician	45 (60.81)	27 (36.49)	0 (0.00)	2 (2.70)	4 (3-4)
8. People without expertise in cultivating marijuana may struggle to control the proportions of CBD and THC, leading to patients being at risk of dangerous side effects	39 (52.70)	30 (40.54)	4 (5.41)	1 (1.35)	4 (3-4)
9. Treatment with marijuana according to indications should be clearly supported by robust observational evidence and limited to individuals who have undergone training in medical marijuana usage	39 (52.70)	31 (41.89)	1 (1.35)	3 (4.05)	4 (3-4)
10. Common neurological side effects such as confusion, neuropathy, or sedation may create confusion for treating physicians	26 (35.14)	42 (56.76)	5 (6.76)	1 (1.35)	3 (3-4)

Table 3 Question for evaluation of attitudes toward medical cannabis (con.)

N = 74	Strongly Agree N (%)	Agree N (%)	Disagree N (%)	Strongly disagree N (%)	Median (IQR)
11. There should be a systematic monitoring and reporting system for undesirable outcomes from medical marijuana usage	45 (60.81)	29 (39.19)	0 (0.00)	0 (0.00)	4 (3-4)
12. There should be stringent controls on advertising or promoting the sale of marijuana extracts	50 (67.57)	21 (28.38)	1 (1.35)	2 (2.70)	4 (3-4)

Discussion

This study serves as a pioneer in investigating the knowledge and attitudes surrounding medical cannabis use following its recent legalization in Thailand. Amidst this period, there is heightened concern regarding cannabis abuse and the escalating social side effects associated with its use. Notably, within the initial first week post-legalization, the Ramathibodi Poison Centre in Bangkok reported a significant surge in cannabis-related cases, with 64% comprising first-time recreational users.[10]

This study specifically targets healthcare providers, encompassing all occupations related to cannabis use. Healthcare providers play a pivotal role as frontline therapists and counselors within the general population and community.[11] Their instrumental contribution lies in significantly enhancing health literacy concerning cannabis use by providing evidence-based education, conducting screenings, and advocating harm reduction strategies. Through effective communication, they disseminate accurate information pertaining to cannabis pharmacology, associated risks, and potential benefits, while also integrating systematic screening protocols for identifying problematic use. Furthermore, healthcare providers actively promote responsible consumption practices and facilitate referrals to support services as necessary. Continual professional development ensures that healthcare providers remain abreast of the latest developments in this field, while fostering cultural competence enables effective communication with diverse populations. Collaboration with stakeholders and advocacy efforts are integral components of comprehensive strategies aimed at addressing public health issues associated with cannabis use.[12] It is imperative that healthcare providers exhibit a strong foundation of knowledge and possess favorable attitudes towards this subject matter.

The proportion of individuals demonstrating high knowledge in medical cannabis use is 86.49%, surpassing that reported in a previous study.[7] Moreover, the distribution of individuals favoring cannabis use is evenly split, with a ratio of 50 : 50 in this study. Variations in prevalence observed between this study and previous study can be attributed to methodological differences, variations in study populations, and shifts in societal attitudes and behaviors over time.[13]

This study has several limitations. It is conducted within a single institution in northern Thailand. Therefore, while our findings may be applicable to healthcare settings with similar clinical contexts and patient or healthcare characteristics, caution should be exercised when generalizing them to other settings. External validation of our results in diverse countries or settings is

warranted. Future research should consider conducting multicenter or national surveys involving a more diverse range of healthcare professions to ensure a representative sample and enhance the robustness of the findings.

Conclusion

This study highlights the limited basic knowledge about cannabis among healthcare providers, alongside an even split in attitudes towards its medical use. Given the pivotal role of healthcare providers in advising patients and the public on medical cannabis, promoting training initiatives to ensure their proper understanding and utilization of medical cannabis is imperative. Enhancing the knowledge and competence of healthcare providers in this area is crucial for facilitating informed decision-making and promoting responsible use of medical cannabis within healthcare settings.

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Conflict of Interest

The authors have no conflicts of interests.

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