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Review Article

The Effects of Intermittent Fasting on Cardiovascular Health of Prediabetes and Type 2 Diabetes Mellitus (T2DM) Patients: A Systematic Review

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ABSTRACT

Background: Type 2 diabetes mellitus and prediabetes are showing an increasing number of cases and are predicted to keep increasing over the years. Both conditions have a 2-3-fold increased risk of developing cardiovascular diseases. Intermittent fasting is one of the forms of dietary intervention being practiced in preventing and managing these conditions and their associated complications. There are many reported benefits of intermittent fasting for health, but no specific studies discussed regarding the effect of this practice on cardiovascular health of these patients. **Objective:** This study is aiming to systematically review the benefits of intermittent fasting on cardiovascular health of these groups of patients. **Methods:** This systematic review followed the guideline of PRISMA and was registered at PROSPERO with the registration number of CRD42022378349. A systematic literature search was conducted in PubMed, Scopus, Cochrane Library, Google Scholar and by using snowball technique. The articles retrieved were independently screened by two authors against the eligibility criteria in stages of title, abstract and full text. The risk of bias in the included studies were assessed independently by two authors using the Joanna Briggs Institute critical appraisal checklist. Data from all the included articles were extracted using a predetermined template. **Results:** Twelve studies were included in this study. Six studies recorded low risk of bias while the other six studies had moderate risk of bias. Three studies recorded significant reduction for both systolic and diastolic blood pressure, and 3 studies showed significant improvement in the lipid profiles at the end of intervention. **Conclusion:** Intermittent fasting is beneficial for cardiovascular health of prediabetes and type 2 diabetes mellitus patients mainly by improving both systolic and diastolic blood pressure and the lipid profiles. Randomized controlled trials that investigate specifically the benefits of this practice on the cardiovascular health of these populations are needed to confirm the findings.

INTRODUCTION

Diabetes mellitus is one of the chronic diseases that has high prevalence worldwide. In 2021, there are 537 million (10.5%) adults living with diabetes worldwide and this number is predicted to increase to 643 million by 2030 [1]. In Malaysia, there are about two million (9.4%) adults aged 18 years and above are diagnosed with diabetes as of 2019, increased 1.1% compared to what have been reported in year 2015 (8.3%) (National Health Morbidity Survey, 2019). The fact that the number of diabetes patients will increase by years is very worrying as it costs a huge socio-economic burden due to increased mortality because of accelerated vascular complications and premature death [2]. It is estimated that the total annual cost of diabetes in Malaysia is USD 600 million (RM 2.6 billion) which covers the direct, indirect, intangible, capital, recurrent and per diem costs [3]. The International Diabetes Federation (IDF) in their most recent research reported that 352 million adults between the ages of 20 and 79

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(or 7.3% of the population) may have prediabetes. This figure is expected to increase to 481 million by 2040. Compared to Malaysia, the report from National Health Morbidity Survey (NHMS) 2019 reported that the prevalence of those with abnormal fasting plasma glucose (FPG) was 23.6% at the time of the survey. This means that there are approximately 5 million individuals in Malaysia with probable prediabetes in Malaysia and future risk of developing diabetes. Prediabetes is defined by blood glucose levels that are higher than normal but fall short of the diagnostic criteria for Type 2 diabetes mellitus (T2DM) [4]. People with prediabetes have a 30% chance of developing T2DM over a 10-year period [5].

It is well established that patients with prediabetes and T2DM will commonly have other coexist non-communicable diseases such as obesity and have a 2-3-fold increased risk of developing cardiovascular diseases (CVD) like hypertension and dyslipidemia. Thus, it is very important to assess the CV profile of the patients at the time of diagnosis and during each clinic visit to prevent the development and progression of the any CVD. Patients may present with CVD such as peripheral arterial disease, heart failure, angina, and non-fatal myocardial infarction [6]. In the National Diabetes Registry Report (2020), it was reported that 80%, 75.7% and 84% of T2DM patients are also diagnosed with hypertension, dyslipidemia, and obesity, respectively. In addition, Newman et al. (2017) reported that the most common cause of death among adults with T2DM is due to cardiovascular diseases [7]. This is proven where 60% of patients with T2DM will eventually die from cardiovascular (CV) complications [2].

Currently there are many strategies being practiced for managing prediabetes and T2DM patients, pharmacologically and non-pharmacologically. Oral hypoglycemic agents (OHA) such as metformin and gliclazide and insulin therapy are used as one of the pharmacological treatments for T2DM patients. Besides, non-pharmacological intervention such as medical nutrition therapy (MNT), exercise, and tobacco cessation also help to prevent and manage prediabetes and T2DM [2]. In addition, weight management is also essential in the management of prediabetes and T2DM patients as obesity is one of the main factors that contribute to the development of T2DM. This is because, excess adipose tissue in obese patients cause an increase in the secretion of proinflammatory cytokines such as interleukin 6 (IL-6), synthesis of leptin (a proinflammatory protein) and decrease secretion of adiponectin (an anti-inflammatory protein) which results in a higher ratio of proinflammatory cytokines and protein which are associated with insulin resistance [8]. One of the strategies to reduce body weight is by having daily negative calorie balance or calorie deficit meals [9].

Many clinical practice guidelines and diabetes associations stated that one of the crucial methods in the treatment and prevention of prediabetes and T2DM, and its associated complications is through dietary interventions [10]. Intermittent fasting (IF) is one of the common approaches that is being practiced for reducing the daily calorie intake and as a form of dietary intervention [10,11]. One of the reasons behind it is that IF is a regime that could be tolerated by majority of the people for a long period of time and at repeated intervals as practiced by the Muslim community worldwide during the holy month of Ramadhan yearly and voluntary fasting on Monday and Thursday weekly [12]. According to Abdellatif and Sedej (2020), IF is characterized by dietary restriction for prolonged periods that is interrupted by periods of ad libitum eating [13]. There are several types of IFs being practiced nowadays such as alternate day fasting (ADF) (36 hours of fasting separated by 12 hours ad libitum eating) and time restricted feeding (TRF) (limiting intake of food for several hours within a specific timeframe, around 6 to 12 hours) [14].

There are many reported benefits of IF for health including on the cardiovascular system. For instance, IF is cardioprotective helps to reduce blood pressure and resting heart rate in healthy patients [13,14]. IF also is also reported to help in reducing the body weight [15], enhancing insulin receptors sensitivity [16], and promoting mitochondrial biogenesis, cellular stress resistance and synaptic plasticity due to increased level of brain-derived neurotrophic factor (BDNF) during IF period [17]. However, there are no studies discuss specifically / in depth on the benefits of IF on cardiovascular system T2DM patients. Therefore, this study aims to investigate the benefits of IF on cardiovascular health of prediabetes and T2DM patients by systemically review available published articles and research.

MATERIAL AND METHOD

Protocol and registration

The current systematic review followed the statement and general principle of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [38] and was registered at the International Prospective Register of Systematic Reviews (PROSPERO) with the number CRD42022378349.

Eligibility criteria

This systematic review examined the intermittent fasting studies with T2DM and prediabetes and the effect of practicing it on their cardiovascular health. To be considered for inclusion within this review, study subjects needed to be aged between 18 to 80 years and have prediabetes or are diagnosed with T2DM. The diagnostic criteria for T2DM according to IDF 2021 are: (1) fasting venous or capillary plasma glucose $\geqslant$7.0 mmol/L (126 mg/dL); (2) 2-hour venous plasma glucose after

loaded with 75g oral glucose ⩾11.1 mmol/L (200 mg/dL); (3) Random plasma glucose in the presence of hyperglycemia symptoms ⩾11.1 mmol/L (200 mg/dL); (4) HbA1c ⩾ 6.5% (48 mmol/mol). T2DM is diagnosed if one or more of the following criteria are met. For prediabetes, the American Diabetes Association (ADA) recommends diagnosing prediabetes with HbA1c values between 39 and 47 mmol/mol (5.7–6.4%) or impaired fasting glucose when the fasting plasma glucose is between 5.6 and 6.9mmol/L (100–125mg/dL) or 2 hour plasma glucose during 75-g oral glucose tolerance test (OGTT) between 140 mg/dL (7.8 mmol/L) to 199 mg/dL (11.0 mmol/L), known as impaired glucose tolerance (IGT).

Study design was not limited to only randomized controlled trial (RCT) and other study designs such as cohort studies, case series and quasi-experimental studies are also included in the review. However, only studies published in English and between 2000 to 2022 were included to extract data sources. This review excluded studies that included participants who are diagnosed with type 1 diabetes mellitus (T1DM), pregnant or breastfeeding, smokers, active athletes, with history of psychiatric or eating disorders and diagnosed with chronic kidney diseases stage 4 and 5 (eGFR <30 ml/min). Further exclusion criteria include animal or non-human studies and study protocols and review articles. Since many individuals with T2DM are already receiving pharmacological interventions for managing glycemic control, subjects on an oral hypoglycemic agent or insulin were not excluded from the review.

As this review investigated the effects of IF on cardiovascular health of prediabetes and T2DM patients, studies had to include an intermittent fasting intervention, or any regimen associated with intermittent fasting such as ADF or TRF. The primary outcome measure was improvement in total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high- density lipoprotein cholesterol (HDL-c), triglycerides (TG), systolic and diastolic blood pressure (SBP and DBP), heart rate (HR) or pulse rate (PR) in the participants. Secondary outcomes included measures of glycemic control and metabolic parameters such as HbA1c, fasting blood glucose (FBG), fasting serum insulin level, and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). Studies that do not include the targeted primary and secondary outcomes will not be included in this review.

Search strategy

Studies or articles were searched from four databases which include SCOPUS, Cochrane Library, PubMed, and Google Scholar. The search strategy included a combination of domain and sub-domain that were combined with Boolean operators "OR" and "AND". Keywords within the same domain were connected using Boolean operator "OR" while "AND" operator was used to connect keywords between other domains. The search terms included five main domains from the title of this study in alignment with the inclusion and exclusion criteria which were: (1) effects: list of all keywords that are synonym to effect such as benefits and advantages; (2) intermittent fasting and its associated terms; (3) cardiovascular health: a general term for cardiovascular system and its associated diseases; (4) type 2 diabetes mellitus: a term describing patients who have been diagnosed with T2DM; and (5) prediabetes. The list of keywords that were used for each domain are listed in Table I. Besides, snowball technique, a technique to search for articles from the reference list of articles retrieved from the databases, were also used to add more articles for selection. Results of the search strategy were then added, stored, and managed in Mendeley, including removal of duplicates. Microsoft Excel was used to support document study selection and data extraction.

Table I: The keywords used in search of articles from databases.

Title Components/Domains	Related keywords/Subdomains
Effect	Effect, effects, benefit, benefits, beneficial effect, beneficial effects, advantage, advantages, positive effect, positive effects, positive impact, positive impacts.
Intermittent fasting	Intermittent fasting, time-restricted feeding, time- restricted eating, alternate day fasting, early time-restricted eating, early time-restricted feeding, fasting-mimicking diet, intermittent fasting 5:2, periodic fasting.
Cardiovascular health	Cardiovascular health, heart rate, pulse rate, blood pressure, systolic blood pressure, diastolic blood pressure, LDL, HDL, VLDL, cholesterol, triglyceride, heart, cardiovascular system, cardiovascular systems, circulatory system, circulatory systems.
Prediabetes	Prediabetes, impaired glucose tolerance, impaired fasting glucose.
Type 2 Diabetes Mellitus	Diabetes mellitus, diabetes mellitus type 2, type 2diabetes mellitus, T2DM, hyperglycemia, glucose intolerance, high blood sugar, insulin resistance.

Study selection

Two authors independently examined the studies against predetermined eligibility criteria in the stages of title, abstract, and full-text study selection. None of the screening authors were blinded to any details of the studies. Two independent authors independently reviewed study titles and abstracts until convergence was reached. Discrepancies were then discussed

between the two authors and if an agreement was not met between the authors, the discrepancies were then discussed with the third author and criteria refined in the process where necessary. Studies that satisfied the inclusion criteria were retrieved for full-text assessment. Full-text screening was performed in full independent double screening by the two authors. Only full-text records selected for inclusion by both the authors were included in this study. Any disagreements during this stage were resolved through discussion and consensus between the two authors and if agreement was not met between authors, the disagreements were discussed with the third author. The authors were contacted, where appropriate, to clarify missing or inadequate information to determine study eligibility. The primary reasons for the exclusion of articles were documented at all stages of the study selection. The study selection was conducted by using Microsoft Excel. A final list of articles was prepared and stored in Mendeley and Microsoft Excel for data extraction.

Data collection/extraction

Eligible studies were independently reviewed, and relevant data were extracted. The information that was gathered included authors, publication year, study region, study design, total number of participants recruited, study intervention or type of IF being implemented, IF duration or duration of the study and the readings or results of targeted primary outcomes and second outcomes. Data from each study was entered into a table in Microsoft Excel to ease the comparison process between the included studies. Numerical data were also extracted and expressed as mean ± standard deviation, or mean (SEM), and p-values, and the differences were considered statistically significant when $p < 0.05$.

Risk of bias in individual studies

Two investigators independently assessed the risk of bias for all included studies by assessing their methodological quality using the Joanna Briggs Institute (JBI) critical appraisal checklists. Score of '1' were given if the studies fulfil the stated criteria of the checklist and '0' if not. After that, the total score was calculated and converted into percentage. Studies with percentage of <50% were considered as having low risk of bias, moderate risk, and high risk of bias if the percentage were between 50% - 69% and $\geqslant$ 70%, respectively [18]. Any disagreements will be resolved through discussion between the two investigators and later with the third investigator if an agreement was not met between the initial two investigators. The results of risk of bias for each type of studies included were then visualized as traffic-light plots by using Risk-of-bias VISualization (robvis) tool [19].

RESULTS

Study selection

A total of 353 studies were identified within the databases used. Additional 23 studies were discovered by using snowball technique, by searching related studies within the references of the studies identified earlier in the databases. PubMed located 77 studies, Scopus located 163 studies, Cochrane Library located 71 studies and Google Scholar located 42 studies, which provided a total of 376 studies. From the databases, 61 studies were excluded due to being duplicates. After title and abstract screening have been done, 272 studies were excluded because they were review articles (n = 125), animal studies (n = 40), recruited wrong study samples (n = 68), did not have the targeted primary and secondary outcomes (n = 36) and used interventions other than IF (n = 3). From the remaining 20 articles, three articles were excluded as the full-text articles were not accessible and eight articles were excluded during full-text reading, with the following reasons: study excluded T2DM patients (n = 2), recruited healthy participants as study subjects (n = 2), has high risk of bias (n = 1) and not all participants recruited in the study have prediabetes or T2DM (n = 4).

After full-text screening, eight studies left from the databases and were included in the review. For the studies identified by using snowball technique, four studies were removed as there was no access to the full-text articles. From the remaining studies, four studies were excluded due to being duplicates, five studies did not report the outcomes of interest, three studies recruited participants that are not diagnosed with prediabetes or T2DM and three other studies used interventions other than IF, leaving four studies behind to be included in this study. As a result, this search produced a total of 12 studies that met the inclusion criteria and were deemed eligible to be included within this systematic review, with 8 of them obtained from the databases and 4 studies from the snowball technique. A PRISMA flow diagram for all database results and article selection can be found in Figure I. prediabetes or T2DM (n = 4). After full-text screening, eight studies left from the databases and were included in the review. For the studies identified by using snowball technique, four studies were removed as there were no access to the full-text articles, leaving 19 studies. From the remaining studies, four studies were excluded due to being duplicates, five studies did not report the outcomes of interest, three studies recruited participants that are not diagnosed with prediabetes or T2DM and 3 other studies used interventions other than IF, leaving 4 studies behind to be included in this study. As a result, this search produced a total of 12 studies that met the inclusion criteria and were deemed eligible to be included within this systematic review, with eight of them obtained from the databases and four studies from the snowball technique. A PRISMA flow diagram for all database results and article selection can be seen in Figure I.

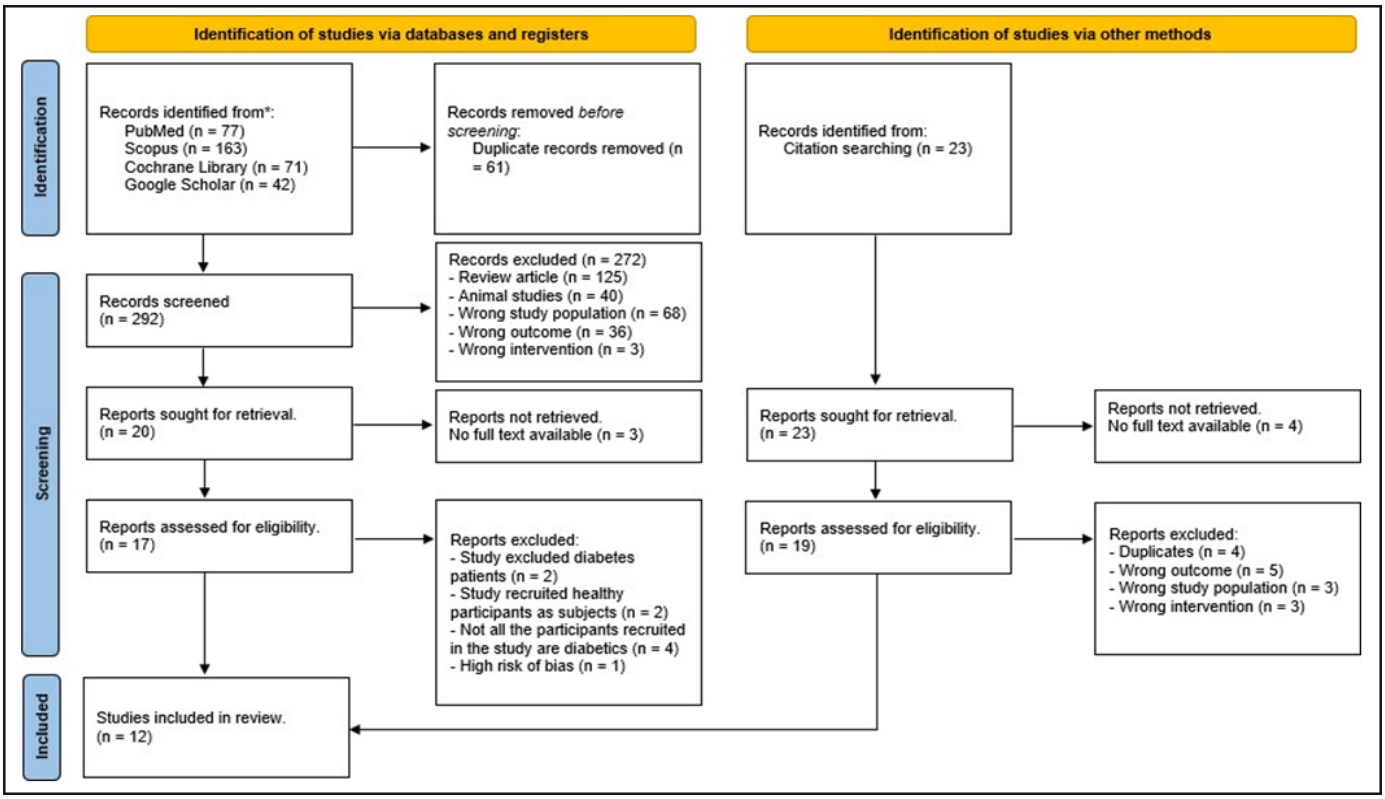

Figure I: Flowchart of the study selection process.

Study characteristics

All included studies were published between the year 2010 to 2022 and comprise of four study designs which were RCT (n = 6), cohort studies (n = 3), case series (n = 2) and a quasi-experimental study. Three of the studies were conducted in China, two in Australia and one each in the United States of America, Canada, New Zealand, Yemen, Germany, Algeria, and India. The studies used certain types of IF with Ramadan IF were the most intervention used among the studies (n = 3). The other types of IF being implemented include 10-hour TRF (n = 1), fasting-mimicking diet (FMD) (n = 1), early-time restricted feeding (eTRF) (n = 1), 18-20 hour IF (n = 1), intermittent energy restriction for two days (n = 1), two consecutive days of very low calorie diet (n = 1), periodic fasting (n = 1), 16/8 TRF (n =1) and one study comparing between ADF and 16/8 TRF. Two of the studies recruited prediabetes participants as the subjects while the other ten studies recruited T2DM patients.

In addition, most studies included both genders except for the study by Sutton et al. (2018) and Abdullah, Al-Habori, and Al-Eryani, (2020) where they recruited only male participants and the study by (Ait Saada et al., 2010) where they recruited female participants only [20-22]. Moreover, seven of the studies included participants who were on OHA as stated in Table II below.

Risk of bias within studies

From all of the 12 studies included in the review, some articles demonstrated low risk of bias (showing high percentage of positive answers to the questions of JBI tool) [21-26,30] and six articles [22, 20, 27-29] had moderate risk of bias. For all the RCT studies, there were high risk of bias for domain four, five and six (except for the study conducted by Che et al. (2021), where they mentioned that outcome assessors were blinded to treatment assignment) all the participants, and those delivering treatment and the outcomes assessors from the studies were not blinded to treatment assignment [30]. One of the articles [30] mentioned that it was not possible to mask the intervention to those delivering intervention as they will need to explain to the participants how to practice the type of IF being assigned to them. According to the same study, it is also impossible to blind or mask the intervention from the participants because they need to know which group they are assigned, as they will need to adhere to the meal timing for any types of IFs being assigned to them.

For the cohort studies (Table II), there was overall high risk of bias for domain five, which is the strategies used to deal with confounding factors. This is because none of the studies mentioned in their report on how they dealt with the confounding factors. In addition, there was also overall high

Table II: Summary of the main characteristics of the included studies

Author, year	Study design	Study region	Total participants	Types of IF implemented/Intervention used	Study duration	
Tang and Lin (2020)	RCT	Wuhan, China	100 T2DM patients	FMD	4 months	Metformin
Hua et al. (2022)	RCT	Changsha, China	101 overweight and obese adults with prediabetes	ADF and 16/8 TRF	- Intervention lasted for 3 weeks. Data on body weight, body mass index, waist circumference, blood glucose, and lipid profile were collected at baseline, at the end of the intervention, and at the 3-month follow-up.	-
Che et al. (2021)	RCT	Tianjin, China	120 T2DM patients, 104 participants completed the study	10-hour TRF	14 weeks	-
Carter, Clifton, and Keogh (2018)	RCT	Adelaide, Australia	137 T2DM patients, only 97completed the study	Intermittent and continuous energy restriction	12 months	Metformin (n = 43), DPP-4 inhibitors (n = 11), SGLT2 inhibitors (n = 4), GLP-1 agonists (n = 4), sulphonylureas (n = 12) and insulin (n = 14).
Sutton et al. (2018)	RCT	Louisiana, USA	8 men with prediabetes	eTRF	5 weeks	-
Corley et al. (2018)	RCT	Wellington , New Zealand	37 T2DM patients	2 consecutive days of very-low calorie diet (VLCD) compared with 2 non-consecutive days of very-low calorie diet	12 weeks	-
Abdullah et al.(2020)	Cohort study	Sana'a, Yemen	98 Yemeni male (30 T2DM patients, 31 healthy first-degreerelatives, 31 healthy control)	Ramadan IF	A couple of days prior to Ramadan (baseline) and a follow-up visit after 3 weeks of fasting.	Metformin
Li et al. (2017)	Cohort study	Berlin, Germany	32 T2DM patients	Periodic (one week) fasting	16 ± 2 weeks	-
Ait Saada et al.(2010)	Cohort study	West Algeria	66 Muslim women with T2DM	Ramadan IF	A week before Ramadan and at thethird week of Ramadan fasting.	Metformin
Kovil and Shaikh, (2020)	Case series	Mumbai, India	50 T2DM patients	Ramadan IF	Immediately before the starting of fastfollowed by after 45 days.	Insulin (n = 15), sulphonylureas (n = 30) and DPP-4 inhibitors and SGLT2 inhibitors (n = 20)
Tang and Lin (2020)	RCT	Wuhan, China	100 T2DM patients	FMD	4 months	Metformin
Hua et al. (2022)	RCT	Changsha, China	101 overweight and obese adults with prediabetes	ADF and 16/8 TRF	- Intervention lasted for 3 weeks. Data on body weight, body mass index, waist circumference, blood glucose, and lipid profile were collected at baseline, at the end of the intervention, and at the 3-month follow-up.	-

risk of bias for domain ten which examined the utilization of strategies to address incomplete follow-up. Same as domain five, none of the articles of the cohort studies reported this item. This is because, all the studies stated that they have successfully completed the follow-up. The study conducted by [21] and [22] reported that they completed the follow up in the third week of Ramadan fasting while the study by Li et al. (2017) completed the follow-up 16 weeks after starting the intervention [25].

Moving on to case series, an overall high risk of bias for domain four (except the study by Kovil and Shaikh, 2020), five, six, and nine were found for all the studies. For domain four, the overall risk of bias was high because the study by Arnason et al. (2017) did not have consecutive inclusion of participants [10]. This is because the study did not mention the range of time when the participants were recruited as needed by the JBI critical appraisal checklist tool. For domain five, the overall risk of bias was also high as none of the studies reported that they included all the patients with prediabetes or T2DM presented at their clinic or available within the used patients' databases. Furthermore, domain six also had overall high risk of bias because all the articles did not report the demographics of participants in their studies such as their age, sex, education, geographic region, and ethnicity. Next, the overall risk of bias for domain nine was also high as the studies did not explain in depth regarding the study population. The first and second domain were not applicable for the article by Ait Saada et al. (2010) as the study did not have a control group [22]. Finally, the quasi-experimental study has an overall low risk of bias, except for one domain which was domain four. This is because the study did not recruit any participants into a control group and monitor only the effect of TRE on 19 T2DM participants. The assessment of bias within studies is presented in Figure IIa until Figure IId.

Individual outcomes of the studies

The targeted primary outcomes for this review are cardiovascular markers such as SBP, DBP and lipid profiles which include TG, TC, LDL-c, and HDL-c. Meanwhile, the targeted secondary outcomes are BMI or body weight, waist circumference, and glycemic parameters such as HbA1c, FBG, glucose level, insulin level and insulin resistance measured as HOMA-IR. For the primary outcomes, all studies reported the effects of IF on the lipid profiles of the study subjects except for the study by Arnason et al. (2017) and Kovil and Shaikh (2020) [22,28]. From 10 studies reported the lipid profiles of the participants, 3 studies recorded significant improvement ($p < 0.05$) for all the lipid profiles which include TG, TC, LDL-c and HDL-c, after completed the intervention [22, 29, 31]. Che et al. (2021) in their study also reported significant reduction ($p < 0.05$) in all lipid profiles of the intervention group except for HDL-c, where the level was not significantly reduced after the intervention. On the other hand, the study conducted by Li et al. (2017) and Parr et al. (2020) found that there was not significant improvement ($p > 0.05$) in the lipid profiles of the participants in the IF group when compared to the control or baseline level [25,26]. However, the study by Sutton et al. (2018) recorded a significant increase in the level of TC ($p = 0.02$) and TG ($p = 0.0007$) at the end of intervention [20]. They explained that circulating TG might increase due to a longer fasting period (18 hours) prior testing and most likely reflects triglyceride re-esterification following lipolysis, as well as hepatic and intramuscular triglyceride storage. In addition, Carter et al. (2018) reported that HDL-c level of the participants reduced due to weight loss ($p < 0.001$) [23].

Furthermore, 7 out of 12 studies included recorded the effects of IF on SBP and DBP in the recruited participants at the end of interventions. From the seven studies, three studies (Li et al., 2017; Sutton et al., 2018; Tang and Lin, 2020) recorded significant reductions ($p < 0.05$) in both SBP and DBP readings after the intervention, three studies (Abdullah et al., 2020; Arnason et al., 2017; Corley et al., 2018) recorded reductions in both SBP and DBP but not statistically significant when compared to the baseline readings or control groups and one study (Kovil and Shaikh, 2020) recorded no changes happened for both SBP and DBP for the recruited participants with respect to the baseline readings. The targeted primary and secondary outcomes of the eligible studies included are described in detail in Table III [25, 20, 29, 21,10, 27, 28].

DISCUSSION

To the best of our knowledge, this is the first study that systematically review the effects of intermittent fasting on cardiovascular health of prediabetes and T2DM patients. In this study, the important findings are IF helps to reduce both SBP and DBP readings and improve the blood lipid profiles at the end of intervention, which are beneficial for the cardiovascular health for the prediabetes and T2DM patients. This is because prediabetes and T2DM patients have a 2-3 times higher risk of developing CVD as mentioned earlier. One of the possible mechanisms that IF benefits the cardiovascular health of the prediabetes and T2DM patients is by reducing the insulin resistance or blood glucose level. This hypothesis might be true as the pathogenesis of CVD in the prediabetes and T2DM patients revealed that insulin resistance and hyperinsulinemia play important roles in the process. Che et al. (2021) in their study recorded significant reduction in the HOMA-IR, HbA1c and FBG values improved significantly ($p = 0.02$, $p < 0.001$, $p < 0.001$, respectively) and thus all the lipid profiles (except HDL-c, $p = 0.33$) improved significantly (all $p < 0.05$) [30]. This is the same result reported by Ait Saada et al. (2010) and Carter et al. (2018) where they reported significant reduction in all lipid profiles ($p < 0.05$, $p < 0.001$, respectively) with significant reduction ($p < 0.05$) in the HbA1c readings [22,23].

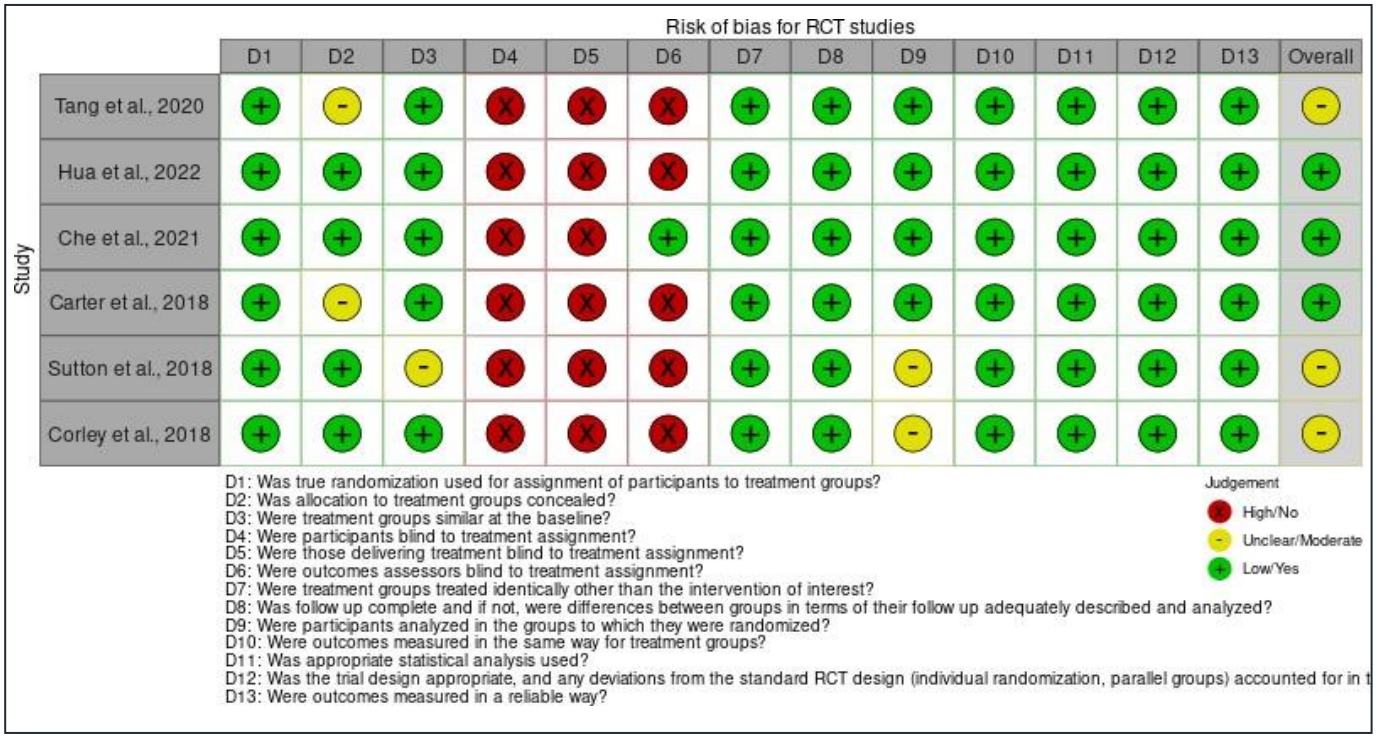

Figure IIa: Risk of bias for RCT studies.

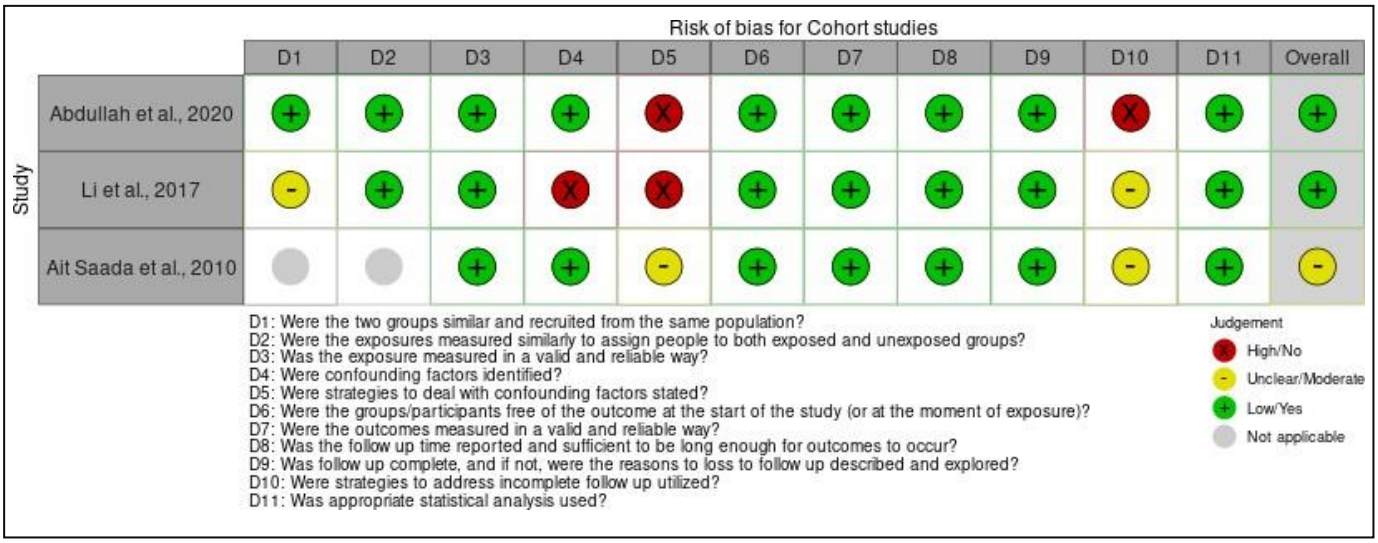

Figure IIb: Risk of bias for cohort studies.

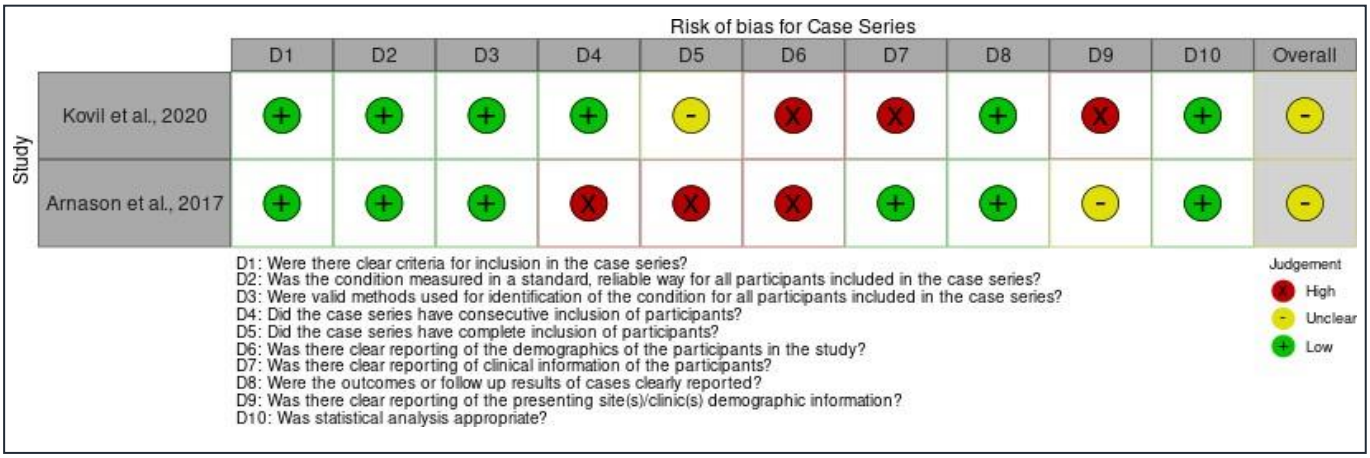

Figure IIc: Risk of bias for case series.

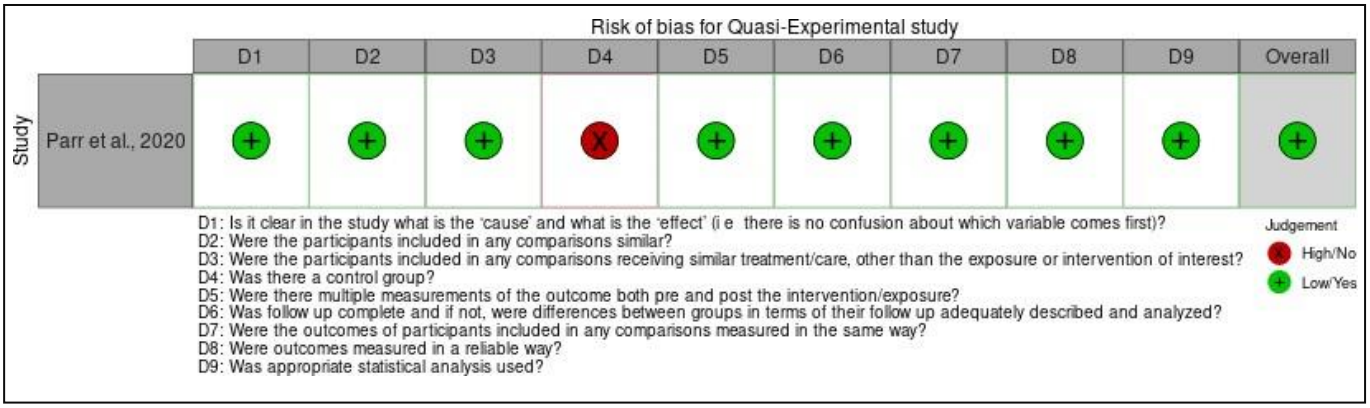

Figure IId: Risk of bias for quasi-experimental study.

Tang and Lin (2020) in their study also found that SBP, DBP and all lipid profiles improved significantly (all $p < 0.05$) with significant reduction in the HbA1c and FBG levels (both $p < 0.05$) [29]. Furthermore, Hua et al. (2022) recorded that all lipid profiles improved significantly (all $p < 0.05$) with significant reduction in the FBG readings ($p < 0.05$) [31]. On the other hand, Corley et al. (2018) reported that SBP, DBP and all lipid profiles (except for a clinically insignificant difference in LDL-c level) were not significantly improved (all $p > 0.05$) as the FBG and HbA1c readings were not significantly reduced ($p > 0.05$) [27]. Based on these results, the SBP, DBP and all the lipid profiles will improve with significant reduction in the blood glucose or insulin resistance. This fact can be supported from a cross-sectional study conducted by Wang et al. (2022) where 1747 T2DM patients were recruited, and study reported that the blood glucose levels were positively associated with TG ($p < 0.01$) and LDL ($p < 0.01$) levels and elevated TG/HDL-c ($p < 0.01$) and LDL-c/HDL-c ($p < 0.01$) ratios. They also reported that the TG levels were increased by 0.34 mmol/L with every 1 mmol/L increase in blood glucose levels.

However, the study by Sutton et al. (2018) found that the fasting TG and TC levels increased significantly ($p = 0.0007$, $p = 0.02$), even the insulin resistance and decreased significantly ($p = 0.005$) at the end of eTRF intervention [20]. This might have happened due to long duration of fasting time (18 hours) prior testing and this most probably reflects triglyceride re-esterification following lipolysis occurring in hepatic and intramuscular triglyceride storage [33]. Nevertheless, they found that the SBP and DBP significantly reduced (both $p = 0.03$) at the end of intervention, probably due to reduction in the insulin resistance and insulin level. One other possibility is that eTRF enhances natriuresis by shifting salt intake relatively early in the day, when the circadian system increases sodium excretion [35]. Moreover, their study found that there was no significant reduction in FBG levels ($p = 0.49$) even though the fasting insulin levels and insulin sensitivity significantly improved (both $p= 0.05$). This is in line with several other studies that reported IF may be more effective improving the insulin sensitivity than reducing the blood glucose levels [36-38]. This is also proven with the study conducted by Ait Saada et al. (2010) where they reported that the FBG values were significantly increased ($p < 0.05$) even the HbA1c levels significantly reduced ($p < 0.05$) at the third week of Ramadan fasting [22]. They suggest that the increase in FBG levels might be contributed by increased gluconeogenesis at that time to compensate for the fall in the glycogenolysis.

Next, Li et al. (2017) in their studies found that periodic fasting causes reduction in the HOMA-IR, insulin and FBG levels but

were not significant (p = 0.08, p = 0.42, p = 0.15, respectively), but the SBP and DBP significantly reduced (p = 0.04, p = 0.02, respectively) [25]. The lipid profiles on the other hand have not significantly improved. The results might be an indicator that even if the insulin resistance is not significantly reduced, it is enough to cause significant reduction in the blood pressure readings. Contrarily, the study conducted by Abdullah et al. (2020) found that the HOMA-IR increased significantly (p = 1.3 x 10−7) during the follow- up in the third week of Ramadan fasting, while the FBG did not significantly reduce (p = 0.651) [21]. As a result, they reported that the DBP increased slightly and the SBP and all lipid profiles were improved, but not statistically significant (all p > 0.05). They stated that the HOMA-IR was increased significantly due sleep deprivation during Ramadan fasting as it leads to elevation of proinflammatory cytokines like IL-6 and is a stressor leading to hypercortisolism. In addition, they mentioned that the leptin concentration was also increased during Ramadan fasting as the feeding patterns were shifted to nighttime where total calorie intake was higher at night, resulting in increased leptin concentration after meal at night. Consequently, both sleep disturbance and changes in feeding pattern during Ramadan fasting lead to decreased adiponectin concentration and thus promote insulin resistance [8]. This fact can be supported by a study conducted by Spiegel et al. (2005) which stated that recurrent sleep loss or short sleep led to an increased risk of developing insulin resistance as sleep has significant modulatory effects on glucose metabolism [39].

From this study, it is not possible to determine which type of IF is the best or most beneficial for the cardiovascular health of prediabetes and T2DM patients as the study included were only investigating a type of IF for their study and with different study periods. However, there are studies included comparing the effects between ADF and 16/8 TRF [24], intermittent and continuous energy restriction [23] and 2 consecutive days of very-low calorie diet (VLCD) compared with 2 non-consecutive days of very-low calorie diet [27]. The study conducted by Hua et al. (2022) found that there were no significant differences in FBG and LDL-c reduction between ADF and 16/8 TRF at the end of intervention. Moreover, ADF showed more significant improvement in HDL-c levels compared to the 16/8 TRF. On the other hand, 16/8 TRF was better in reducing the triglyceride level when compared to the ADF. Next, the study conducted by Carter et al. (2018) found that there were no significant differences between continuous and intermittent energy restriction in terms of HbA1c and FBG reduction, and improvement in all of the lipid profiles [23]. Moreover, Corley et al. (2018) reported that there were no significant differences between two consecutive and non-consecutive days of VLCD in HbA1c, FBG, HDL-c, and TG level while significant differences between the two interventions were seen for TC and LDL-c level at the end of the study [27]. However, since there are a limited number of studies comparing different types of IF, it is difficult to compare these results with other studies.

In this systematic review also, the included studies did not investigate whether the duration of study plays an important role for determining the benefits for cardiovascular health for both prediabetes and T2DM patients. Nonetheless, there is an interesting finding by Sutton et al. (2018) from their study where they found that 5 weeks of eTRF reduced the blood pressure significantly, as effective as antihypertensive medications such as angiotensin-converting enzyme (ACE) inhibitors [20]. However, we believe that a continuous intermittent fasting scheme should be practiced or followed to achieve more pronounced and significant effects for the cardiovascular health of prediabetes and T2DM patients. This fact can be proven by the study conducted by Arnason, Bowen, and Mansell (2017) where they found that the beneficial effects of intermittent fasting such as reduction in the blood pressure readings were not sustained after the IF was completed. These readings inflected back towards the baseline values once participants resumed to the normal diet [10]. This might be due to long periods of fasting that induce a strong physiological stimulus and pronounced hormonal changes such as stimulation of the hypothalamus-pituitary (HPA) axis, which translated into a hermetic stress reaction [25]. It is also important to consider the usage of OHA in the studies included as 8 of the included studies recruited participants who were on OHA. This is because certain groups of OHA which include biguanide (metformin), SGLT2-I, and GLP1-RA, have cardiovascular benefits for the T2DM patients (Clinical Practice Guideline, Management of T2DM, 2020). Thus, this factor must be taken into consideration to rule out the possibility of OHA effects instead of the IF itself.

In the CPG Management of T2DM (2020), it is stated that one of the recommended non- pharmacological interventions in preventing and managing prediabetes and T2DM is by reducing the calorie intake. IF fasting is one of the common approaches that is being practiced reducing the calorie intake [40]. However, it is also stated in that CPG that the effectiveness of IF in diabetes management is uncertain and not significantly different from conventional calorie-restricted diets due to limited evidence and small-scale studies. Nevertheless, based on the findings in this study, it is proven that IF can improve the cardiovascular health of prediabetes and T2DM patients as it can significantly lower the blood pressure and improve the lipid profiles at the end of intervention with respect to the baseline readings or control groups.

However, this study possesses some possible limitations. Firstly, this study included cohort studies, case series and a quasi-experimental study which recruited a relatively small number of participants. Next, there was only 3 included studies [23,24,27] that compared between 2 different types of IF. Thus, there is insufficient data to determine which type of IF is the most beneficial for cardiovascular health of the prediabetes and T2DM patients. In addition, this study also included only 2 studies [20, 24] that recruited prediabetes participants as their study subjects. Hence, studies that recruited larger sample sizes

of prediabetes participants are needed to confirm the benefits of IF on their cardiovascular health. Based on all the studies included most of them reported cardiovascular benefits of IF as their secondary outcomes except for the studies conducted by Ait Saada et al. (2010), Arnason et al. (2017) and Kovil and Shaikh (2020) [22, 10, 28]. However, these 3 studies were only observational. Thus, RCTs investigating the effect of intermittent fasting on cardiovascular health of prediabetes and T2DM patients which makes the cardiovascular health as the primary outcome are warranted to confirm the benefits stated and discussed in this study. Once the benefits are confirmed based on the studies conducted, IF can be integrated into practice as one of the non-pharmacological interventions in preventing and managing prediabetes, T2DM and their associated complications. Besides, IF can be practiced by both patients that had already received their pharmacological treatment or not as part of disease management. In addition, future research can be done to compare the benefits of different types of IF for cardiovascular health and which type is the most practical and suitable to be practiced by prediabetes and T2DM patients. This is because there were only 3 studies included [23,24,27] in this systematic review that compared different types of IF.

CONCLUSIONS

Based on all the data obtained from the studies, most of the study reported that IF such as TRF, ADF and eTRF improved the blood pressure readings and lipid levels for the participants intervened with the IF regimen as compared to the baseline levels/readings or the control group. These cardiovascular benefits might be achieved due to reduction in the insulin resistance and the blood glucose level at the end of IF intervention. By improving the blood pressure readings and lipid profiles of prediabetes and T2DM patients, the risk of developing cardiovascular diseases such as hypertension and ischemic heart disease in the future can be reduced for prediabetes and T2DM patients. IF has a high potential to be used as one of the non-pharmacological interventions in managing and preventing prediabetes and T2DM and their associated complications. This systematic review reported only the effects of IF on cardiovascular health as the secondary outcomes after glycemic control, weight, BMI, and waist circumference, thus RCTs that focus on the benefits of IF on cardiovascular health of prediabetes and T2DM patients as their primary outcomes are warranted to confirm the findings in this study.

CONFLICT OF INTEREST

The authors do not have any financial interests or commercial associations to disclose. All authors have viewed and agreed to the submission of this article.

ACKNOWLEDGEMENTS

Not available.

Table III: Targeted primary and secondary outcomes of the included studies

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Tang and Lin (2020)	• **SBP:** Decreased 14.18 ± 0.71 mmHg and 6.14 ± 0.71 mmHg for the IF and control groups, respectively, with $p < 0.05$, at the end of intervention. • **DBP:** Decreased 10.98 ± 0.84 mmHg and 5.42 ± 0.55 mmHg for thefor the IF and control groups, respectively, with $p < 0.05$, after 4 months. • **TC:** Decreased 2.78 ± 0.09 mmol/L in IF group and 0.58 ± 0.02 mmol/L in control group. • **TG:** Decreased 1.75 ± 0.06 mmol/L in IF group and 0.42 ± 0.06 mmol/L in control group. • **HDL-c:** Increased 1.05 ± 0.1 mmol/L in IF group and 0.21 ± 0.02 mmol/L in control group. • **LDL-c:** Decreased 1.96 ± 0.24 mmol/L in IF group and 0.58 ± 0.04 mmol/L in control group. • All lipid profiles showing significant statistical intergroup differences (all $p < 0.05$)	• **HbA1c:** Decreased by 1.36 % in the IF group and by 0.43% in thecontrol group. • **FPG:** Decreased 2.31 ± 0.33 mmol/L in IF group and 1.3 ± 0.15mmol/L in control group. • All glycemic parameters showed significant statistical intergroupdifferences ($p < 0.05$) • **BMI:** Decreased 5.11 ± 0.18 kg/m^2, and 1.13 ± 0.1 kg/m^2 for the IFand control groups, respectively. • **Waist circumference:** Decreased 14.28 ± 0.94cm and 5.94 ± 0.57cm for the IF and control groups, respectively. • Both BMI and waist circumference showing significant statistical intergroup differences (all $p < 0.05$).
Hua et al. (2022)	• **TC:** ADF group showed more significant reduction in TC at the end of intervention than the control ($p = 0.032$) and 16/8 TRF group ($p = 0.029$) • **TG:** 16/8 TRF showed significant reduction of TG compared to the control group at the end of intervention ($p = 0.015$) and at 3 months follow-up ($p < 0.001$). In contrast, ADF showed significant increase in TG compared to the 16/8 TRF compared to the control group at the end of intervention ($p = 0.032$) and at 3 months follow-up ($p < 0.041$). • **HDL-c:** ADF group showed more significant improvement in HDL- c in all periods (all $p < 0.05$) while 16/8 TRF only showed significant improvement at 3 months follow-up ($p = 0.004$) • **LDL-c:** 16/8 TRF group recorded a significant decrease in LDL-c at 3 months follow-up ($p = 0.04$) while no significant differences in LDL-c reduction between ADF and 16/8 TRF.	• **FBG:** ADF group showed significant reduction in FBG at the end of intervention ($p = 0.027$) and at 3 months follow-up ($p = 0.02$) while 16/8 TRF group only showed significant reduction at 3 months follow-up ($p < 0.001$). No significant differences in FBG reduction between the two intervention groups. • **BMI:** Both intervention groups showed significant reduction compared to the control group (all $p < 0.01$) across the study period. ADF group showed more significant reduction compared to the 16/8 TRF group both at the end of intervention and at 3 months follow-up (all $p < 0.01$) • **Waist circumference:** Both intervention groups showed significant reduction compared to the control group (all $p < 0.01$) across the studyperiod. TRF group showed significant reduction at the end of intervention ($p < 0.001$) a and at 3 months follow-up ($p < 0.001$)compared to baseline readings.

Table III: Targeted primary and secondary outcomes of the included studies (continued)

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Che et al. (2021)	• **TG:** TRF group resulted in significant reduction of TG compared to the control group (− 0.23 ± 0.08 mmol/L, [9%] vs.0.13 ± 0.06 mmol/L, [5%]; $p = 0.03$) over 12 weeks. • **TC:** TRF group resulted in significant reduction of TC compared to the control group (− 0.32 ± 0.07 mmol/L [6%] vs. − 0.15 ± 0.06 mmol/L [3%], $p = 0.01$) over 12 weeks. • **LDL-c:** TRF group resulted in significant reduction of TC compared to the control group (− 0.42 ± 0.13 mmol/L [11%] vs. − 0.21 ± 0.13 mmol/L [6%], $p = 0.02$) over 12 weeks. • **HDL-c:** TRF group did not show any significant differences inHDL-c level compared to control group (− 0.16 ± 0.04 mmol/L vs. − 0.15 ± 0.05 mmol/L, $p = 0.33$) over 12 weeks.	• **HbAIc:** TRF group showed significant decrease (− 1.54% ± 0.19) compared to control group ($p < 0.001$) over 12 weeks. • **FBG:** Decreased significantly in TRF group compared to control group (− 1.47 ± 0.25 mmol/L [15%] vs. − 0.78 ± 0.21 mmol/L [8%], $p < 0.001$) over 12 weeks. • **HOMA-IR:** Decreased significantly in TRF group compared to control group (− 0.51 ± 0.08 [14%] vs. − 0.12 ± 0.06 [3%], $p = 0.02$) over 12 weeks. • **BMI:** TRF group showed significant decrease compared to control group (− 1.64 ± 0.38 kg/m^2 [6%] vs. 0.42 ±0.24 kg/m^2 [2%], $p < 0.001$) over 12 weeks.
Carter, Clifton, and Keogh, (2018)	• **TG:** The mean (SEM) TG level was reduced by 0.4144 (0.22) mmol/L with weight loss ($p <$ 0.001), with no differences between groups. • **TC:** The mean (SEM) TC level was reduced by 0.3367 (0.10) mmol/L with weight loss ($p <$ 0.001), with no differences between groups. • **LDL-c:** The mean (SEM) LDL-c level was reduced by 0.288 (0.085)mmol/L with weight loss ($p < 0.001$), with no differences between groups. • **HDL-c:** The mean (SEM) HDL-c level was reduced by 0.0363 (0.039) mmol/L with weight loss ($p < 0.001$), with no differences between groups.	• **HbA1c:** The mean (SEM) HbA1c level was reduced significantly, with no difference between treatment groups (– 0.5% [0.2%] in the continuous energy restriction group vs – 0.3% [0.1%] in the intermittent energy restriction group; P = 0.65). • **Body weight:** Weight reduction was significant over time but not by treatment (–5.0 [0.8] kg in the continuous energy restriction group vs –6.8 [0.8] kg in the intermittent energy restriction group; P = 0.25).

Table III: Targeted primary and secondary outcomes of the included studies (continued)

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Sutton et al. (2018)	• **LDL-c:** eTRF did not affect LDL-c level after 5 weeks of intervention (Δ = 2 ± 6 mg/dL; p = 0.75). • **HDL-c:** eTRF did not affect HDL-c level after 5 weeks of intervention (Δ = −0.6 ± 0.9 mg/dL; p = 0.48). • **TG:** eTRF group have a significant increase (p = 0.0007) in the level of TG by 57 ± 13 mg/dL after 5 weeks of intervention. • **TC:** eTRF group have significant increase (p = 0.02) in the level of TC by 13 ± 5 mg/dL after 5 weeks of intervention. • **SBP:** Morning levels of SBP reduced by 11 ± 4 mm Hg (p = 0.03) in eTRF group compared to the control group after 5 weeks of intervention. • **DBP:** Morning levels of SBP reduced by 10 ± 4 mm Hg (p = 0.03) in eTRF group compared to the control group after 5 weeks of intervention.	• **FBG:** 5 weeks eTRF did not show significant reduction in FBG (Δ = −2 ± 2 mg/dL; p = 0.49) • **Fasting insulin:** 5 weeks eTRF decreased fasting insulin by 3.4 ± 1.6mU/L (p = 0.05). • **Insulinogenic index (a marker of β cell responsiveness):** 5 weeks of eTRF increased the insulinogenic index by 14 ± 7 U/mg (p = 0.05). • **Insulin resistance:** Decreased by 36 ± 10 U/mg (p = 0.005), bymeasured by the 3-hr incremental area under the curve (AUC) ratio.
Corley et al. (2018)	• **SBP:** There was reduction in SBP readings from baseline in both non-consecutive (133 ± 15 mmHg vs. 129 ± 12 mmHg) and consecutive VLCD groups (132 ± 12 mmHg vs. 129 ± 16 mmHg) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.77). • **DBP:** There was reduction in DBP readings from baseline in both non-consecutive (78 ± 11 mmHg vs.75 ± 10 mmHg) and consecutiveVLCD groups (74 ± 11 mmHg vs. 72 ± 10 mmHg) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.80). • **TC:** There was reduction in TC levels from baseline in the non- consecutive (4.2 ± 1.0 mmol/L vs. 3.8 ± 0.8 mmol/L) but slightly increased in the consecutive VLCD groups (3.9 ± 0.8 mmol/L vs. 4.0 ± 1.0 mmol/L) at 12 weeks of intervention. TC showing significant statistical intergroup differences (p = 0.01). • **HDL-c**: There was no effect on HDL-c levels from baseline in the non-consecutive (1.1 ± 0.2 mmol/L vs. 1.1 ± 0.2 mmol/L) but slightly increased in the consecutive VLCD groups (1. ± 0.2 mmol/L vs. 1.1 ± 0.2 mmol/L) at 12 weeks of intervention. HDL-c shows no significant statistical intergroup differences (p = 0.37). • **LDL-c**: There was reduction in LDL-c levels from baseline in the non-consecutive (2.1 ± 0.8 mmol/L vs. 2.0 ± 0.7 mmol/L) but slightly increased in the consecutive VLCD groups (2.1 ± 0.8 mmol/L vs. 2.25 ± 0.81 mmol/L) at 12 weeks of intervention. TC showing significant statistical intergroup differences (p = 0.03). • **TG**: There was reduction in TG levels from baseline in both non- consecutive (1.8 ± 0.6 mmol/L vs.1.7 ± 0.4 mmol/L) and consecutive VLCD groups (1.8 ± 0.7 mmol/L vs. 1.7 ± 0.8 mmol/L) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.80).	• **BMI:** There was reduction in BMI from baseline in both non- consecutive (36.8 ± 5.2 kg/m^2 vs. 36.0 ± 5.2 kg/m^2) and consecutive VLCD groups (36.6 ± 5.3 kg/m^2 vs. 36.1 ± 5.5 kg/m^2) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.96). • **Waist circumference:** There was reduction in waist circumference from baseline in both non-consecutive (122.5 ± 13.6 cm vs. 119.1 ± 14.2 cm) and consecutive VLCD groups (120.4 ± 17.0 cm vs. 118.8 ± 16.6 cm) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.99). • **HbA1c:** There was reduction in HbA1c levels from baseline in both non-consecutive (8.2 ± 1.3% vs. 7.5 ± 1.5%) and consecutive VLCD groups (8.4 ± 1.8% vs. 7.8 ± 1.8%) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.53). • **FBG:** There was reduction in FBG readings from baseline in both non-consecutive (9.0 ± 2.1 mmol/L vs. 7.9 ± 1.7 mmol/L) and consecutive VLCD groups (8.2 ± 2.8 mmol/L vs. 6.9 ± 2.1 mmol/L) at 12 weeks of intervention, but there are no significant statistical intergroup differences (p = 0.21).

Table III: Targeted primary and secondary outcomes of the included studies (continued)

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Abdullah etal. (2020)	• **SBP:** Decreased slightly at the follow up but not statistically significant (p = 0.811) with respect to baseline level prior to Ramadan fasting (124.30 ± 4.11 mmHg vs. 124.00 ± 6.16 mmHg). • **DBP:** Increased slightly at the follow up but not statistically significant (p = 0.09) with respect to baseline level prior to Ramadanfasting (83.30 ± 2.49 mmHg vs. 84.30 ± 2.59 mmHg). • **TG:** Decreased but not significant (p = 0.06) in T2DM group by 6%with respect to the baseline prior to Ramadan fasting (124.64 ± 50.75 mg/dL). • **TC:** Decreased but not significant (p = 0.08) in T2DM group by 5.1% with respect to the baseline prior to Ramadan fasting (178.38 ± 29.57 mg/dL). • **LDL-c:** Decreased slightly at the follow up but not statistically significant (p = 0.395) with respect to baseline level prior to Ramadan fasting (38.30 ± 9.55 mg/dL vs. 37.34 ± 7.11 mg/dL). • **HDL-c:** Decreased slightly at the follow up but not statistically significant (p = 0.371) with respect to baseline level prior to Ramadan fasting (111.15 ± 28.12 mg/dL vs. 106.03 ± 30.15 mg/dL).	• **BMI:** Decreased slightly at the follow up but not statistically significant (p = 0.293) with respect to baseline level prior to Ramadan fasting (25.55 ± 4.49 kg/m^2 vs. 25.51 ± 4.38 kg/m^2). • **Waist circumference:** Significantly (p = 0.024) decreased in T2DM group with respect to the baseline prior to Ramadan fasting (99.83 ± 14.20 kg/m^2 vs. 99.61 ± 14.27 kg/m^2). • **FBG:** Decreased at the follow up but not statistically significant (p = 0.651) with respect to baseline level prior to Ramadan fasting (7.63 ± 2.23 mmol/L vs. 7.47 ± 1.80 mmol/L). • **HbA1c:** Decreased significantly (p = 1 x 10^{-6}) in T2DM group with respect to the baseline prior to Ramadan fasting (8.26 ± 1.7% vs. 7.40 ± 1.49%). • **Insulin:** Increased significantly (p = 1.4 x 10^{-9}) in T2DM group with respect to the baseline prior to Ramadan fasting (226.95 ± 75.05 pmol/L vs. 263.90 ± 86.25 pmol/L). • **HOMA-IR:** Increased significantly (p = 1.3 x 10^{-7}) in T2DM group with respect to the baseline prior to Ramadan fasting (4.43 ± 1.55 vs. 5.11 ± 1.74).
Parr et al.(2020)	• **TC:** Decreased after 4 weeks of TRE (4.5 ± 0.8 mmol/L) compared to the 2 weeks habitual period (4.6 ± 0.9 mmol/L), but not statistically significant (p = 0.16). • **HDL-c:** Did not change after 4 weeks of TRE (1.1 ± 0.3 mmol/L) compared to the 2 weeks habitual period (1.1 ± 0.3 mmol/L) with p = 0.16. • **LDL-c:** Decreased after 4 weeks of TRE (2.5 ± 0.8 mmol/L) compared to the 2 weeks habitual period (2.6 ± 0.9 mmol/L), but notstatistically significant (p = 0.22). • **TG:** Did not change after 4 weeks of TRE (1.8 ± 0.8 mmol/L) compared to the 2 weeks habitual period (1.8 ± 0.7 mmol/L) with p= 0.78.	• **HbA1c:** Decreased but not significant (p = 0.053) after 4 weeks of TRE (7.4 ± 1.0%) compared to the 2 weeks habitual period (7.6 ± 1.1%). • **FBG:** No differences were measured in FBG after 4 weeks of TRE compared to the 2 weeks habitual period (p = 0.29). • **Insulin:** No differences were measured in insulin after 4 weeks of TRE compared to the 2 weeks habitual period (p = 0.09). •

Table III: Targeted primary and secondary outcomes of the included studies (continued)

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Li et al.(2017)	• **SBP:** Significantly reduced (p = 0.04) in fasting group after the intervention with respect to the baseline level, with the mean change of −13.9 ± 15.3 mmHg. There were significant statistical (p = 0.01) intergroup differences between the fasting and control groups (mean change = 0.4 ± 15.8 mmHg; p = 0.25). • **DBP:** Significantly reduced (p = 0.02) in fasting group after the intervention with respect to the baseline level, with the mean change of −9.0 ± 12.3 mmHg. There were significant statistical (p = 0.003) intergroup differences between the fasting and control groups (mean change = 3.2 ± 11.9 mmHg; p = 0.17). • **TG:** Reduced after the intervention with respect to the baseline levelbut not statistically significant (p = 0.2), with the mean change of −26.6 ± 88.5 mg/dL. There were no significant statistical (p = 0.5) intergroup differences between the fasting and control groups (mean change = −2.5 ± 81.9 mg/dL; p = 0.9). • **HDL-c:** Increased after the intervention with respect to the baseline level but not statistically significant (p = 0.2), with the mean change of 6.5 ± 23.3 mg/dL. There were no significant statistical (p = 0.14) intergroup differences between the fasting and control groups (mean change = −2.3 ± 6.9 mg/dL; p = 0.2). • **LDL-c:** Decreased after the intervention with respect to the baselinelevel but not statistically significant (p = 0.9), with the mean change of −2.6 ± 26.9 mg/dL. There were no significant statistical (p = 0.2) intergroup differences between the fasting and control groups (mean change = −7.8 ± 17.3 mg/dL; p = 0.41). • **TC:** Decreased after the intervention with respect to the baseline level but not statistically significant (p = 0.5), with the mean change of 0.5 ± 27.1 mg/dL. There were no significant statistical (p = 0.14) intergroup differences between the fasting and control groups (mean change = −15.5 ± 27.4mg/dL; p = 0.84).	• **BMI:** Reduced after the intervention with respect to the baseline level but not statistically significant (p = 0.16), with the mean change of 1.2 ± 1.7 kg/m^2. There were significant statistical (p = 0.03) intergroup differences between the fasting and control groups (mean change = − − 0.6 ± 2.6 kg/m^2; p = 0.08). • **Waist circumference:** Significantly reduced (p = 0.001) in fasting group after the intervention with respect to the baseline level, with the mean change of −9.0 ± 12.3 cm. There were significant statistical (p = 0.001) intergroup differences between the fasting and control groups (mean change = − 0.3 ± 2.0 cm; p = 0.72). • **FBG:** Reduced after the intervention with respect to the baseline levelbut not statistically significant (p = 0.15), with the mean change of −10.6 ± 30.4 mg/dL. There were significant statistical (p = 0.03)intergroup differences between the fasting and control groups (mean change = − 38.4 ± 46.0 md/dL; p = 0.02). • **Insulin:** Reduced after the intervention with respect to the baseline level but not statistically significant (p = 0.42), with the mean change of −3.5 ± 9.3 μU/ml. There were no significant statistical (p = 0.09) intergroup differences between the fasting and control groups (mean change = −0.2 ± 5.4 μU/ml; p = 0.05). • **HOMA-IR:** Reduced (p = 0.08) in fasting group after the intervention with respect to the baseline level, with the mean changeof −1.5 ± 4.6. There were no differences (p = 1.0) between the fasting and control groups (mean change = −1.5 ± 2.1; p = 0.06).

Table III: Targeted primary and secondary outcomes of the included studies (continued)

Author (year)	Targeted primary outcomes	Targeted secondary outcomes
Ait Saada et al. (2010)	• **TC:** Levels during the fasting period (1.836 ± 0.473 g/L) weresignificantly lower ($p < 0.05$) than the non-fasting period (2.101 ±0.473 g/L) • **TG:** Levels during the fasting period (1.489 ± 0.213 g/L) weresignificantly lower ($p < 0.05$) than the non-fasting period (1.769 ± 0.304 g/L) • **LDL-c:** Levels during the fasting period (0.906 ± 0.374 g/L) weresignificantly lower ($p < 0.05$) than the non-fasting period (1.219 ± 0.527 g/L) • **HDL-c:** Levels during the fasting period (0.671 ± 0.285 g/L) weresignificantly higher ($p < 0.05$) than the non-fasting period (0.557 ± 0.165 g/L)	• **BMI:** Reduced at the week of Ramadan (27.40 ± 03.55 kg/m^2) compared to the baseline, a week before fasting (27.57 ± 03.08 kg/m^2), but not statistically significant ($p = 0.36$). • **FBG:** The average FBG levels recorded were significantly ($p < 0.05$) higher 1.98 ± 0.17 g/L during fasting when compared to the baseline level, a week before fasting (1.85 ± 0.20 g/L). • **HbA1c:** Levels of HbA1c reduced slightly during the fasting period at $p < 0.05$, reduced from 9.52% to 8.74%. • **Insulin:** The average level of plasma insulin in the participants duringfasting (11.97 ± 2.48 µU/ml) were significantly reduced ($p < 0.05$) from the baseline levels (14.62 ± 2.79 µU/ml).
Kovil and Shaikh, (2020)	• **SBP** and **DBP** of the participants did not change during the Ramadan fasting (121/80 mmHg) when compared with the baseline value (121/80 mmHg).	• **BMI:** Decreased significantly ($p < 0.001$) in the patients during follow up session (27.45 ± 5.06 kg/m^2) when compared to baseline value (27.90 ± 5.11 kg/m^2). • **HbA1c:** Slightly decreased but statistically significant ($p < 0.05$) inthe patients during follow up session (7.62 ± 0.99 mmol/mol) when compared to baseline value (7.90 ± 1.24 mmol/mol).
Arnason, Bowen, and Mansell, (2017)	• **SBP:** Decreased after 2 weeks of IF (127.0 ± 21.4 mmHg) comparedto the baseline level (130.0 ± 17.8 mmHg), with the mean change of −3 mmHg, but not statistically significant ($p = 0.83$). However, the reading was increased by 1.5 mmHg (128.5 ± 14.3 mmHg; $p = 1.0$) after 2 weeks of ending the intervention when participants returned to normal diet intake. • **DBP:** Decreased after 2 weeks of IF (79.8 ± 15.7 mmHg) compared to the baseline level (80.5 ± 13.2 mmHg), with the mean change of −0.72 mmHg, but not statistically significant (p = 1.0). However, the reading was increased by 1.9 mmHg (81.7 ± 12.2 mmHg; p = 0.76) after 2 weeks of ending the intervention when participants returned to normal diet intake.	• **BMI:** Decreased significantly ($p = 0.01$) after 2 weeks of IF (36.4 ± 8.1 kg/m^2) compared to the baseline level (36.9 ± 8.3 kg/m^2), with themean change of −0.52 kg/m^2. However, the reading was increased by 0.1kg/m^2 (36.5 ± 8.1 kg/m^2; $p = 1.0$) after 2 weeks of ending theintervention when participants returned to normal diet intake. • **Waist circumference:** Decreased after 2 weeks of IF (107.8 ± 11.1 cm) compared to the baseline level (109.6 ± 11.1 cm), with the mean change of −1.75 cm, but not statistically significant (p = 0.086). The reading did not change at the end of study, after 4 weeks of completing the intervention (p = 1.0). • **HOMA-IR**: There were no differences in the HOMA-IR value for all phases of the study (all p = 1.0).

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Original Research Article

Deferoxamine injection practice assessment and common administration errors among adult thalassaemia patients

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ABSTRACT

Background: Thalassaemia is a hereditary blood disorder characterised by defective globin chains, which results in haemolysis and impaired erythropoiesis. Routine blood transfusion is required, which may lead to iron overload in a long run. Deferoxamine (DFO) provides effective iron chelation in thalassaemia patients, however, challenges were faced among patients, especially in ensuring proper drug administration techniques. **Objective:** The study aim is to describe the current DFO administration practice, common administration errors, patient compliance towards iron chelation therapy and to identify factors associated with serum ferritin levels. **Methods:** A cross-sectional study among adult thalassaemia patients was done from January 2019 until November 2020. Patients were assessed for DFO injection practices and compliances. A DFO score was used to assess steps of dilution and administration of DFO, categorised as good (score 75-100%), satisfactory (score 60-74%), and fail (score <60%). Compliance was assessed using a validated 7-questions survey; a score of less than 27 marks dictated non-compliance. A multivariate linear regression was used to identify factors associated with high serum ferritin level (>1000 µg/L). **Results:** Twenty-four patients were included in the study. For administration practice, 79.2% were able to administer independently. Abdomen was the most favoured injection site and most patients administered DFO at night. Although 75% of the patients had a good DFO score, DFO administration errors persist. The compliance rate was low (29.2%), and majority reported more than 4 times per month of missed doses. Working status (4664.13, 95% CI 1609.41-7718.86, p=0.005) and frequent missed dose (1152.39, 95% CI 382.18-1922.6, p=0.005) were identified as factors associated with serum ferritin level, controlling for other factors. **Conclusion:** DFO administration errors persist among thalassaemia patients. Patients who are working and non-compliant were identified to be associated with high serum ferritin level. Effective counselling method may be needed to properly educate patients on DFO administration and improve patients' compliance.

INTRODUCTION

Thalassaemia is a hereditary blood disorder characterised by defective synthesis of globin chains, namely α and β globin chains which function as oxygen carrier in the haemoglobin [1]. The absence of haemoglobin subunit α or β synthesis will cause imbalance of the globin chains which results in haemolysis and impaired erythropoiesis [2]. Approximately 240 million people worldwide who are detected as β-thalassaemia heterozygous and approximately 200,000 people are annually born as homozygotes [3]. The highest prevalence of β-thalassaemia is reported in Cyprus, Sardinia, and Southeast Asia. It is also common in populations of Middle East, Central Asia, African heritage and Indian Subcontinent [4]. Lately, thalassaemia is also common in Europe, South America, North Central, and Australia due to worldwide migration [5]. Thalassaemia patients often req uire routine blood transfusion as part of mainstaytherapy in managing thalassaemia. Frequent

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transfusion subsequently may lead to iron overload due to gradual iron accumulation over the time, since the naturally occurring physiological mechanism of iron removal is impaired. Therefore, life-long treatment of iron chelation therapy is necessary in thalassaemia patient to prevent mortality and morbidity associated with iron accumulation in the body [6].

Iron overload causes devastating effects towards organ functions. Iron accumulation can be toxic to most of human tissues in the body and may cause complications such as cardiomyopathy, liver cirrhosis, stunted growth and endocrine abnormalities [7]. One of the means to monitor iron status is by measuring serum ferritin. Ferritin is a protein substance that encapsulates the iron stores in the body. However, serum ferritin is a non-specific marker, which can be elevated in various clinical conditions such as malignancy, iron overload, arthritis, and hemophagocytic lymphohistiocytosis [8]. In the absence of inflammation, the ferritin concentration is positively correlated with total iron stores [9].

Serum ferritin is relatively inexpensive and widely available in healthcare facilities, which makes it a preferred marker among clinicians to monitor iron stores. Several studies had demonstrated an association between serum ferritin level and clinical outcome. A study found that high serum ferritin was a significant predictor for cardiac mortality [10]. Another study found that serum ferritin of ≤1000 ng/mL was significantly associated with lower incidence of heart failure and prolonged survival [11]. A Cochrane review was reported on the accuracy of ferritin concentrations towards detection of iron deficiency and iron overload. The study concluded that blood ferritin concentration was reasonably sensitive in detecting iron deficiency, however for iron overload, it is very low certainty that high serum ferritin was sensitive for iron overload [12]. Nevertheless, serum ferritin is still relevant in the current practice, as suggested by recent study which mentioned that serum ferritin is a good predictor for hepatic iron overload in TDT [13].

Deferoxamine (DFO) is the oldest standard iron chelation therapy in managing iron overload. It is administered through subcutaneous or intravenous infusion, typically 8–12 hours per day for 5–7 days per week [14]. This medication works by protecting the cell from iron mediated toxicity in two ways which are through removal of excess iron as well as neutralization of free iron in the body. Long term chelation by DFO has proven to improve functional complication due to iron accumulation such as liver fibrosis, arrhythmias and abnormalities detected by echocardiogram. However, it might not be effective to reverse the complications due to extensive tissue damage, like frank diabetes, hypothyroidism and myocardial sclerosis [15]. Introduction of DFO has provide a longer and better quality of life of thalassaemia patients in comparison to few decades ago whereby patients died even before or during adolescence due to various complications. However, there are several limitations on DFO administration. The time-consuming nature of DFO administration has imposed several restrictions on patient's life including work, social activities, sleep, and emotional well-being especially in adult patient. This leads to suboptimal compliance which in turn increases risk of iron overload complications and poor prognosis [6, 16]. Furthermore, repeated daily administration of DFO may cause emotional burden as well as economic burden in patients who cannot afford such therapy.

Oral iron chelators such as Deferasirox (DFX) is expensive which causing accessibility issue [17]. DFX is one of the iron chelators that allows once daily administration, which is convenient to patients. A report showed that patients who received DFX had a significantly higher rate of compliance compared to those who were treated with DFO, however it was not shown to improve patient satisfaction [18]. There were several reports that prove the efficacy of DFX in reduction of serum ferritin, liver iron concentration (LIC) [19-22], as well as improvement in cardiac magnetic resonance imaging T2* (MRI T2*) [23]. However, the cost of drug remains a concern among healthcare professionals.

In clinical practice, DFO is self-administered at home. On the first encounter, patients were taught on subcutaneous injection technique, administration, and handling of DFO. Most of the thalassaemia patients, particularly those transfusion-dependent thalassaemia (TDT) was already started on DFO during childhood. As a result, they might be still dependent on their caretakers when they grew up. In thalassaemia clinic, patients were periodically assessed on the injection techniques to ensure proper administration of the medication. However, there is a paucity of information on how patients prepare and administer DFO at home, and some of the administration errors might not be encountered by the healthcare providers. Based upon our literature search, there are no studies that thoroughly describe DFO administration practice and identify common administration errors among thalassaemia patients. Some of the studies reported mainly on the compliance of DFO but did not elaborate specifically on the administration technique [14, 16, 24, 25]. Therefore, it is crucial to identify the current DFO self-administration practice among thalassaemia patients at home. Besides compliance, administration techniques are also important to ensure success of medication therapy. In this study, besides identifying the current DFO administration practices among thalassaemia patients and common errors made during administration have also been studied. In addition, patient compliance towards iron chelation therapy and factors associated with high serum ferritin levels were also investigated.

METHODS

Study Design

This is a cross-sectional study, including all adult thalassaemia patients using DFO as iron chelators from Thalassaemia Medication Adherence Clinic (TMTAC) in Hospital Tengku Ampuan Afzan (HTAA) Kuantan. Potential samples were screened using Pharmacy Information System (PhIS). Data were traced from January 2019 until November 2020 and a registry was generated from the system. Patients were identified during thalassaemia clinic follow-up.

We used Raosoft Sample Size Calculator for sample size calculation [26]. According to Malaysian Thalassaemia Registry Report, there were a total of 2084 patients who were on DFO therapy in Malaysia [27]. However, based on the registry in Pahang, only 95 patients were on DFO therapy [28]. With margin of error (E) set at 0.05 and response rate (r) set at 0.5, the minimum sample size of 77 is needed to represent the state. However, we did not perform a multicentre study, hence inadequate samples.

Purposive sampling method was used. This is a population study, all identified patients were included. After the initial screening, a total of 40 patients were included in the registry. All adult thalassaemia patients who were followed-up in adult thalassaemia clinic and prescribed with DFO from January 2019 until November 2020 were included. Patients who were not on DFO for more than 6 months and newly started DFO for less than a month were excluded. Data collection form was used to collect patient characteristics, details of DFO administration, and patient compliance assessment.

There were two main parts in the data collection. First part was patient characteristics, DFO injection practices and DFO dilution steps. The second part was a validated questionnaire for compliance assessment, adapted from Ramli et al. [29]. The interview session was conducted for 10-15 minutes. Informed consent was obtained from the patients. The interview of DFO administration practice at home was carried out according to the checklist in Thalassaemia Medication Adherence Clinic (TMTAC) Protocol, Ministry of Health (MOH) Malaysia.

Questions asked including drug dose, frequency, DFO injection independence, dilution of drugs, injection site, injection technique, needle handling, DFO machine settings, infusion duration and steps taken prior administration. DFO dilution steps was adapted from DFO administration guide by Northern California Comprehensive Thalassaemia Centre [30]. Patients were asked to describe on step-by-step approach in DFO administration, each of the correct step were given 1 mark. There were 15 steps in total, and total marks were calculated as percentages. The scores were defined as good (score 75-100%), satisfactory (score 60-74%), and fail (score <60%). No validation was involved, as the guide was only for checklist purpose to assess patient's knowledge in DFO administration.

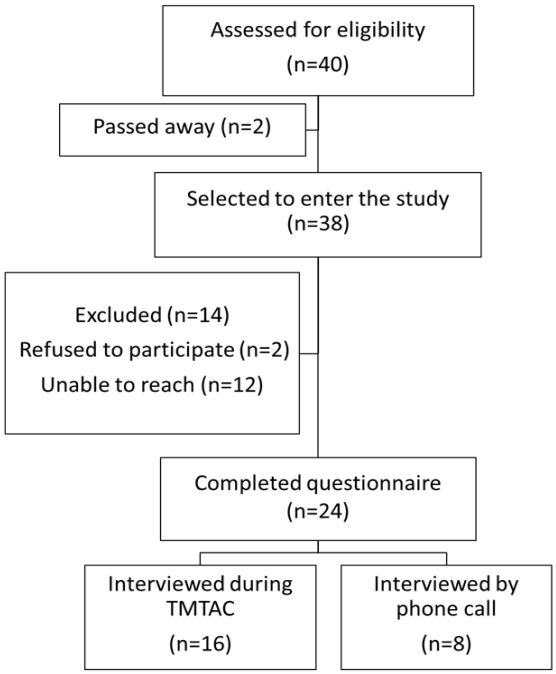

Figure I. Study Flow.

The second part was compliance assessment. For this part, a validated questionnaire consisting of seven questions was given, and patients were required to answer all seven questions. The questionnaire was validated in previous study, mentioning that it has good internal consistency and reliability [29]. Patient compliance was assessed based on the answers provided, and scores were calculated. Patients were considered compliant to medications when the compliance score obtained was at least 27 out of 28. For patients whose clinic appointment falls outside of data collection period, the session was completed through a phone call. Figure I outlined the flow of study.

Ethical Approval and Consent to Participate

This study had obtained ethical approval from the Head of Medical Department, the Hospital Director, and the National Medical Research and Ethics Committee (MREC) of the Ministry of Health (MOH) Malaysia. The study was conducted in compliance with ethical principles outlined in the Declaration of Helsinki and Malaysian Good Clinical Practice (GCP).

Statistical Analysis

The study data were analysed using the SPSS statistical software version 25. Descriptive statistics were used for demographic data. All categorical data were presented as

Table I. Patient demographic data. TDT, transfusion-dependent thalassaemia. [a]Mann-Whitney U test, [b]Fisher's Exact test.

Variables	Compliance		Statistical Test	p Value
	Yes (n=7)	No (n=17)		
Median age, years (IQR)	22 (21 to 43)	25 (23 to 28.5)	-0.383[a]	0.702
Gender, n (%)				
Male	2 (28.6)	6 (35.3)	0.101[b]	1.00
Female	5 (71.4)	11 (64.7)		
Median Weight, kg (IQR)	45.5 (41.25 to 49.75)	50.4 (45.5 to 57.4)	-1.508[a]	0.132
Working Status, n (%)				
Yes	2 (28.6)	8 (47.1)	0.697[b]	0.653
No	5 (71.4)	9 (52.9)		
Median Income, RM (IQR)	600 (0 to 2225)	600 (0 to 1875)	-0.25[a]	0.802
Education, n (%)				
Secondary and below	3 (42.9)	6 (40)	0.016[b]	1.00
Tertiary and above	4 (57.1)	9 (60)		
Diagnosis, n (%)				
B-Thalassaemia Major	1 (14.3)	4 (23.5)	2.262[b]	0.495
Hb E β-Thalassaemia	5 (71.4)	12 (70.6)		
HbH Constant Spring	1 (14.3)	0 (0)		
Splenectomy, n (%)				
Yes	5 (71.4)	10 (58.8)	2.246[b]	0.193
No	2 (28.6)	6 (35.5)		
TDT, n (%)				
Yes	7 (100)	15 (88.2)	0.457[b]	1.00
No	0 (0)	1 (5.9)		

Table II. Iron overload status and total iron chelators used. IQR, interquartile range; MRI T2*, magnetic resonance imaging T2*. aMann-Whitney U test, bFisher's Exact test, *significance at <0.05.

Variables	Compliance		Statistical Test	p Value
	Yes (n=7)	No (n=17)		
MRI T2* Heart, n (%)				
Normal	2 (28.6)	10 (58.8)		0.245
Moderate	0 (0)	0 (0)		
Severe	2 (28.6)	2 (11.8)		
MRI T2* Liver, n (%)				
Normal	2 (28.6)	1 (5.9)	0.782[b]	1.00
Moderate	2 (28.6)	6 (35.3)		
Severe	4 (57.1)	4 (23.5)		
Serum ferritin range, n (%)				
<1000 µg/L	3 (42.9)	4 (23.5)	2.03[b]	0.425
1000 – 2500 µg/L	2 (28.6)	3 (17.6)		
>2500 µg/L	2 (28.6)	10 (58.8)		
Median serum ferritin, mcg/L (IQR)	1358 (239.9 to 3361.4)	5244.3 (1929.55 to 8318.45)	-2.537[a]	0.011*
Number of iron chelators used, n (%)				
1	2 (28.6)	3 (17.6)	0.728[b]	0.816
2	4 (57.1)	11 (64.7)		
3	1 (14.3)	2 (11.8)		

frequencies and percentages, whereas continuous data were presented as median and interquartile range. Univariate analyses were performed using Mann-Whitney, Chi-square test and Fisher's exact test, as appropriate. Univariate linear regression was used to identify factors associated with high serum ferritin levels, and multivariate linear regression (MLR) was performed for significant variables. P values <0.05 were considered as statistically significant

RESULTS

Demographic Data

A total of 38 patients were identified. Out of 38 patients, only 24 were able to complete the study. Fourteen patients were excluded, 2 refused to join the study, and 12 were unreachable. Summary of demographic data is shown in Table I. Majority of patients were female (16, 66.7%), median age of 24.5 years old

(interquartile range (IQR) 21 to 43). Most of the patients had tertiary education level (13, 54.2%), had median income of RM 600 per month (IQR 0 to 1625), and more than half were not working (14, 58.3%). The most common diagnosis was Hb E β-Thalassaemia (17, 80%), with half of the patients underwent splenectomy. Majority of the patients were transfusion dependent thalassaemia (TDT) (22, 91.7%). Sixteen percent had severe cardiac MRI T2*, and 8 (25%) had severe LIC. Half of the patients had serum ferritin of >2500 µg/L, with median serum ferritin of 3361.4 µg/L (IQR 1385.9 to 6214.55). Majority of the patients received at least 2 iron chelators (15, 72.75%). A significant difference was found in the median serum ferritin between compliant and non-compliant patients (U=15, z=-2.537, p=0.011). Summary of iron overload status and total iron chelators used are shown in Table II.

Deferoxamine Injection Practice

Median of mean daily dose (MDD) of DFO received by the patient per day was 18.63 mg/kg (IQR 11.7 to 47.5). In terms of DFO practice, most of the patients administered the drug independently (19, 79.2%) with more than half applied eutectic mixture of local anaesthetics (EMLA®) cream before injection (15, 62.5%). The most common injection site was abdomen (19, 79.2%), followed by arm (3, 12.5%) and outer thigh (2, 8.3%). Majority of the patients administered DFO at night (23, 95.8%). Most of them missed dose more than four times per month (10, 41.6%). Summary of DFO injection practice is as shown in Table III.

Table III. DFO injection practice and common administration errors. DFO, deferoxamine; EMLA, eutectic mixture of local anesthetics; IQR, interquartile range. [a]Mann-Whitney U test, [b]Fisher's Exact test.

Variables	Compliance		Statistical Test	p Value
	Yes (n=7)	No (n=17)		
Median MDD DFO, mg/kg/day (IQR)	18.63 (11.7 to 47.5)	19.9 (17.68 to 27)	0.000[a]	1.00
EMLA® cream application, n (%)				
Yes	4 (57.1)	11 (64.7)	0.974[b]	0.393
No	3 (42.9)	6 (35.3)		
Injection Site, n (%)				
Arm	0 (0)	3 (17.6)	1.774[b]	0.406
Abdomen	7 (100)	12 (70.6)		
Thigh	0 (0)	2 (11.8)		
Time setup, n (%)				
Morning	0 (0)	1 (5.9)	0.43[b]	1.00
Night	7 (100)	16 (94.1)		
Errors made, n (%)				
Yes	1 (14.3)	16 (94.1)	0.458[b]	0.507
No	6 (85.7)	1 (5.9)		
DFO score, n (%)				
Good	5 (71.4)	13 (76.5)	1.50[b]	0.587
Satisfactory	2 (28.6)	2 (11.8)		
Fail	0 (0)	2 (11.8)		

Common Administration Errors

Most of the patients made errors during administration of DFO (22, 91.7%). Common DFO practice errors found were divided into three parts which are dilution error, administration error and disposal error. Among these 3 errors, the most common error was dilution error (83.3%), followed by administration error and disposal error. Overall, 18 (75%) of the patients had good DFO score, however majority of the patients treated with DFO were non-compliant (17, 70.8%). These are the examples of dilution errors identified:

- Dilution of DFO with smaller amount of diluent which resulted in concentrated DFO solution and may cause irritation during infusion.
- Did not fully syringe out DFO solution from the vials, which cause inadequate dose administered.
- Administration of DFO without waiting for the drug powder to fully dissolve, which may cause precipitation.
- Wrong method of dilution: using one of the reconstituted DFO to dilute another vial, instead of using pure water for injection (WFI) to reconstitute all vials. This may lead to
- inaccurate measurements and incomplete dissolution of drug powder.

Factors Associated with Serum Ferritin Level

Univariate linear regression was performed on each variable to identify factors associated with serum ferritin level. Variables included were age, gender, working status, income, education level, MRI T2* liver, MRI T2* cardiac, number of iron chelators, injection site, and frequency of missed dose. Variables that showed a trend towards significance were selected for MLR which are working status, MRI T2* liver, injection site, and frequency of missed dose in a month. Compliance was not included for MLR due to its relationship with frequency of missed dose in a month. Summary of linear regression is shown in Table IV. MLR was done using stepwise method, and the results showed that MRI T2* liver and injection site were not statistically significant (p >0.05).

However, the results revealed that there was a statistically significant interaction between working status and frequency of missed dose. Result shows that 57.4% of the variance in serum ferritin can be accounted for by two predictors, F (2,11) = 9.773, p=.004. In this model, interaction was found between working and frequency of missed dose. The predicted serum ferritin is equal to 3475.48 + (1152.39 × frequency of missed dose) + (-5557.06 × working status) - 1637.28 (working status × frequency of missed dose), per one unit increase in each factor. No multicollinearity was found, and the final model met all the assumptions.

Table IV. Factors affecting serum ferritin level among adult thalassaemia patients. DFO, deferoxamine; CI, confidence interval; MDD, mean daily dose; MRI T2*, magnetic resonance imaging T2*; TDT, transfusion-dependent thalassaemia. [a]Method: Multivariate logistic regression, stepwise method, *variables that were included for multivariate linear regression.

Variables	Univariate Linear Regression			Multivariate Linear Regression		
	B	95% CI	p Value	B[a]	95% CI	p Value
Age	-28.37	-363.44 to 306.7	0.861	-	-	-
Gender	-1692.8	-6288.87 to 2903.27	0.450	-	-	-
Working Status						
Yes	5044.88	1365.2 to 8724.55	0.010*	4664.13	1609.41 to 7718.86	0.005
No						
Income	0.32	-1.078 to 1.722	0.629	-	-	-
Education				-	-	-
Primary	-6399.59	-14928.89 to 2129.71	0.131			
Secondary	-5700.93	-13487.08 to 2085.22	0.140			
Tertiary						
MRI T2* Cardiac				-	-	-
Normal	3083.85	-2992.97 to 9160.67	0.293			
Severe						
MRI T2* Liver				-	-	-
Normal	-15640.31	-21938.63 to -9341.99	<0.001*			
Moderate	-16986.56	-23491.44 to -10481.68	<0.001*			
Severe						
Number of Iron Chelators	538.226	-3060.5 to 4136.95	0.758	-	-	-
Injection Site				-	-	-
Arm	-8799.68	-15118.97 to -2480.39	0.009*			
Abdomen	-10332.05	-18785.44 to -1878.66	0.019*			
Thigh						
Frequency of missed dose in a month	1107.57	284.18 – 1930.95	0.011*	1021.51	341.84 to 1701.17	0.005

DISCUSSIONS

Median serum ferritin was 3361.4, which is more than usual target in most facilities that requires serum ferritin level to be less than 2500 µg/L. Half of the patient had serum ferritin level of more than 2500 µg/L. A study reported mean serum ferritin of 2767.52 µg/L, which is lower than our current findings [31]. Another study reported mean serum ferritin level of 4236.5 µg/L among thalassaemia major patients, which are expected to have higher levels than HbE β-thalassaemia [32].

The high median serum ferritin could be explained by its association with compliance status, as majority of the patients were non-compliant to iron chelation. As a result, 16.7% of the patients had severe cardiac iron overload, and 25% had severe liver iron overload, based on MRI T2* findings. These findings were correlated with high prevalence of non-compliance among the patients studied. A systematic review revealed that serum ferritin levels were achieved with increased adherence to iron chelators [33].

There were several benefits gained by achieving low level of serum ferritin. A report by Liu et al. mentioned that, those patients who had serum ferritin level of less than 2500 µg/L would have cardiac disease free survival outcome of 91% after 15 years of chelation with DFO, compared to patients who had serum ferritin of 2500 µg/L and above [34]. Another report showed lower serum ferritin of less than 1000 µg/L were significantly associated with reduction in heart failure event and prolonged survival [11].

Our findings showed a significant association between median serum ferritin and compliance status, whereby patients who had higher serum ferritin level were associated with non-compliance. This association can be explained by a direct effect of iron chelators in reducing iron burden, which is showed as reduction serum ferritin levels. Similar findings were demonstrated by other study which showed significantly higher serum ferritin level, cardiac and severe liver iron overload among non-adherent patients [24]. Although serum ferritin is not accurate to determine patient iron overload status and MRI T2* is preferred, it can be used as a surrogate marker to represent iron overload. A study showed that cardiac and hepatic MRI T2* had significant correlation with serum ferritin level [35]. This was supported by another study that demonstrated higher serum ferritin can predict the severity of cardiac and liver iron overload [36]. Another study also found that serum ferritin was highly predictive for hepatic iron overload and fairly predictive for myocardial iron overload [13].

In this study, we were unable to identify number of iron chelators as a predictive factor that may contribute to serum ferritin level, which is related to patient compliance. This finding is similar to results from Cochrane systematic review that was also unable to ascertain whether medication interventions such as DFX will have impact on adherence. It was also uncertain whether patients who received combination therapy would affect their compliance differently compared to

monotherapy. There was only one trial in the review that reported combination of DFX plus DFO may improve adherence compared to combination of DFP plus DFO [25]. The review advocates for more real-world studies to investigate adherence issues, as the adherence in clinical trials may be contributed to increased attention and involvement of clinicians. Overall adherence reported in the systematic review was high, ranged from 70% to 100% [25]. In contrast, our study reported medication adherence rate of only 29.2%, which is very low.

In MLR analysis, working status and frequency of missed dose were identified as factors that were associated with serum ferritin level. Interaction was found between these two variables; this interaction can be explained by patients who are working tend to miss dose more frequently due to busy schedule, compared to those who did not work. A qualitative study had explored lack of integration of routine as one of the barriers towards medication adherence, whereby participants claimed that they were unable to inject DFO due to busy schedule [37]. This could also explain why patients still low compliance have despite having good knowledge on DFO administration.

We would like to highlight common administration errors among patients recruited. While performing the interview, a few of injection practices that were unexplored before was revealed, such as cleaning the needle with hot water to be used again for next administration, injecting DFO less than 45°, did not fully syringe out DFO solution from the vials, dilution for DFO which was too concentrated, and administered DFO without waiting for the drug to dissolve properly. The same qualitative study explored the barriers of medication adherence towards iron chelation therapy. Five barriers had been classified in the study: patient-related, medication-related, sociocultural-related, patient-provider relationship, and environmental context. For patient-related barriers, lack of knowledge was reported to be one of patient-related barriers, apart from negative attitude, negative emotion, and lack of integration into routine [37].

Having a good insight on the disease is very important to ensure that patient is aware on the complications of iron overload, hence will appreciate the value of medicine and adhere to them. In our results, although most of the patients made errors, they still had good insight on DFO injection. However, the compliance rate was low. Another study similarly found no correlation between disease knowledge and adherence to iron chelation therapy [24]. In contrast, another study found positive association between knowledge and treatment adherence [38]. There are a lot of other factors other than knowledge that were reported in previous studies, such as socioeconomic status, number of siblings who had thalassaemia, age, and thalassaemia complications [33]. Hence, we cannot deduce that patients who had good insight of the disease and medication will have greater compliance.

The limitations of this study mainly were small sample size, as we did not proceed with multicentre study. Furthermore, we were having limited face-to-face sessions due to postponement of clinic visits due to COVID-19 pandemic. Although we shifted the strategy to phone calls, it is more difficult to obtain data compared to face-to-face sessions. There were limited number of patients ongoing DFO therapy; some of them stopped taking a long time ago, and a few of the patients newly started on DFO. Most patients who refused to participate in the study were those who were contacted through phone calls. Furthermore, the self-reported compliance may be potential for recall bias among patients.

CONCLUSIONS

As a conclusion, DFO administration errors persist among thalassaemia patients. Dilution error was the most common error among thalassaemia patients. Although most of the patients had good DFO administration practice, the overall compliance rate was low. Patients who are working and non-compliance were identified to be associated with high serum ferritin level. Effective interventions such as support groups, educational interventions, are needed to improve DFO administration and patients' compliance. Further studies are needed to identify in depth about working status and non-compliance to medications.

CONFLICT OF INTEREST

None

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Not available.

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Original Research Article

Knowledge, Practices & Attitudes Towards Adverse Drug Reaction Reporting among Public Healthcare Professionals in West Pahang

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ABSTRACT

Background: The World Health Organization identifies adverse drug reactions (ADRs) as unintentional and harmful reactions to drugs, which are among the leading causes of death in many nations. ADR under-reporting is a threat to pharmacovigilance. **Objective:** The objectives of this study were to evaluate the knowledge, practices & attitudes towards ADR reporting among public healthcare professionals and to determine factors that lead to underreporting of ADR in public health facilities (hospitals and clinics) in West Pahang, Malaysia. **Methods:** This was a cross-sectional, questionnaire-based observational study conducted in 2021 with respondents consisting of healthcare professionals from all hospitals and health clinics in West Pahang. Data was collected from March 2021 to April 2021 via a validated questionnaire. **Results:** A total of 400 questionnaires were distributed and 344 healthcare professionals took part in the study, with an 86% response rate. The majority of survey respondents selected positive responses in the area of knowledge and attitude, but not in the area of practice for ADR reporting. It was found that 95.3% of respondents had good knowledge, 11% had good practices and 52% had good attitudes towards ADR reporting. Over half of respondents (53.2%) believed that it is nearly impossible to conclude that a drug is the cause of a particular adverse event. Fifty percent of respondents believed they would be held accountable for the ADR which occurred once they reported it. The majority of the respondents (68%) thought that really serious ADRs were already documented when the drugs were marketed. **Conclusion:** The majority of healthcare professionals in West Pahang showed good knowledge and attitude, but poor practice in ADR reporting. Insecurity, fear of admitting harm, complacency, and ignorance were the discouraging factors that led to the underreporting of ADR among healthcare professionals from West Pahang.

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INTRODUCTION

The World Health Organization identifies adverse drug reactions (ADRs) as unintentional and harmful reactions to drugs, which are among the leading causes of death in many nations [1]. ADRs may cause a loss of confidence or evoke negative emotions towards physicians, leading patients to seek self-treatment options, which, in turn, can compromise patients' health further [2]. Approximately 5% of all hospital admissions are attributed to ADRs, and around 10%-20% of those admitted into wards will experience at least one ADR during their hospital stay [2]. In the United States, the full adverse reaction profile of a new drug approved by the Food and Drug Administration (FDA) for marketing may not be known due to limitations in pre-approved clinical trials [3]. Therefore, all drugs that are approved for sale are subject to safety monitoring through the post-marketing surveillance (PMS) program, and timely reporting of ADRs by healthcare professionals is essential to ensure the success of the program [3]. PMS is a part of pharmacovigilance, an activity associated with detecting, evaluating, understanding, and preventing occurrences of ADRs or other problems associated with drugs or vaccines [2,3].

However, ADR under-reporting is a threat to pharmacovigilance [4] and is known as a major obstacle to the success of pharmacovigilance programs attributed to factors related to the knowledge, practices and attitudes of healthcare professionals [5]. As proposed by Inman [6] in 1976, the 'seven deadly sins' which lead to underreporting of ADR include Ignorance, Diffidence, Complacency, Fear and Guilty, Lethargy, Greed, as well as Indifference [6,7].

Healthcare professionals should consider ADR reporting as their professional responsibility because the efficient ADR reporting scheme is important in improving patient care and safety [4]. According to a study by Sen S et al. in evaluating knowledge, attitude, and practice with regards to pharmacovigilance among physicians in India, approximately 89.62% of public practitioners correctly identified the WHO definition of pharmacovigilance, whereas only 77.5% of private practitioners achieved the same accuracy [8]. Among public practitioners, only 19.81% documented a suspected ADR using any surveillance form, while the percentage was even lower for private practitioners at 3.75% [8]. Around 59.43% of physicians in government hospitals published ADR case reports in medical journals, and this figure was much lower in private practice settings, a mere 18.75% did so [8].

A lack of knowledge was observed in Southern India where 28.3% of respondents reported having heard the name pharmacovigilance, while only 3.3% knew the meaning of pharmacovigilance [9]. In Hospital Sikkim, 37% responded to the definition of pharmacovigilance correctly, the majority by the professors were the most accurate (71.4%), and lower by postgraduates or residents (19.2%) [10]. Most of the respondents knew that doctors, pharmacists, and nurses could report ADRs, while 63% of the respondents could correctly identify the spontaneous reporting system as the most commonly employed method to monitor ADRs [10]. Among interns at a tertiary healthcare centre in India, 52.0% of the respondents did not know about the reporting system in the hospital [11]. Only 20.0% had reported an ADR and 97.0% had never filled out the ADR notification forms [11].

Apart from that, a study in Pakistan showed that most of the respondents (83.1%) indicated poor ADR reporting knowledge [12]. The majority of respondents (78.2%) expressed a positive attitude towards ADR reporting, but only a few hospitals (12.3%) demonstrated good ADR reporting practices. Factors such as the seriousness of ADR, unusualness of reactions, involvement of new drugs, and confidence in diagnosing ADR encouraged respondents to report ADR [12]. However, lack of knowledge regarding where and how to report ADR, limited access to ADR reporting forms, prioritizing patient management over-reporting ADR, and concerns about legal liability were the major factors that discouraged respondents from reporting ADR [12].

In a study conducted in Saudi Arabia, 95.0% of the respondents stated that ADRs should be reported for both newly marketed and already marketed agents [13]. When it came to the types of ADRs that should be reported, 75.0% of the respondents believed that all reactions should be reported, while 16.0% stated that only serious reactions should be reported [13]. Physicians and pharmacists had nearly identical responses (80.0%) in favour of reporting all reactions [13]. However, 20.0% of nurses believed that serious ADRs should be reported for more than one type of reaction, which differed from the views of physicians (10.0%) and pharmacists (15.0%) [13].

Locally, a study conducted in the Klang Valley involving private practitioners reported that almost half (46.2%) of the participants believed that only confirmed reactions needed to be reported. In comparison, only 58.6% provided the correct response that all suspected reactions to established drugs in new combinations or for new indications should be reported [14]. Concerning new products, 45.5% of the participants held the opinion that only serious reactions needed to be reported, and 89.7% responded that all serious reactions to both new and established products should be reported [14]. On the other hand, another study involving community pharmacists in Selangor reported a positive attitude towards ADR, but less than half (34.2%) had ever reported an ADR [15]. The main obstacles to ADR reporting were lack of patient information, inadequate

clinical knowledge, and uncertainty regarding the relationship between the adverse reaction and the drug [15].

According to a study conducted by Belton et al. to evaluate the attitudes of European Union (EU) healthcare professionals towards their national spontaneous reporting system, issues that appeared to discourage reporting included the lack of available report forms, absence of contact details for the reporting agency, insufficient information on the reporting process, and limited time to report [16]. On the contrary, issues that did not seem to discourage reporting included concerns about patient confidentiality, fear of legal liability or embarrassment, hesitation to acknowledge the harm caused to a patient, and a desire to accumulate and publish personal case series [16].

Previous studies have shown ADR to be under-reported globally [8,9,12,16]. It was therefore worth investigating the level of knowledge, attitude, and practice in ADR reporting among healthcare professionals in West Pahang. To our knowledge, no research related to the assessment of knowledge, attitude, and practice in ADR reporting was conducted for a combination of public hospitals and public health clinics in Pahang State. The primary objective of this study was to evaluate the knowledge, practices, and attitudes towards ADR reporting among Public Healthcare Professionals (hospitals and clinics) in West Pahang while the secondary objective was to determine factors that lead to underreporting of ADR in public health facilities (hospitals and clinics) in West Pahang.

MATERIAL AND METHOD

West Pahang consists of 4 districts including Kuala Lipis, Bentong, Raub and Cameron Highlands. Each district has a hospital and a district health office, which is the headquarters of several primary health clinics. Approvals were obtained from the National Medical Research Registry (NMRR) and Medical Research Ethics Committee (MREC) before data collection (Registration number: NMRR-20-2615-57129). Consent forms were signed by respondents before filling out the questionnaires.

A "principal investigator at the site" was nominated in each hospital and district health office. These on-site principal investigators were responsible for collecting data from healthcare providers at their respective sites. The healthcare professionals who participated in the study were doctors (specialists/ medical officers/ houseman officers), dentists, pharmacists, medical assistants, pharmacy assistants and nurses. Stratified systematic sampling was used to select the participants in each study site. All health professionals in West Pahang were stratified by occupational group, and then every fifth person (5th, 10th, 15th and so on) from the list was selected to participate in the survey. The inclusion criteria were West Pahang public healthcare professionals who were available to participate in the study and were able to complete the English version of the questionnaire. Individuals who were off duty during the study period were excluded from the study.

There were 2,326 public health professionals working in the West Pahang area entitled to this study. The sample size needed for this study was 330 and was calculated using the Raosoft sample size calculator (with a margin of error of 5%, a confidence interval of 95% and a response distribution of 50%) [17]. Another 70 participants were intentionally added to account for missing data or dropouts, with the final target sample size for this study being 400 participants. Data collection was conducted from March 2021 to April 2021, using a validated questionnaire previously used in a similar study in Klang Valley, Malaysia [14]. Written permission was obtained from the Malaysian Journal of Medical Sciences to utilize the questionnaire and protocol from the study [14].

The questionnaire was divided into two sections. The first section consisted of questions on demographic characteristics. The second section had three domains, in which two questions with 11 sub-items, two questions with 7 sub-items, and three questions with 22 sub-items were used to evaluate knowledge, practices, and attitudes towards ADR reporting, respectively [14]. Each favourable answer for each sub-item was scored as 1, and participants who obtained a domain score of 70% or more were assessed as having good knowledge, attitude, and practice, respectively [14]. In addition, participants with a score of 70% or higher across all sub-items in all three domains were considered to have a good overall knowledge, attitude and practice (KAP) towards ADR reporting. The self-administered questionnaires were distributed to the participants by principal investigators at their respective sites. Each respondent took approximately 30 minutes to complete the survey.

Statistical Package for Social Sciences (SPSS) program version 26 was used to analyse the collected data in this study. The data were analysed using descriptive statistics as well as the chi-square test. This study was conducted in compliance with the ethical principles outlined in the Declaration of Helsinki and the Malaysian Good Clinical Practice Guidelines.

Ethical Approval and Consent to participate

Approvals were obtained from the National Medical Research Registry (NMRR) and Medical Research Ethics Committee (MREC) before data collection (Registration number: NMRR-20-2615-57129). Consent forms were signed by respondents before filling out the questionnaires.

Table I: Demographic Data of the Study Participants (n = 344)

Characteristics	n (%)
Gender	
Male	111 (32.3)
Female	233 (67.7)
Age (years)†	31 (7.8)
District	
Lipis	120 (34.9)
Bentong	116 (33.7)
Raub	78 (22.7)
Cameron Highlands	30 (8.7)
Facility	
Hospital	167 (48.5)
Health Clinics (PKD)	177 (51.5)
Department	
Outpatient	114 (33.1)
Emergency	32 (9.3)
Pharmacy	53 (15.4)
Inpatient	73 (21.2)
Others	72 (20.9)
Professional Status	
Doctors	86 (25.0)
Medical Assistants	61 (17.7)
Pharmacists	34 (9.9)
Pharmacy Assistants	22 (6.4)
Nurses	127 (36.9)
Dentists	14 (4.1)

† ***Data are shown as median (IQR)***

RESULT

A total of 400 questionnaires were distributed and 344 healthcare professionals took part in the study, with an 86% response rate. The majority of the participants were female, 67.7% (n=233), from the outpatient department, 33.1% (n=114) and nurses, 36.9% (n=127). The data are presented in (Table I).

The majority of the study participants chose favourable answers to the questions regarding knowledge in ADR reporting as shown in Table II. However, most of them chose unfavourable answers regarding practices of ADR reporting. More than half of the participants had suspected an ADR before. Out of 180 participants who had ever suspected an ADR, 80% of them sent an ADR report to the national reporting agency (Table III). Concerning the attitude of participants towards ADR reporting, most participants chose favourable answers in this domain. The factors discouraging ADR reporting among participants in West Pahang include the perception that serious ADRs are already well established by the time a drug is marketed (68.0%) and it is nearly impossible to determine the causal relationship between a drug and a particular adverse event (53.2%, Table IV). In terms of scoring, it was found that 95.3 per cent of participants had good knowledge, 11 per cent had good practices and 52 per cent had good attitudes towards ADR reporting.

Chi-square tests of homogeneity were conducted to check a difference in proportion between each group of sociodemographic characteristics and Overall KAP in ADR Reporting. All expected cell frequencies were greater than five, and post hoc analysis involved pairwise comparisons using the z-test of two proportions with a Bonferroni correction. In total, 52.9 per cent of all healthcare professionals in West Pahang had good overall KAP towards ADR reporting. A breakdown of the data is presented in (Table V).

DISCUSSION

Knowledge

The current study found that 65% of the participants showed good knowledge of ADR reporting as the majority chose favourable answers. This finding is consistent with a local study conducted in northern Malaysia, where 72.1 per cent of healthcare professionals demonstrated good knowledge of ADR reporting [18]. However, it was found that over 40% of participants felt that only proven responses should be reported. This is in line with the previous study by Belton et al., which found that more than 20% of respondents in Italy, Portugal and Spain were unwilling to report suspected reactions (as opposed to proven ones) [16]. According to the Malaysian Adverse Drug Reaction (ADR)/ Adverse Event Following Immunization (AEFI) Reporting: Manual for Healthcare Providers, all suspected ADRs should be reported for all products registered with the Drug Control Authority (DCA), even if the products are not used following the authorised dosage. [19].

Attitude

According to the result in Table IV, 64.5% of respondents knew how to report an ADR and 72.1% of respondents knew where the form is available from. This is contrary to the study by Adegbuyi et al., where only 38.3 percent of those surveyed had ever seen an adverse drug reaction reporting form [20]. According to a study by Survase et al. involving nurses in a tertiary care centre, 65% of the participants responded that the non-availability of forms was one of the major factors that have deterred nurses from reporting ADRs [5].

More than two-thirds of the respondents in this study disagreed that reporting ADR is time-consuming or too busy to report. This is encouraging as the attitude survey on the reporting of adverse reactions to medicinal products by health professionals in the European Union in 1997 showed that lack of time was one of the key issues that prevented suspected ADRs from being reported [16].

Table II: Responses about Knowledge in ADR Reporting (n = 344)

Variable	Yes, n (%)	No, n (%)
Regarding type of ADR to be reported:		
All suspected reactions to established drugs in new combination or for new indication should be reported.	334 (97.1)	10 (2.9)
All suspected reactions to new products should be reported.	329 (95.6)	15 (4.4)
Only serious reactions to new products should be reported.	75 (21.8)	269 (78.2)
All serious reactions to old & new products should be reported.	336 (97.7)	8 (2.3)
Only proven reactions should be reported.	146 (42.4)	198 (57.6)
Following is/are the purpose(s) of the national ADR reporting scheme in Malaysia:		
For identification of previously unrecognized ADRs.	328 (95.3)	16 (4.7)
To recognize factors predisposing to ADRs.	327 (95.1)	17 (4.9)
To characterize ADRs.	324 (94.2)	20 (5.8)
To enable toxicity of drugs in similar therapeutic classes to be compared.	303 (88.1)	41 (11.9)
To identify safe drugs.	312 (90.7)	32 (9.3)
To measure the incidence of ADRs.	329 (95.6)	15 (4.4)

More than half of the respondents (53.2%) believed that it is nearly impossible to conclude that a drug is the cause of a particular adverse event thus resulting in low practice in ADR reporting. This is following findings from a systematic review of 45 articles, where 67% of the studies identified insecurity as one of the factors related to under-reporting [21]. Notably in this section, half of the respondents believed they would be held responsible for the ADR that happened once they reported it. This is much higher than the findings from a study by Belton et al., where only under 10% of respondents in each European Union countries agreed on the fear of admitting harm as a deterrent for ADR reporting [16].

The majority of the respondents (68%) thought that really serious ADRs were already documented when the drugs were marketed. This is also consistent with results from two-thirds of studies with a higher level of evidence in a systematic review, where complacency was found to be a factor associated with ADR under-reporting [21].

Practice

While respondents demonstrated a good attitude, their practice of reporting ADR was not satisfactory. According to the 70% threshold rating for whether respondents had good practices, only 11% of respondents were considered to have good practices for ADR reporting. The low percentage of practice among respondents may be attributable to most health professionals in West Pahang who opted to report ADR only if the reaction is severe, unusual, caused by a new product, or had been observed previously. The reaction is confidently caused by the medication (Table III). These factors correspond to the findings of a study by Aziz et al., in which 'ADR considered to be too trivial or too well known to report' and 'uncertainty that the reaction had been caused by a drug' was among the top three predictors of physicians not reporting ADR in a multivariate logistic regression model [22]. It was nonetheless encouraging to note that, although most participants opted for

Table III: Responses regarding Practices of ADR reporting (n = 344)

Variable	Yes, n (%)	No, n (%)
Have you ever:		
1. *Sent an ADR report to your national reporting agency or a pharmaceutical company.*	144 (41.9)	200 (58.1)
2. *Suspected an ADR.*	180 (52.3)	164 (47.7)
I will report an ADR, only if:		
1. *The reaction is serious.*	241 (70.1)	103 (29.9)
2. *The reaction is unusual.*	263 (76.5)	81 (23.5)
3. *I have observed similar reactions to the drug class before.*	253 (73.5)	91 (26.5)
4. *The reaction is to a new product.*	256 (74.4)	88 (25.6)
5. *I am confident that the reaction is an adverse reaction to the drug.*	287 (83.4)	57 (16.6)

Table IV: Responses regarding Attitudes towards ADR reporting (n = 344)

Variable	Agree, n (%)	Disagree, n (%)
Attitudes towards ADR reporting.		
1. *The ADR form is not available and I do not know from where I can get the form.*	96 (27.9)	248 (72.1)
2. *The ADR form is too difficult to fill up.*	101 (29.4)	243 (70.6)
3. *The space provided in the ADR form to describe the ADR is too little or inadequate/insufficient.*	139 (40.4)	205 (59.6)
4. *It requires stating my identity, which I do not wish to provide.*	105 (30.5)	239 (69.5)
5. *It requires stating patient's identity, which I do not wish to provide.*	67 (19.5)	277 (80.5)
6. *Filling this ADR form will hold me responsible for ADR related harm to the patient.*	172 (50.0)	172 (50.0)
7. *I do not know the relevant phone numbers.*	130 (37.8)	214 (62.2)
8. *I do not know the address of the agency to which I should report.*	129 (37.5)	215 (62.5)
9. *I am not sure about how to report.*	122 (35.5)	222 (64.5)
10. *I am not sure whether the patient's confidentiality will be maintained.*	126 (36.6)	218 (63.4)
11. *I feel I will appear foolish.*	49 (14.2)	295 (85.8)
12. *I am worried about legal liabilities.*	131 (38.1)	213 (61.9)
13. *I am too busy to report.*	83 (24.1)	261 (75.9)
14. *I wish to publish a personal series of cases rather than reporting ADRs.*	53 (15.4)	291 (84.6)
15. *Really serious ADRs are well documented by the time a drug is marketed.*	234 (68.0)	110 (32.0)
16. *It is nearly impossible to determine if a drug is responsible for a particular adverse event.*	183 (53.2)	161 (46.8)
17. *The one case an individual physician might see cannot contribute to medical knowledge.*	127 (36.9)	217 (63.1)
18. *I should be financially reimbursed for providing the reports of ADRs.*	75 (21.8)	269 (78.2)
19. *I have a professional obligation to report ADRs.*	231 (67.2)	113 (32.8)
20. *Reporting ADRs puts my career at risk.*	45 (13.1)	299 (86.9)
21. *It takes too much time to report ADRs.*	120 (34.9)	224 (65.1)
22. *I will be asked to provide more information which I don't want to do because I am busy, it will take too much time (or other reasons).*	115 (33.4)	229 (66.6)

Table V: Difference in Overall KAP in ADR reporting by sample characteristics (n = 344)

Variable	*Good Overall KAP*	*Poor Overall KAP*	
	n (%)	n (%)	$\chi 2$ (p)*
Gender			
Female	137 (58.8)	96 (41.2)	10.059 (**0.002**)
Male	45 (40.5)	66 (59.5)	
Age Group			
21-30	87 (54.4)	73 (45.6)	1.474 (0.479)
31-40	73 (54.1)	62 (45.9)	
>40	22 (44.9)	27 (55.1)	
Facility			
Hospital	89 (53.3)	78 (46.7)	0.019 (0.889)
PKD	93 (52.5)	84 (47.5)	
District			
Lipis	65 (54.2)	55 (45.8)	21.237 (<**0.0005**) [Δ]
Bentong	77 (66.4)	39 (33.6)	
Raub	32 (41.0)	46 (59.0)	
Cameron Highlands	8 (26.7)	22 (73.3)	
Professional Status			
Doctors	59 (68.6)	27 (31.4)	41.575 (<**0.0005**) [§]
Medical Assistants	17 (27.9)	44 (72.1)	
Pharmacists	29 (85.3)	5 (14.7)	
Pharmacy Assistants	12 (54.5)	10 (45.5)	
Nurses	57 (44.9)	70 (55.1)	
Dentists	8 (57.1)	6 (42.9)	

**Chi-square test. P < 0.05 indicates a statistically significant difference in proportions.*

*[Δ]Post hoc analysis showed statistically significant differences in proportion between groups of **Lipis vs Cameron Highlands**, **Bentong vs Raub**, and **Bentong vs Cameron Highlands**.*

*[§]Post hoc analysis showed statistically differences in proportion between groups of **Medical Officer vs Medical Assistant**, **Medical Officer vs Nurse**, **Pharmacist vs Medical Assistant**, and **Pharmacist vs Nurse**.*

unfavourable responses in the field of practice, 80% of those who previously suspected an ADR had submitted a report to the national reporting agency.

Overall KAP

The results showed that 52.9% of respondents had an overall score above 70% and were considered to have a good overall KAP concerning ADR reporting.

The proportion of doctors and pharmacists who have good overall KAP is significantly higher than that of nurses and medical assistants, which coincides with results from a study by Palaian S et al. in Nepal, where total KAP scores obtained by doctors and pharmacists were higher than those of nurses [23]. Another finding of this study was that the proportion of female respondents with a good overall KAP was significantly higher than that of male respondents. This is in line with the results from a study by Burud et al., where female respondents were 1.86 times and 2.74 times more likely to have satisfactory knowledge and desirable practices, respectively for ADR reporting than male respondents [24]. The study also showed that there were significantly fewer female respondents with perceived attitude barriers to the reporting process compared to male respondents [24].

In terms of comparison between the 4 districts, the Bentong and Lipis districts showed a greater proportion of health professionals with good overall KAP than the Cameron Highlands district. This could be due to the fact that the Cameron Highlands Hospital is a non-specialist hospital, whereas Kuala Lipis Hospital and Bentong Hospital are hospitals with specialists. According to findings from Agarwal et al., practitioners with a post-graduate degree demonstrated higher levels of knowledge with respect to ADR reporting than practitioners with only a basic medical degree because of greater clinical experience [14].

In order to further improve overall KAP among health professionals in West Pahang, educational interventions concerning pharmacovigilance may be necessary. According to Leena et al., ongoing educational interventions, especially with respect to how to identify and report ADRs, are often required to increase KAP scores in ADR reporting [11]. Further studies may be carried out to compare the KAP scores before and after educational interventions among healthcare professionals in West Pahang.

Limitations

Given that this was a self-reported survey, there might be response bias and the accuracy of the recall by respondents may be questioned. In addition, as this study was conducted only in Ministry of Health facilities, the results may not be generalized to other healthcare professionals in different practice contexts.

CONCLUSIONS

The majority of healthcare professionals in West Pahang showed good knowledge and attitude, but poor practice in ADR reporting. Insecurity, fear of admitting causing harm, complacency, and ignorance were found to be the discouraging factors that led to ADR underreporting among healthcare professionals in West Pahang. Proper education and training should be provided for healthcare professionals regularly to further improve their overall KAP in ADR reporting.

CONFLICT OF INTEREST

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Original Research Article

Antibiofilm Activity of Activated Carbon Film Wound Dressing

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ABSTRACT

Background: Biofilm is a structured community of bacterial cells enclosed in a self-produced matrix containing 80% extracellular polymeric substances (EPS). When biofilms are present on the wound surface, they impair optimal penetration of antimicrobials resulting in delayed healing. Chronic wounds are likely to be impacted since the wound may be moist and purulent which are conducive for biofilm growth. **Objective:** To develop and characterise hydrogel films containing AC at various concentrations and to study the antibiofilm activities of the produced films through in vitro approach on two common biofilm strains, Staphylococcus aureus, and Pseudomonas aeruginosa. **Methods:** An approach was suggested in this study by utilising the high adsorptive capacity of activated carbon (AC) loaded into PVP/CMC hydrogel film wound dressing to disrupt and inhibit the biofilms present on the wound surface. **Results:** The results obtained showed that the developed hydrogel films containing AC at different concentrations (0.1%, 0.5%, 1.0%, 1.5% and 2.0%) provided a balanced moist environment to the wound surface with adequate swelling properties. Characterisation of the AC films also showed satisfactory mechanical properties indicating the films were durable and flexible, thus suitable for wound application. Antibiofilm study was conducted via XTT assay by treating biofilms of two common bacterial strains, Staphylococcus aureus and Pseudomonas aeruginosa with the AC hydrogel films. Findings showed AC exerted antibiofilm activity by inhibiting more than 50% of biofilm growth on P. aeruginosa biofilm, with AC 0.5% achieving the highest biofilm inhibition (62.41±8.48%). However, there was lack of evidence to support AC hydrogel films in inhibiting biofilm produced by S. aureus because there was no significant difference ($p<0.05$) in biofilm inhibition when compared to AC 0%. **Conclusion:** Although AC hydrogel films showed promising results on P. aeruginosa biofilm inhibition, its application in wound dressing as antibiofilm agent may be limited especially on multispecies biofilm due to its lack of activity towards S. aureus. Further investigations are required to assess antibiofilm activity of AC hydrogel films on other bacterial strains and its mechanism of biofilm inhibition.

INTRODUCTION

Wound is formed when there is any disruption to the epithelial integrity of the skin resulting in the impairment of normal tissue function and structure. Wound can be categorised into acute or chronic wound depending on their ability to heal. Acute wounds are wounds that occur recently and heal in a short period of time within four weeks such as traumatic wounds and surgical wounds whereas chronic wounds are wounds that fail to heal under a normal expected healing period and require more than 12 weeks to heal [1]. Examples of chronic wounds include diabetic ulcers, pressure ulcers and venous ulcers. Nevertheless, when a wound becomes infected, the natural healing process is impaired resulting in delayed wound healing commonly seen in chronic wounds [2, 3]. Bacterial biofilms pose a significant issue in chronic wound management. It was reported that up to 75% of microbial infections are due to the

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colonisation of bacteria in the form of biofilm [3]. Biofilm is a structured community of bacterial cells enclosed in a self-produced matrix and adherent on an inert or living surface. It is made up of 80% extracellular polymeric substances (EPS) consisting of polysaccharides, proteins, and nucleic acids. Bacteria in biofilms behaved differently than planktonic bacteria since mostly are sessile. Multi-species bacteria can also adhere to a surface and form biofilm. Due to the nature of biofilms, they are recalcitrant to antimicrobials, including antibiotics [4]. It is estimated that bacteria in a biofilm are 50-1000 times more resistant to conventional antibiotic treatment [5]. Wound infection persists because biofilms are resistant to killing by host defence mechanisms and antibacterial treatments. The wound is unable to resolve from the inflammatory stage resulting in poor wound healing, and the formation of chronic wounds [4]. The current management of biofilms consists of physical debridement and chemical approaches. Physical debridement involves the removal of the tissue layer containing biofilm, which causes pain on the wound surface. hence chemical approaches were favoured over physical debridement. Chemical approaches include the development of antibiofilm agents such as antimicrobials, ethylenediaminetetraacetic acid (EDTA) and xylitol.

A more specific approach is required to manage biofilms present in chronic wounds. In this research, hydrogel films were chosen as a wound dressing of choice. Hydrogel dressings are insoluble hydrophilic materials made up of natural or synthetic polymers that can provide a moist environment due to their high-water content promoting granulation of tissues and epithelium. They are also skin-friendly and easy to apply to wounds [6-8].

Novel approaches are intensively studied to find ways to disrupt the biofilm layer and improve the penetration of antimicrobials. Activated carbon (AC) was studied to investigate its antibiofilm activities in vitro. Activated carbon is also known as activated charcoal. Activated charcoal is characterised by a carbon matrix. It incorporates a large number of pores of various diameters and lengths. The available surface for adsorption ranges from 400 to 1200 m2 per gram of charcoal and can be up to 5000 m2 [9]. Clearly, AC possessed powerful adsorptive capability resulting in its uses in the biomedical field for the removal of toxins and malodour [10]. It is hypothesised that AC may also have potential antibiofilm activity aimed at combating biofilms present on the wound surface.

Thus, this research aimed to develop and characterise hydrogel films containing AC at various concentrations and to study the antibiofilm activities of the produced films through in vitro approach on two common biofilm strains, Staphylococcus aureus, and Pseudomonas aeruginosa. These objectives were made to find out the potential of AC to be loaded into hydrogel films since the majority of the wound dressing containing AC available in the market was either in fibre or cloth form indicated for malodour management of wounds. Nevertheless, there was also a lack of study in investigating the ability of AC in inhibiting biofilm growth. Therefore, this research was conducted to address these issues and produced a practical wound dressing with advantages to wound healing.

MATERIALS AND METHODS

Materials

Formulation of hydrogel films consist of two polymers, namely low viscosity sodium carboxymethylcellulose from Merck (Darmstadt, Germany) and polyvinylpyrrolidone K-90 from Acros Organics (Massachusetts, USA). Agar powder bacto grade and Glycerol 99.5% were obtained from Sisco Research Laboratories Chemicals (Mumbai, India). A premium grade activated carbon was purchased from Noble Healthcare (Kuala Lumpur, Malaysia). Next, potassium chloride was obtained from Merck (Darmstadt, Germany). Gelatine from bovine skin was obtained from Sigma Aldrich (Missouri, USA).

For antibiofilm study, Gram-positive bacteria, Staphylococcus Aureus (ATCC 25923) and Gram-negative bacteria, Pseudomonas aeruginosa (ATCC 27853) were used. Mueller-Hinton agar (MHA) and Mueller-Hinton broth (MHB) were purchased from Merck (Darmstadt, Germany). Phosphate-buffer saline (PBS) tablet was obtained from Sigma Aldrich (Missouri, USA). The chemical reagent used consists of tetrazolium sodium (XTT) purchased from Bio Basic (Toronto, Canada), menadione crystalline and acetone from Sigma Aldrich (Missouri, USA).

Preformulation and Film Preparation

Preformulations of hydrogel consist of incorporating two polymers, polyvinylpyrrolidone (PVP) and sodium carboxymethylcellulose (Na-CMC) based on a method described by by varying the ratios of the polymers 80:20 and 20:80. A total of six formulations were prepared with different ratio of the polymers and glycerol concentration (Table I). Then, the formulation that possessed the best physical attributes such as satisfactory appearance and flexibility was chosen to be incorporated with activated carbon (AC) at different concentrations (0%, 0.1%, 0.5%, 1.0%, 1.5% and 2.0% w/v).

The hydrogel films were prepared by heating 30 mL of distilled water to 80°C. PVP and Na-CMC were then poured slowly into the heating water and mixed with a magnetic stirrer for 20 minutes until all powders were completely dissolved and no lumps were present. Then, glycerol was added slowly into the solution while stirring. Next, 2 g of agar powder was added to the solution and stirred for another 30 minutes. The solution

Table I. Preformulation compositions of hydrogel films.

Composition (%W/V)	Formulations					
	F1	F2	F3	F4	F5	F6
PVP	0.2	0.2	0.2	0.8	0.8	0.8
Na-CMC	0.8	0.8	0.8	0.2	0.2	0.2
Glycerol	1.0	3.0	5.0	1.0	3.0	5.0
Agar	2.0	2.0	2.0	2.0	2.0	2.0
Distilled water	100	100	100	100	100	100

was made to 100 mL by adding distilled water. A cloudy solution was formed and placed under moist heat treatment at a condition of 121°C and 103 kPa for 20 minutes to allow crosslinking of the polymers resulting in a transparent gel solution [11]. Then, the hydrogel solution (20 g) was carefully poured into a petri dish (85 mm diameter) following the solution casting method and allowed to solidify at room temperature for 30 minutes. A total of four films was obtained from the preparation of 100 mL hydrogel solution. The hydrogels were set to dry in the oven at 50 ± 2°C for 24 hours resulting in the formation of hydrogel film. The dried films were peeled gently from the petri dish and conditioned in a desiccator (50 – 60% RH, 27 ± 2°C) prior to testing for temperature stabilisation as described by Ng and Leow [12].

The most satisfactory formulation was chosen based on physical observation and incorporated with AC at different concentrations. AC was added into the chosen formulation after moist heat treatment by continuous stirring at 350 rpm for 10 minutes until a homogenous, black-coloured gel solution was formed. Characterisation and further studies were performed on AC-containing hydrogels at different concentrations.

Physical Assessment

Physical assessments of the films were done macroscopically which included assessing their appearance, colour, odour, and flexibility. The film thickness and diameter were also measured at four points at every 45-degree angle by using a digital vernier calliper, Absolute Digimatic Calipers from Mitutoyo (Kanagawa, Japan). The measurements were expressed as the mean ± standard deviation (SD).

The moisture content of each film was also assessed by measuring the weight of the films before drying and after drying at 50 ± 2°C in the oven for 24 hours. The weight before drying was taken when pouring the hydrogel solutions (20 g) into the petri dish whereas the weight after drying was taken after storing the films in the desiccator for 30 minutes for temperature stabilisation. The moisture content was determined as the percentage of the initial film weight lost during drying using the following equation:

$$\text{Moisture content (\%)} = \frac{W_0 - W_1}{W_0} \times 100 \qquad \text{Eq. 1}$$

Where W_0 is the initial weight of the film (g) and W_1 is the weight of the film after drying (g). All tests were done in triplicate.

Swelling Ratio Test

A method described by Matthews, Stevens [13] was adapted with some modifications. It consisted of using a simple non-animal model to demonstrate swelling properties of hydrogel films on suppurating wounds. The swelling medium consisted of 4% w/v gelatine that was prepared by adding 4 g of gelatine powder into 100 mL of distilled water that had been heated to 60°C. The mixture was slowly stirred for 10 minutes until homogenous. When a clear solution was formed, 20 g of the gelatine solution was poured into petri dish and allowed to cool at room temperature. Once cooled, the petri dishes were covered and allowed to be set overnight at room temperature. Next, the films were cut into a circular shape with a diameter of 20.50 mm and weighed accurately. Then, the films were placed at the centre and on top of the gelatine medium. The weight of the films was measured at different time intervals (1 hour, 3 hours, 5 hours, and 24 hours). The swelling properties of the films were calculated as shown in Eq. (2). The experiment was done in triplicate for each formulation.

$$\text{Swelling ratio} = \frac{\text{Weight of film after swelling (g)}}{\text{Initial weight of film (g)}} \times 100 \qquad \text{Eq. 2}$$

Moisture Absorption Study

A method described by Ng and Leow [12] was adapted with modifications. Films of known diameter (20.50 mm) were cut, weighed accurately, and placed in a desiccator containing silica beads for 24 hours (50 – 60% RH, 27 ± 2°C). Another desiccator containing saturated potassium chloride solution was prepared and left to condition for 24 hours to form a humid environment (84% RH, 27 ± 2°C). The films were then placed inside the desiccator and left for 24 hours. After 24 hours, the films were weighed, and the percentage of moisture absorption was computed as the difference in the initial and final weight of the films with respect to the initial weight. The equation used to compute the percentage was shown in the following equation.

$$\text{Percentage moisture uptake (\%)} = \frac{W_f - W_0}{W_0} \times 100 \qquad \text{Eq. 3}$$

Where W_f is the final weight of the film (g) and W_0 is the initial weight of the film (g).

Moisture Vapour Transmission Rate (MVTR) Study

Films of known diameter (20.50 mm) were cut and gently placed on an opened glass vial that contained 2 g of silica beads. A double-sided tape was used to ensure the films adhered

firmly to the top of the glass vial. The exposed film diameter was measured (15.50 mm) and used in the calculation of the exposed surface area. An opened glass vial without any film and a glass vial sealed with aluminium foil acted as controls in this study. Then, the glass vials with films and controls were placed in a desiccator containing saturated potassium chloride solution (84% RH, 27 ± 2°C). The weight of each glass vial was measured including the silica beads and film every hour for the first 6 hours followed by every 24 hours for two days. The moisture transmitted through the films was determined gravimetrically by the increment of weight of the glass vials. The rate of moisture transmission was calculated from the difference in mass in a period of time divided by the exposed film area. The equation used to calculate the MVTR was as follows:

$$\text{MVTR } (\text{g}/cm^2/h) = \frac{(W_t - W_0)(t)}{A} \quad \text{Eq. 4}$$

Where W_t is the weight of the glass vial at a given time (g), W_0 is the initial weight of the glass vial (g), t is the time presented in hours, and A is the exposed surface area of film in cm^2. The test was run in triplicates.

Mechanical Properties

The mechanical properties of the films were evaluated by utilising the Universal Testing machine model 5567 from Instron (Warwick, UK). The computed value obtained consists of the maximum load at break withstand by the film and the extension of film at break. These parameters were used to assess the tensile strength (TS), percentage of elongation at break (E%) and Young's modulus.

To prepare the film, firstly the films were trimmed according to the ASTM standard dumbbell shape template with a length of 30 mm and width of 5 mm. The films were stretched to break at a crosshead speed of 5 mm/min. The test was run in triplicates. The calculation of TS, E%, and Young's modulus were demonstrated below:

$$\text{TS (MPa)} = F_{\max}/A \quad \text{Eq. 5}$$

Where $F_{\max}$ is the maximum force at break (N) and A is the cross-sectional area of the film (mm^2).

$$E\ (\%) = \left(L_f/L_0\right) \times 100 \quad \text{Eq. 6}$$

Where L_f is the extension of the film at the point of break (mm) and L_0 is the initial length of the film prior to stretching (30 mm).

$$\text{Young's modulus (MPa)} = \left(\text{TS}/\left(L_f/L_0\right)\right) \quad \text{Eq. 7}$$

Where TS is the tensile strength computed in Eq. (5), L_f and the initial length of the film prior to stretching (30 mm), L_0

Fourier-Transform Infrared Spectroscopy (FTIR)

The dried hydrogels were analysed by using a Perkin Elmer Spectrum 100 FT-IR spectrophotometer using a Perkin Elmer universal attenuated total reflection (ATR) sampling assembly. The samples that were tested include hydrogel films containing AC 0%, 0.1%, 0.5%, 1.0%, 1.5% and 2.0% as well as AC powder only.

Firstly, the hydrogel films were cut into a circular shape with a diameter of 10 mm and placed on top of the ATR crystal. Adequate force was applied to the ATR pressure clamp to ensure optimal contact of samples on the ATR crystal. As for the AC powder, the powder was gently loaded on top of the crystal and ATR pressure clamp was firmly placed on the powder. The FTIR spectra of samples were recorded with a uniform resolution of 2 cm^{-1} and the selected range was between 4000 and 650 cm^{-1}.

Antibiofilm Study (XTT assay)

The antibiofilm activities of the AC-containing films were quantified by performing XTT assay. Aseptic techniques were practised throughout the study and the biosafety cabinet class II (BioAir, Italy) were utilised in carrying out this experiment. The methods described by Peeters, Nelis [14] were adapted with some modifications.

Firstly, two bacterial strains (Staphylococcus Aureus and Pseudomonas Aeruginosa) were grown in MHB to form bacterial suspensions with turbidity equal to 0.5 McFarland standard. Adjustments were done accordingly to achieve the desired turbidity. The adjusted suspensions were used within 30 minutes. In the preparation of treatments, the hydrogel films containing AC were trimmed into a circular shape, with a diameter of 5 mm and placed carefully at the bottom of the wells of a 96-well-flat-bottom tissue culture plate (Biofil Chemicals and Pharmaceuticals Ltd., India). 100 µL of the bacterial suspension was added to each well by using a micropipette. Untreated wells were also prepared to contain MHB only and bacterial suspension only. The plates were incubated at 37°C for 24 hours under static conditions. After 24 hours, the remaining bacterial suspension and the hydrogel films in the wells were removed. The plates were then rinsed with PBS twice to remove planktonic bacteria and any residue from the films.

Next, XTT assay was conducted to study the antibiofilm activities of the films. The light-yellow colour of the XTT solution prepared in this experiment will be reduced to a dark orange water-soluble formazan salt facilitated by bacterial

enzymes. In this antibiofilm study, the first step was to prepare fresh XTT solution prior to use by dissolving 4 mg of XTT powder in 10 mL of prewarmed (37°C) PBS followed by 100 µL of menadione stock solution. Menadione stock solution was prepared beforehand by dissolving 55 mg of menadione in 100 mL of acetone. Next, 100 µL of PBS and 100 µL of XTT-menadione solution were added to each well, then the plates were incubated in the dark for 5 hours at 37°C. After 5 hours, the plates were taken out of the incubator and the supernatant contained in the wells was withdrawn (100 µL) and transferred to a newly opened 96-well-flat-bottom tissue culture plate. The new tissue culture plate containing the supernatant was analysed by using Thermo Scientific™ Multiskan™ GO microplate reader and the absorbance was measured at 490 nm. The percentage of biofilm inhibition was calculated by using the following equation:

$$\text{Biofilm inhibition (\%)} = \left(\frac{OD490_{Control} - OD490_{Treated}}{OD490_{Control}}\right) \times 100 \quad \text{Eq. 8}$$

Statistical Analysis

All the collected data are presented as the means ± standard deviation and analysed using Microsoft Excel 2019. Statistical tests such as *t*-test and one way-ANOVA were also computed using IBM SPSS® for Windows version 22. The significant difference was set to be $p < 0.05$.

RESULTS AND DISCUSSION

Preformulation and Film Preparation

In the preformulation stage, six formulations (F1-F6) were prepared by varying the ratio of PVP/CMC and the amount of glycerol. The films produced were thin, transparent, odourless and had good adherence to the skin without leaving any sticky residue. A study by Roy, Saha [11] reported that PVP/CMC ratios of 20:80 and 80:20 appeared to have better swelling and viscoelastic properties compared to other ratios of PVP/CMC (0:100, 50:50, 100:0). F1 and F4 were the formulations adopted from Roy, Saha [11] . Modifications were made to the amount of glycerol to investigate the effects of glycerol concentration on film flexibility. The amount of glycerol (%w/v) used were 1%, 3% and 5%. It was found that when the concentration of glycerol increased, the films produced seemed to be more flexible. This was due to the effect of glycerol as plasticizer that reduced the intra-molecular attraction between the polymeric chains, by favourably forming hydrogen bonds resulting in reduced polymer-polymer interactions and improve mobility of the polymer chains [15, 16]. Therefore, among the six formulations, F6 had the most satisfactory physical appearance, flexibility, and adherence to the skin. F6 was further incorporated with AC at various concentrations (0%, 0.1%, 0.5%, 1.0%, 1.5% and 2.0%).

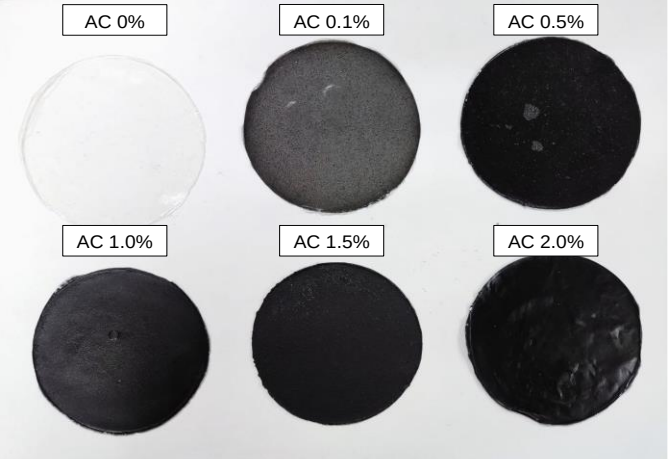

Figure I. Photograph of AC hydrogel films at different concentrations.

The AC films prepared were opaque and exhibited an increasingly intense black colour when the concentration of AC increased (Figure I). Hydrogels are well known for their transparency because of the ease of observing the wound without detaching the dressing [17]. However, the addition of AC led to an opaque appearance that impaired the ability to observe the wound during application. It was also observed that the films became drier when the concentration of AC increased. The moisture content of AC films at different concentrations was significantly different ($p < 0.05$) from one another, with AC 2.0% exhibiting the lowest moisture content (89.65 ± 0.31). Nevertheless, the films remained flexible and pliable at all AC concentrations. They were easily trimmed into desired shapes and further characterisations of the films were conducted.

Physical Assessments

The weight, thickness, diameter, and moisture content of AC films were measured and summarised in Table II. Based on the measurements obtained, the weight and thickness of the AC films gradually increase when the concentration of AC increases. This was relatively due to the increased mass of AC added into the formulation. As for the thickness of the films, only AC 1.5% and AC 2.0% showed a significant increase ($p < 0.05$) when compared to AC 0%. On the other hand, shrinkage was observed as the diameter of AC 1.5% and AC 2.0% films were significantly smaller than AC 0% ($p < 0.05$). A study by Illsley, Akhmetova [10] also reported similar findings where there was shrinkage when incorporating AC in plasticised agarose film composites. This was due to a surface adsorption effect on AC resulting in the carbons interacting with and retaining water, plasticizer (glycerol) or the polymeric chain during drying [10]. The moisture content of AC films was significantly higher ($p < 0.05$) than AC 0% due to the high

Table II. Physical properties of AC films at different concentrations.

Formulations	Physical properties			
	Weight (g)	Thickness (mm)	Diameter (mm)	Moisture content (%)
AC 0%	1.69 ± 0.01	0.45 ± 0.14	80.64 ± 1.10	91.57 ± 0.04
AC 0.1%	1.83 ± 0.06*	0.52 ± 0.01	80.34 ± 1.58	90.90 ± 0.32*
AC 0.5%	1.78 ± 0.01*	0.45 ± 0.05	80.21 ± 2.76	91.13 ± 0.08*
AC 1.0%	1.85 ± 0.01*	0.56 ± 0.04	80.67 ± 1.52	90.77 ± 0.05*
AC 1.5%	1.90 ± 0.01*	0.75 ± 0.09*	76.94 ± 0.34*	90.51 ± 0.04*
AC 2.0%	2.08 ± 0.06*	0.84 ± 0.10*	68.70 ± 2.58*	89.65 ± 0.31*

All measurements were expressed as mean ± SD (n = 4).
***Significantly different compared with AC 0% ($p < 0.05$)**

adsorptive capacity and high pore volume of AC [18]. Generally, hydrogels possess high moisture content (70-90%) due to their complex hydrophilic polymers that facilitate autolytic debridement by providing hydration to necrotic tissues [17, 19]. Hence, the AC films prepared exhibited high moisture content that had potential in wound healing applications targeted for rehydrating the wound bed and facilitating debridement for improved wound healing.

Swelling Ratio Test

The wound model used to investigate the swelling properties of the AC films was gelatine medium due to its comparable characteristics to a suppurating wound, as described by Matthews, Stevens [13]. According to their methods, the diameter of the films was measured at time intervals and the degree of swelling was computed. However, as for the AC films, measuring the diameter may not provide an accurate representation of their swelling properties, because they did not expand uniformly. Instead, the film swells by increasing both its thickness and diameter. Hence, the weight of the films was measured to increase the accuracy in studying the swelling properties of the films.

The percentage of swelling of AC film preparations was presented in Figure II. As an overview, all films swelled at least twice their weight. The film that had the highest swelling capacity after 24 hours was AC 0% (373.33 ± 15.28%). The films containing AC were unable to retain their swelling capacities as a gradual decrease in the percentage of swelling was seen after 3 hours. At 24 hours, AC 1.5% and AC 2.0% swelling reduced to 194.97 ± 26.53% and 191.67 ± 9.55% respectively in comparison to its swelling at the first hour, 256.67 ± 20.82% and 263.33 ± 5.77% respectively. AC 1.0% reduced its swelling capacity after 5 hours while AC 0.5% was after 24 hours. Only AC 0.1% showed a gradual increase in swelling after 24 hours. These findings suggested that there is a limit to the degree of swelling of films containing AC as a bigger loss in swelling capacity at certain time intervals was seen when AC concentrations increased. A reduction in swelling capacity in the function of time also suggested that the absorbed fluid may be released to the surrounding, which is unfavourable, especially for suppurating wounds. Conversely,

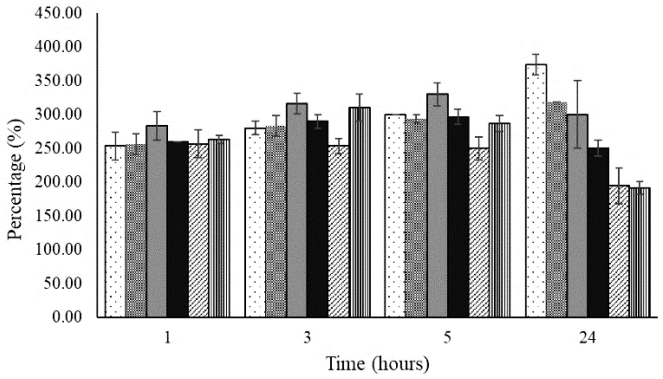

Figure II. The percentage of swelling of different AC film concentrations at different time intervals, n = 3.

a study by Minsart, Mignon [20] also reported similar findings, where PEG-based hydrogels containing AC 0.5% also possessed significantly lower swelling capacities ($p < 0.05$) when compared to hydrogel with no AC. It was deduced that the hydrophobicity of AC contributed to the reduction of swelling capacities [20].

Swelling capacity is an important factor in the prevention of bacterial growth on the wound bed due to fluid accumulation such as exudates [21]. Hence, the AC film wound dressings prepared may be beneficial for low and medium exudates as they are able to absorb exudates with limitations to promote wound debridement [22].

Moisture Absorption Study

Moisture absorption study was conducted to find out the ability of the film to absorb moisture from its surroundings in providing a moist environment to the wound. Figure III showed the percentage of moisture absorption of all films. It was found that films containing AC at different concentrations had a significantly higher percentage of moisture absorption ($p < 0.05$) than film with no AC (15.02 ± 0.63%). AC 0.5% had the highest percentage of moisture absorption (36.66 ± 6.05%). Films containing AC were able to absorb more moisture than the control film due to AC adsorptive properties. AC is highly porous and has a large surface area that enhances the adsorption of moisture from the surrounding (18). Additionally, there were no significant differences ($p < 0.05$) in moisture absorption among different concentrations of AC.

Besides, maintaining a moist environment is important in wound healing. Wound dressings that have higher moisture absorption had an improved healing rate compared to dressings that had lower moisture absorption [23]. Thus, the AC hydrogel films prepared have potential benefits for wounds that require adequate moisture to facilitate healing such as burn wounds, and necrotic wounds [22].

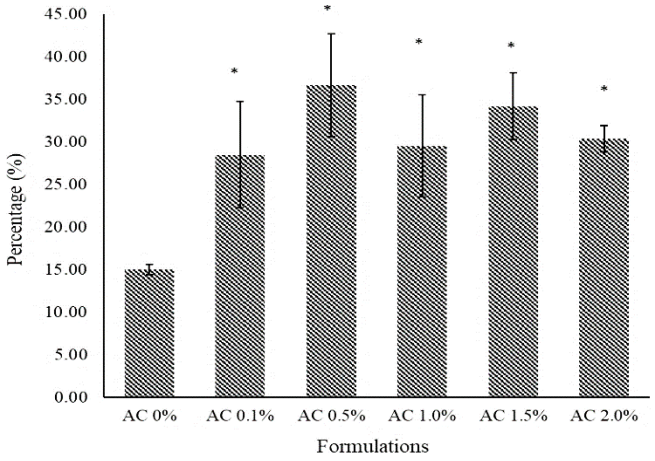

Figure III. The moisture absorption of different film formulations after 24 hours, n = 3. *Significantly different compared with AC 0% (p < 0.05).

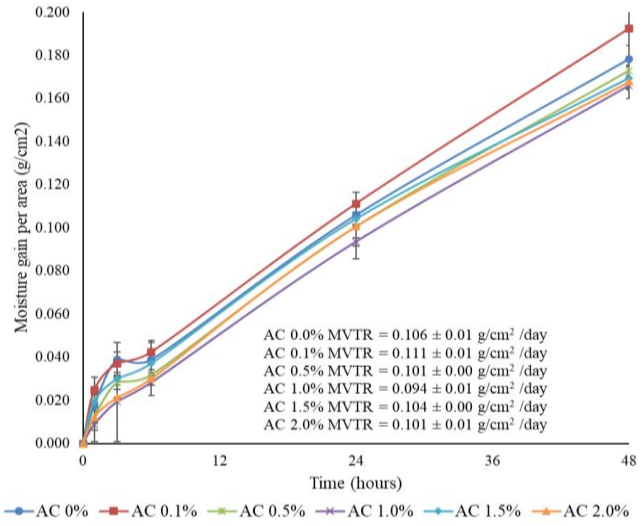

Figure IV. The moisture vapour transmission rate (MVTR) of all formulations, n = 3

Moisture Vapour Transmission Rate (MVTR) Study

Maintaining optimal moisture on the wound surface is one of the determining factors to facilitate wound healing. MVTR study can be utilised to assess the ability of wound dressings in controlling moisture gain and moisture loss (24). Figure IV summarised the findings obtained for all film formulations. It was found that in the first three hours, all film formulations showed a rapid increase in moisture gain per area followed by a gradual increase after 6 hours. AC 0.1% had the highest MVTR of 0.111 ± 0.01 g/cm2/day, although there is no significant difference ($p < 0.05$) of MVTR in all film formulations. MVTR of normal skin is 0.020 g/cm2/day, whereas, for wounded skin, MVTR may rise within the range of 0.028 to 0.514 g/cm2/day due to the disruption of the skin barrier resulting in the release of fluids and electrolytes (25, 26). A study by Xu, Xia (24) also reported that wound dressings that had MVTR of $\leqslant 0.2028 \pm 0.024$ g/cm^2/day were capable of maintaining optimal moisture for proliferation and regular function of epidermal cells and fibroblasts. They also noted that MVTR should not be too low as it will lead to the accumulation of fluids while MVTR that is too high may lead to dehydration of the wound surface. Hence, the prepared film formulations containing AC were considered suitable for wounded skin because MVTR obtained was within the specified range ($\leqslant 0.2028 \pm 0.024$ g/cm2/day) for wounded skin and capable of advancing wound healing.

Mechanical Properties

Factors such as durability and flexibility are important for the application of film wound dressings. An ideal wound dressing should be able to withstand adequate pressure as well as possess suitable flexibility to ensure the film does not tear easily and is able to follow body movements providing optimal protection of the wound towards mechanical stress [27]. The evaluation of mechanical properties of AC-containing films was done by assessing their tensile strength, percentage of elongation at break and Young's modulus. The results obtained were summarised in Figure V. The tensile strength of AC 0% film (0.69 ± 0.02 MPa) was the highest among formulations containing AC. It was also significantly higher than other formulations ($p < 0.05$), although there was a significant difference in TS among concentrations of AC 0.1%, 0.5%, 1.0%, 1.5% and 2.0%. This showed that the addition of AC in the formulation had an impact on the maximum force required to cause breakage per unit area. It took lesser force to inflict tearing on the AC hydrogel films, with AC 1.5% requiring the least force per unit area (0.36 ± 0.08 MPa) to tear.

Nevertheless, the highest percentage of elongation at break was exhibited by AC 2.0% (69.00 ± 8.19 %). It was not significantly different ($p < 0.05$) from AC 0% (57.00 ± 7.55 %). Only AC 1.5% (38.33 ± 2.08 %) was significantly different ($p < 0.05$).

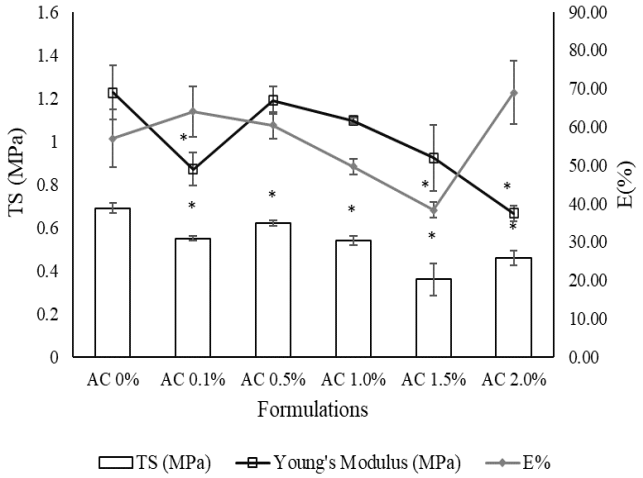

Figure V Mechanical properties of different film formulations, n = 3. *Significantly different compared to AC 0% (p < 0.05).

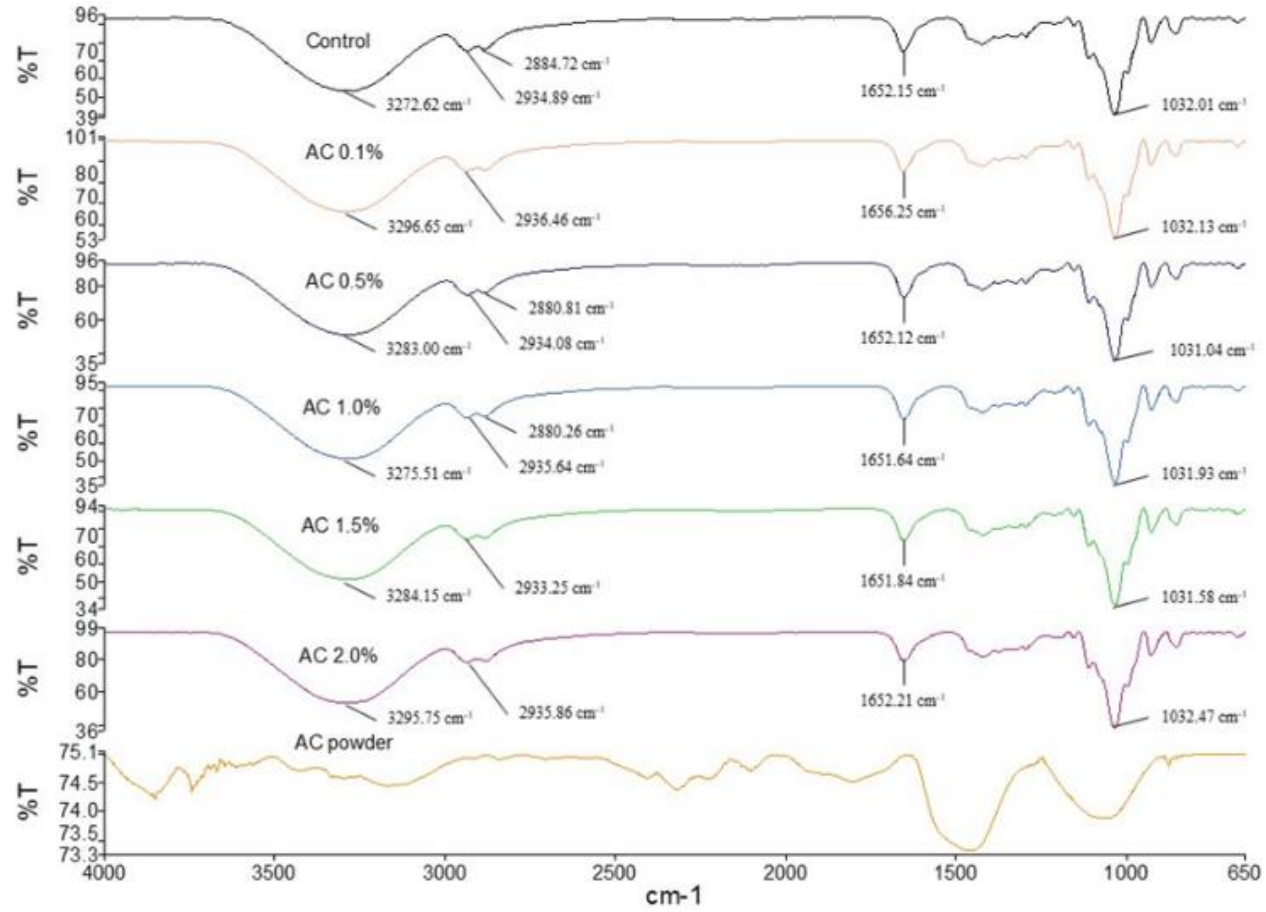

Figure VI. FTIR results for all film formulations with different AC concentrations and AC powder.

These findings suggested that AC may not have a significant impact on the ability of the films to be more elastic that may be due to the low concentration of AC present in the hydrogel films to contribute to a stronger intermolecular interaction. To investigate further on elasticity, Young's modulus was computed. Generally, Young's modulus of the skin is 0.5 – 2.0 MPa [28]. According to Figure V, it showed that Young's modulus of the films containing AC at various concentrations was not significantly different ($p < 0.05$) from AC 0% (1.23 ± 0.13 MPa), other than AC 0.1% (0.87 ± 0.08 MPa) that was significantly lower ($p < 0.05$).

The evaluation of mechanical properties of hydrogel films is important to assess the performance of the films in the presence of external force. A hydrogel film that is easily torn can impair adequate wound protection. Hence, it is crucial that the hydrogel films should be able to withstand satisfactory pressure throughout the application on the wound surface to ensure optimal protection of the wound from contaminants [22, 29, 30]. All AC films prepared were almost as elastic as the human skin. Based on the obtained findings of mechanical properties of the films, it could be summarised that the addition of AC in the formulation may not significantly enhance the mechanical properties of the films.

Fourier-Transform Infrared Spectroscopy (FTIR)

FTIR analysis was utilised in this study to investigate the presence of functional groups in the films, and to observe any possible chemical bonding occurring in the presence of AC. Figure VI showed the FTIR spectra of all film formulations containing different concentrations of AC. Control FTIR spectrum refers to film formulation that had AC 0%. As a recap, the film formulations were adapted from Roy, Saha [11] with some modifications. Hence, the findings from Roy, Saha [11] were used as a reference to compare and contrast the obtained FTIR spectra. Based on the FTIR spectra (Figure VI), it showed that spectra for control, AC 0.1%, AC 0.5%, AC 1.0%, AC 1.5%, and AC 2.0% were identical. A strong broad peak was observed between the range of 3500 – 3000 cm^{-1} indicating O-H stretching due to the presence of hydroxyl group. Then, a weak doublet peak (2830 – 2695 cm^{-1}) were seen representing

aldehyde functional group. According to Roy, Saha [11] findings, the presence of a single broad peak between 1620 – 1650 cm^{-1} showed that there was good interaction among components of the hydrogel film (PVP, CMC, agar and glycerol). Thus, the spectra obtained from the films prepared were coherent with Roy, Saha [11]. As an overview, there is no noticeable difference between the control film and the films containing AC at various concentrations. There were no additional peaks seen in films containing AC. It could be deduced that AC may not have an impact on the physical and chemical interactions that occurred in the films. The nearly identical spectra may be due to the small amount of AC added to the films, which was less than 2% w/v.

Antibiofilm Study (XTT assay)

The XTT assay was conducted under aseptic conditions to maintain sterility. XTT assay is a reproducible and relatively easy method to quantify the presence of biofilm formation by measuring its activity through optical density (OD). A high OD reflects a positive indication of biofilm formation because the XTT solution was reduced from a light-yellow colour to dark orange water-soluble formazan salt by bacterial enzymes [14]. The antibiofilm study was conducted by using two bacterial strains, *Staphylococcus aureus* (Gram-positive) and *Pseudomonas aeruginosa* (Gram-negative). These bacterial strains were chosen in this study because they are known to be high biofilm producers resulting in increased cases of nosocomial infections, delayed wound healing in chronic wounds and resistance towards antimicrobials [3, 31]. Figure VII summarised the findings obtained for all films containing different concentrations of AC. It was found that the films containing AC exhibited antibiofilm activities towards both bacterial strains. The highest percentage of *P. aeruginosa* inhibition was achieved by film AC 0.5% (62.41 ± 8.48%) followed by AC 0.1% (60.33 ± 4.27 %), AC 1.5% (53.99 ± 5.08%), AC 1.0% (51.37 ± 1.61%) and AC 2.0% (44.69 ± 5.51%). The formulations containing AC demonstrated more than 50% of biofilm inhibition and were significantly different ($p < 0.05$) when compared to AC 0%, which showed an increase in biofilm growth indicated by the negative value of biofilm inhibition (-16.72 ± 3.87%). The result obtained also showed that the highest concentration of AC which is AC 2.0% had the lowest percentage of biofilm inhibition. The mechanism of the reduced biofilm inhibition with the increase of AC concentration was not studied.

On the contrary, antibiofilm activities of AC hydrogel films on S. aureus bacteria strain were less promising in comparison to *P. aeruginosa*. The highest percentage of biofilm inhibition was achieved by AC 1.5% (39.83 ± 5.84%) followed by AC 1.0% (34.49 ± 3.22%), AC 0.5% (26.50 ± 5.28%), AC 0.1% (26.50 ± 6.64%) and AC 2.0 (14.43 ± 1.65%). The findings showed that the highest concentration of AC, AC 2.0% exhibited the lowest percentage of biofilm inhibition (14.43 ± 1.65%). Besides, the film that contained no AC (18.81 ± 11.07%) showed some antibiofilm activities which suggest that other components of the hydrogel films (PVP/CMC polymer, glycerol and agar) may be contributed to the antibiofilm activity. Therefore, there is a lack of evidence to indicate that the films containing AC have antibiofilm activity towards S. aureus biofilm since there was no significant difference ($p < 0.05$) of biofilm inhibition of AC hydrogel films when compared to AC 0%. The rationale for the lack of antibiofilm activity towards S. aureus biofilm was not further studied.

Based on the obtained results, it could be deduced that AC had antibiofilm properties towards *P. aeruginosa* bacterial strains. P. aeruginosa, a gram-negative bacteria, was found to be more sensitive towards the inhibitory effect of AC resulting in more than 50% of the biofilms present being disrupted. Gram-negative bacteria are commonly known to be more resistant towards antimicrobials because of the presence of the outer membrane that can undergo alterations by increasing hydrophobicity or mutations [32]. Theoretically, it can be deduced that due to the hydrophobicity properties of AC, it possesses the ability to adsorb the outer membrane of *P. aeruginosa* resulting in the disruption and inhibition of biofilm. However, this research did not study the mechanism of biofilm inhibition of AC and further investigations are required to assess the adsorptive ability of AC films to adsorb the outer membrane of *P. aeruginosa*.

Furthermore, a study by Ng and Leow [12] showed that gentamicin at a concentration of $\leqslant$ 0.06 mg/mL showed an inhibitory effect towards biofilm at $\leqslant$ 60% (*P. aeruginosa*). Gentamicin ($\leqslant$ 0.06 mg/mL) also showed biofilm inhibition towards S. aureus, but at a lower percentage (<50%) because it had lower activity towards gram-positive bacteria. It can be said AC had potential equivalent antibiofilm activities with $\leqslant$ 0.06 mg/mL of gentamicin. Theoretically, antimicrobials that are effective towards gram-positive bacteria include vancomycin which works by inhibiting cell wall formation. It is done so through binding of the hydrophilic part of vancomycin to the alanine residue present on the cell wall linking enzyme [33]. It could be deduced that AC had no antibiofilm activity towards S. aureus due to its high hydrophobicity, and unable to bind and adsorb optimally to the surface of the cell wall of *S. aureus*. However, this hypothesis should be assessed with further investigations on the mechanism of AC in exhibiting its antibiofilm activity.

Next, 1.5% w/w of xylitol had antibiofilm activity of 36.9 ± 6.7% while AC 1.5% showed 53.99 ± 5.08% of biofilm inhibition towards P. aeruginosa. Xylitol had almost the same activity towards both S. aureus and P. aeruginosa. For bacterial strain S. aureus, xylitol 1.5% was able to exhibit 32.9 ± 8.4% of

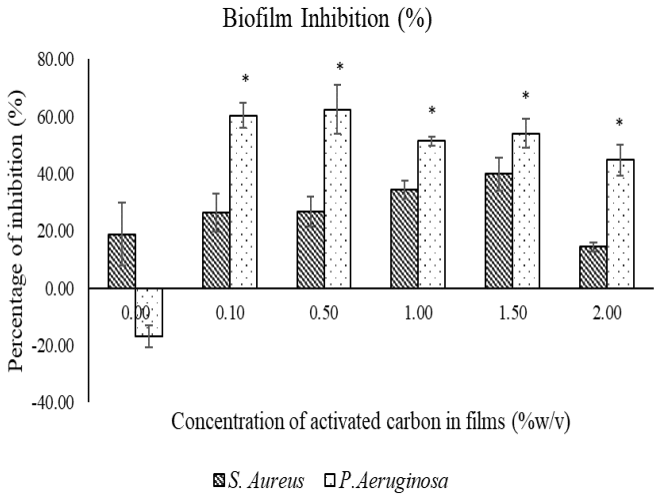

Figure VII. Percentage of biofilm inhibition exhibited by hydrogel films containing AC at different concentrations, n = 3. *Significantly different compared to AC 0% ($p < 0.05$).

antibiofilm activities in comparison to AC 1.5% (39.83 ± 5.84%). Besides, 0.05% w/w of EDTA showed 38.4 ± 4.5% of antibiofilm activity towards *P. aeruginosa* in comparison to AC 0.1% (60.33 ± 4.27 %) [34].

In summary, AC hydrogel films had antibiofilm properties as discussed above and were found to be competitive with other antibiofilm agents available commercially such as antimicrobial (gentamicin), xylitol and EDTA, when in vitro results were compared. It can be theorised that the mechanism behind AC antibiofilm activities was due to its powerful adsorptive capacities with high porosity, and large surface area for the site of adsorption [18]. Previously, AC had shown effectiveness for wounds with malodour [10]. Thus, the results obtained from this study showed the promising potential of AC being an antibiofilm agent towards *P. aeruginosa* biofilm. However, the application of AC as an antibiofilm agent in wound dressing may be limited due to the lack of evidence to indicate antibiofilm activity towards *S. aureus*. Biofilms that are formed on the wound surface may consist of multispecies of bacteria, thus selective inhibition of biofilms by AC may reduce its effectiveness when applied onto the wound.

CONCLUSION

Hydrogel film wound dressings containing activated carbon were successfully developed with satisfactory physical appearance, swelling and mechanical properties. According to the characterisation results, the produced AC hydrogel films were found to be suitable for low to medium-suppurating wounds as they were shown to maintain adequate moisture balance to the wound bed while possessing good swelling properties to absorb exudates. The prepared AC hydrogel films were also found to have good mechanical properties that were flexible and durable with moderate pressure.

Furthermore, AC hydrogel films were also tested on their antibiofilm activities. It was shown that AC had potential antibiofilm activities with more than 50% of inhibition towards *P. aeruginosa* biofilm whereas there was a lack of evidence that showed antibiofilm activity of AC towards *S. aureus* which limits AC application in biofilm-targeted therapies for wound healing. Therefore, this study concluded that the AC hydrogel film produced required further investigations to evaluate the mechanism of AC in biofilm inhibition and understand why AC showed no significant antibiofilm activity towards *S. aureus* prior to establishing AC as an antibiofilm agent.

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PROCEEDINGS *of* MPS-NATIONAL PHARMACISTS CONVENTION 2023

28th-30th July 2023

Venue: Dorsett Grand Hotel, Subang Jaya
Theme: Pharmacists In The Evolving Integrated Healthcare Landscaope: Navigating Change And Driving Quality In Primary Care

Editors

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NPC20230002

Perception and Readiness of Community Pharmacists regarding Online Healthcare Services

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Background and Objective: Community pharmacists as primary healthcare provider has extended their services over the years especially during COVID 19 pandemic. Delivery of online healthcare services by community pharmacists encompasses the use of technologies and telecommunication systems to administer healthcare services to patients remotely. This service is widely implemented in developed countries and termed as telepharmacy which has gained wide recognition among their citizens. However, such service has not been widely implemented among community pharmacies in Malaysia. The study aimed to assess the perception and readiness of community pharmacists regarding online healthcare services. **Method**: This study was a cross sectional survey using an online self-administered questionnaire, conducted among community pharmacists in Malaysia, through convenience sampling for a 3-month period between November 2022 and January 2023**. Results and Discussion**: A total of 105 community pharmacists answered this survey. A majority of community pharmacists had moderately positive perception but high level of readiness. This study reported a statistically significant association between the perception score of community pharmacists regarding online healthcare services and working experience. Community pharmacists who had worked for 10 years or more had high perception regarding online healthcare services. A positive and very strong correlation between perception and readiness of community pharmacists regarding online healthcare services was observed. **Conclusion:** Community pharmacists in Malaysia are ready for the implementation of online healthcare services. This study serves as evidence to policy makers on the feasibility of implementing online healthcare services by community pharmacists.

NPC20230004

Assessing the Impact of an Educational Workshop on Antibiotic Use and Resistance Among Secondary School Students

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Background and Objectives: Antimicrobial resistance poses a significant worldwide public health risk and it is attributed to inadequate knowledge on antibiotic use. It is alarming that awareness, knowledge and practice (AKP) on antibiotic resistance among students and young adults are poor in many countries, including Malaysia. Therefore, this study aimed to investigate the AKP on antibiotic use and resistance and evaluate the impact of a pilot educational workshop titled 'Antibiotic Use and Antimicrobial Resistance' which was adapted from the e-Bug programme by Public Health England among secondary school students. **Method:** A cross-sectional study was conducted through universal sampling using a self-administered questionnaire among Form 4 Science students from Sekolah Menengah Jenis Kebangsaan (SMJK) Yok Bin, Melaka. A validated questionnaire on three main domains: awareness, knowledge and practice towards antibiotic use and resistance was administered before and after the online workshop. **Results and Discussion:** A total of 65 students participated in the study, resulting in a 100% response rate. More than half of the respondents were male (56.9%) and the majority were residing in urban areas (78.5%) and had never attended antibiotic related-workshop, webinar or seminar (98.5%). Prior to the workshop, most respondents exhibited a median positive practice score of 67% (Interquartile range (IQR): 37.5). However, their median scores for awareness and knowledge were moderate (60%, IQR: 25; 50%, IQR: 17, respectively). Nonetheless, there was a significant improvement in both awareness (80%, IQR: 20, $p<0.001$) and knowledge (100%, IQR: 0, $p<0.001$) immediately after the workshop. The awareness level before intervention was significantly higher for respondents with a history of recent use of antibiotics ($p= 0.020$) or those who frequently take antibiotics ($p=0.005$). **Conclusion:** This study emphasized the potential benefits of the educational

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workshop for secondary school students, demonstrating promising outcomes in terms of enhancing their awareness and knowledge regarding antibiotic usage and resistance.

NPC20230006

Compliance of Private Healthcare Facilities Towards the Supply of Diphenhydramine Cough Syrup in Malaysia

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Background and Objectives: Diphenhydramine Cough Syrup (DCS) is classified as a Group C Poison in the Poisons Act 1952 which can be sold or supplied only by registered/ licensed healthcare providers. There has been a 43% increase in the seizure of DCS and kratom by enforcement agencies in Malaysia from 2019 to 2021. This study aimed to determine the compliance rate of private healthcare facilities towards the supply of DCS to the public in Malaysia as well as to identify common offences that were detected by the Pharmacy Enforcement Branch (PEB) during the controlled substance operation. **Method:** A retrospective observational study of the controlled substance operation reports conducted by the PEB in private healthcare facilities which include clinics and pharmacies throughout Malaysia from 2019 to 2021. The record on compliance and the types of offences were extracted and tabulated using a data collection form. **Results and Discussion:** Controlled substance operations were conducted on 156 private healthcare facilities from 2019 to 2021 in which 87.8% (n=137) of the healthcare facilities were found to be non-compliant towards the supply of DCS. Meanwhile, the top three common offences observed were no recording of the sales of DCS in the prescription book (26.5%), the sales of DCS not as a dispensed medicine/not for treatment purposes (25.2%) and no labelling on the DCS (20.6%). **Conclusion:** This study revealed that the majority of private healthcare facilities inspected during controlled substance operation were non-compliant towards the supply of DCS in Malaysia whereby the most common offence found was the sales of DCS not recorded in the prescription book. Hence, raising awareness among healthcare providers and public-private collaboration could be taken to deter non-compliance towards the supply of DCS. Therefore, there is a need for a comprehensive approach that involves multiple stakeholders, including healthcare providers, policymakers, and community organizations.

NPC20230009

3D Printed Transdermal Microneedles: A Painless Approach for the Personalized Treatment of Osteoporosis

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Background and Objectives: 3-Dimensional (3D) Microneedle (MN) is an advanced drug delivery strategy for delivering drugs (for examples, small molecules and macromolecules) to the skin. The main objective of this study as to determine the effectiveness of 3D printed microneedles (3DMNs) as the future treatment strategy for the treatment of chronic osteoporosis. It is a major concern for patients over the age of 50 and hence the treatment strategies should be catered to provide optimum possible patient compliance, efficacy and safety. 3DMNs are a promising alternative to the existing treatment strategies as it is painless, non-invasive, quick, easy to apply and cost-effective hence, can be utilized for mass production (Scale up) commercially. **Method:** Denosumab, a human monoclonal antibody (Denosis 60®; 147 kDa) was used as a model compound. 3DMNs patches were designed and fabricated using the stereolithographic technique of 3D printing. The ability of the 3DMNs to deliver denosumab through the dorsal skin of Swiss albino female mice was examined using Franz diffusion cells in vitro. Approximately 90% of the total amount delivered was detected in the receptor compartment in 1 h. 3DMNs fabricated by 3D printing were also employed to treat osteoporosis in osteoporotic female mice in vivo. During the six-month uninterrupted in vivo studies, the serum concentrations of critical bone minerals were monitored for six months to assess recovery. **Results and Discussion:** It was found that the denosumab administered by the 3D

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printed microneedles induced an enhanced therapeutic effect in restoring bone-related minerals, compared to subcutaneous injections. **Conclusion:** The findings of this study suggest that 3D printed microneedles can efficiently replace typical needles in the clinical setting, with the dynamic to improve treatment outcomes as well as patient compliance.

NPC20230012

Questionnaire Validation on Knowledge and Perception of Prescribers on Generics and Biosimilars in Oncology

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Background and Objectives: The integration of biosimilars and generics in oncology has the potential to considerably enhance the sustainability of cancer care. Nonetheless, their utilization is often constrained due to safety concerns and inadequate knowledge surrounding them. Thus, this study aimed to validate the questionnaire and explore the prescribers' knowledge and perception of generic and biosimilar oncology drugs. **Method:** A 25-item questionnaire was developed and reviewed by five experts, consisting of lecturers and pharmacists. These experts assessed the questionnaire for relevance, simplicity and clarity using a 4-point Likert rating scale. The questionnaire's validity was pilot tested among 16 prescribers in the National Cancer Institute. Internal consistency of the questionnaire was evaluated using Cronbach's alpha coefficient. **Results and Discussion:** All items in the questionnaire scored ≥ 0.8 in terms of relevance, simplicity and clarity. The scale-based content validity scored more than 0.93, which is above the acceptable limit of ≥ 0.90. The pilot study showed satisfactory internal consistency ($\alpha = 0.75$). Among the respondents, 56% were male and 87.5% were 30-40 years old. Overall findings on the knowledge showed that only 40% of the prescribers were able to correctly differentiate between generics and biosimilars while only 30% identified generics as small molecules with simple structures. Half (50%) of the prescribers felt uncomfortable with prescribing generics, perceiving them to have more side effects than innovators. Similarly, 70% of them were uncomfortable with prescribing biosimilars, with 88% believed that they have more side effects than innovators. **Conclusion:** The questionnaire was successfully validated with good internal consistency. Most of the prescribers still hold negative perceptions and concerns for both generics and biosimilars in oncology. Therefore, there is a substantial need for further education and reassurance among the prescribers to promote the use of generics and biosimilars.

NPC20230001

Assessment of Attitudes, Perceptions and Readiness of Community Pharmacists towards Online Health Services for Minor Ailments in Malaysia

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Background and Objectives: Community pharmacist is the most accessible healthcare providers who plays a vital role in managing minor ailments. Developed countries implemented minor ailment management through an online platform as traditional offline health services have been transformed into online health services due to COVID-19. Nevertheless, there is limited study in Malaysia that explored the practice of community pharmacists in providing online health services for minor ailment management. The aim of the study was to assess the attitudes, perceptions and readiness of community pharmacists towards online health services for minor ailments in Malaysia. **Methodology:** This cross-sectional study was conducted online in which questionnaire was distributed to pharmacists working at community pharmacies from September 2022 till December 2022. **Results and discussion:** 71 community pharmacists were involved in this study. Majority of the community pharmacists agreed to 7-attitude, 10-perception and 9-readiness items. This study reported that only working year as community pharmacist and their perception and readiness scores towards online health services for minor ailments showed statistically significant association. Community pharmacists who worked less than 1 year, had the most favourable perceptions and were the most ready towards online health services for minor ailments compared to those

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working 2–10 years and more than 10 years. Such observation could be attributed to community pharmacists with fewer working years were normally younger and they were more comfortable with technology and digital services. **Conclusion:** Community pharmacists have positive attitudes and perceptions towards minor ailments management through an online platform. Also, they are ready to provide online health services for minor ailments.

NPC20230003

Exploring the Willingness of Pharmacy and Medical Students to be Engaged in Community Service Projects

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Background and Objectives: Service learning has been recognized by The Malaysia Education Blueprint 2015-2025 as a valuable approach that addresses real community needs, while simultaneously equipping students with skills necessary for future workplace. This study aimed to explore the willingness of medical and pharmacy undergraduate students to be engaged in community service projects (CSP) and to determine their perception regarding incorporation of community service into their curriculum. **Method:** An online quantitative cross-sectional survey using convenience and snowball sampling methods. All medical and pharmacy undergraduates of a local private university, aged 18 years and above were invited to participate. Informed consent was obtained. A questionnaire with 35 closed-ended items was validated to ensure its reliability. The variations in willingness to be engaged in CSP and the perception of incorporating community service into curriculum were assessed using the Chi-square, Mann-Whitney and Kruskal-Wallis tests. Factors associated with willingness were determined through multivariate regression. A p-value<0.05 was considered statistically significant. **Results and Discussion:** A total of 300 responses were collected, which gave a response rate of 49.8% (300/603). The majority of participants (71.7%) were willing to be engaged in CSP. Significant predictors of willingness were participants' attitude towards CSP (p<0.001), duration participants would like to be engaged in CSP (p=0.012), past experience (p=0.046) and the preferred working mode (p=0.004). However, subjective norms and perceived behavioural control did not exhibit significant predictive effects. Career and professional development opportunities were identified as the strongest motivating factor (87.3%), while time constraints were identified as the main barrier to community service engagement (83.0%). **Conclusion:** Universities should consider the factors that influence students' willingness to be engaged in CSP, key motivating factors, barriers and issues perceived by students to ensure effective CSP implementation. Institutional involvement and support are vital for successful incorporation of community service into the curriculum.

NPC20230005

Netnography Research: Public Attitudes About Covid-19 Vaccine in Malaysia

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Background and Objectives: The Covid-19 pandemic has prompted scientists and researchers worldwide to establish safe and effective vaccines to contain the outbreak. However, there was hesitation from the public when Malaysian government attempted to roll out the vaccination. This qualitative research used a netnography approach to explore Malaysian attitudes towards Covid-19 vaccines during the pandemic. The main objective is to identify the hindrances and facilitators of Covid-19 vaccines. **Method:** Public comments about Covid-19 vaccines were collected from posts published by the Malaysian news publisher "Bernama" on its Facebook page between 2020 and 2021. These comments were analysed using content analysis and were initially categorised into positive and negative towards Covid-19 vaccines. Then, these positive and negative opinions were further categorised into common themes. **Results and Discussion:** Collected data showed more negative than positive comments. Those who commented positively supported the production, importation, and distribution of Covid-19 vaccines in Malaysia. They also encouraged booster vaccination and believed vaccination of children was necessary to achieve herd immunity. People who refused to be vaccinated due to political hindrances also believed that the government was conducting experiments on citizens. They were also sceptical about the safety and efficacy of the vaccines following persevering deaths in intensive care patients who contracted Covid-19. Some parents opposed the government mandating vaccinations for children because the side effects of the vaccines were

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unknown. **Conclusion:** Malaysians remained sceptical of Covid-19 vaccination, mainly due to the lack of accurate information on vaccine development, transparent plans for vaccination programs, and proper channels to obtain information about the disease and vaccination. Therefore, it is crucial to understand this phenomenon and take prior measures to reduce hesitancy toward vaccinations, such as raising awareness about vaccinations in early childhood education, public health campaigns, and developing a national platform to disseminate health information to public.

NPC20230007

Positive Mental Health Among the Malaysian Population and Their Sociodemographic Correlates

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Background and Objectives: Young adults in Malaysia aged between 20 to 40 years old have the most mental health issues as reported in 2015. Studies investigating mental health status among young working adult population in Malaysia are lacking. This study aimed to measure the mental health status and wellbeing of young working adults in Malaysia and its correlation with the sociodemographic factors. **Method:** A cross-sectional observational survey was conducted using the Positive Mental Health (PMH) instrument in all states of Malaysia. **Results and Discussion:** A total of 410 Malaysian young adults completed the questionnaire. The Malaysian young adult population generally showed an average higher PMH score, 4.16 out of 6. The younger population aged 20 to 24 years old showed lower PMH scores compared to the older age group. Participants from Indian ethnicity, Hindu, married participants, higher income and adults working in the government sector had relatively higher total PMH scores. All sociodemographic factors studied except gender and residing state showed statistical significant association with the total PMH scores ($P<0.05$). Spirituality was the most prominent domain which showed significance across all sociodemographic factors, probably due to spirituality being the key resource to help facilitate positive growth mentally. Participants prioritize emotional support, interpersonal skills and spirituality which contribute to a higher psychological wellbeing. **Conclusion:** Young adults working in Malaysia have an average high PMH score and the sociodemographic factors studied were found to correlate with their positive mental health.

NPC20230008

Telepharmacy: Malaysian public perception and willingness

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Background and Objectives: Telepharmacy has emerged as a good solution with cost savings and provides wider coverage. This study aimed to assess Malaysian public perception and willingness towards telepharmacy **Method:** A cross-sectional survey involving the Malaysian public was conducted using a validated questionnaire. Information regarding participants' demographic as well as perception and willingness towards telepharmacy were collected. **Results and Discussion:** A total of 387 Malaysian public participated in this study with 40.6 % who have heard about telepharmacy service and 8.8 % have used the service. In addition, 13.7% preferred to use an online/app-based tool, 8.0% preferred to have a consultation over the phone and 3.9% preferred a video visit for pharmacist consultation. However, 55% still chose in-person visits to the pharmacy. A majority of the public (88.1%) agreed that telepharmacy services helped to save time and energy with a total mean of 4.41 (SD=0.77). The public believed that telepharmacy would enable consultation to be done much faster (Mean=4.32, SD=0.82). Of the participants, 53.5% showed less positive perception and 55.6% showed less willingness towards the implementation of telepharmacy. From the demographic characteristics, there was no association with the participants' perception and willingness regarding telepharmacy. **Conclusion:** Most participants have less positive perceptions towards telepharmacy. More strategies are required to attract more users of telepharmacy and improve their perception.

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NPC20230010

Türkiye Earthquake: Sharing of a Military Pharmacist's Experiences and Roles in Malaysian Field Hospital in Adıyaman, Türkiye

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Background and Objectives: On 6 February 2023, an earthquake with an amplitude of 7.8 on the Richter scale struck southern and central Türkiye and also northern and western Syria, decimating the city of Adıyaman. A military pharmacist and two pharmacist assistants were part of the 110 medical and logistic personnel from the Malaysian Armed Forces who were deployed to set up and run a level 3 field hospital in Çelikhan, Adıyaman. Over the course of six weeks of operation, Malaysian Field Hospital (MFH) treated a total of 2,415 patients. The general objective of this case was to report and share pharmacist experiences and roles in providing pharmaceutical care and managing Pharmaceutical and Medical Logistics (PharmaMedLog) in MFH. **Method**: This is a retrospective observational case report by a military pharmacist who had been deployed for six weeks to support MFH operation. Prescriptions from both inpatient and outpatient departments were collected, analysed and the data documented. **Results and Discussion:** The findings indicated a significant increase in the utilization of painkillers and antibiotic medications for trauma-related injuries and hygiene-related diseases. The requirement of pharmaceutical support in the mission depends on the nature of the disaster, geographical location, climate and socioeconomic status. Pharmacists are trained to provide PharmaMedLog which is vital prior to any medical mission deployment. **Conclusion:** Pharmacists play a critical role to perform the PharmaMedLog support in accomplishing the mission objectives during Human Assistance and Disaster Relief (HADR) operations.

NPC20230011

Pharmacy predesigned medication labels of High Alert Medication (HAM) injections to prevent medication errors in hospitalized patients

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Objective: To evaluate the effectiveness of Pharmacy predesigned medication labelling of high alert medication (HAM) injections in reducing medication errors in the preparation and administration stages of prescribed medications in hospitalized patients. **Methods**: This is a pre and post intervention study. Pre-intervention group: HAM injections administered from 1st January to 30th April 2022, using traditional labelling (adhesive label without preparation and administration instructions on it). Every ward had a hard copy of Medication Dilution & Administration reference. Post-intervention group: HAM injections administered from 1st May to 30th September 2022, using Pharmacy predesigned labelling (adhesive label with pre-set data of preparation and administration notes on it). Outcome of near miss preparation and administration errors were recorded and analysed. **Results and Discussion**: In the pre-intervention group, 516 doses of HAM were administered to 187 patients. There were 27 and 6 near missed errors related to preparations and administrations recorded, respectively. The near missed error rate was 52.33 per 1000 in preparations and 11.62 per 1000 in administrations. The post-intervention group of 544 doses and comprised of 213 patients with similar routes of administration recorded 5 near missed errors in preparations and zero in administrations. The error rate were 9.19 per1,000 in preparations and zero per 1000 for administrations. Patients receiving HAM injections who did not use predesigned labelling had 5.69 times higher risk of medication errors related to preparations than those who had used predesigned labels. The adults in medical ward had the highest probability of medication errors related to preparations and administrations compared to patients in Intensive Care Unit (ICU). **Conclusions**: The use of Pharmacy predesigned labelling for HAM injections reduced medication errors especially in the last two phases of preparation and administration.

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NPC20230013

Views, Barriers and Facilitators of Healthcare Professionals and Patients Regarding Trastuzumab Biosimilar Use in the Treatment of HER2+ Breast Cancer

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Background and Objectives: Trastuzumab biosimilar was introduced to increase the accessibility and affordability for patients with HER2+ breast cancer. However, its uptake has been poor. Therefore, we aimed to explore the views, barriers, and facilitators of healthcare professionals and patients regarding the use of trastuzumab biosimilar in the treatment of HER2+ breast cancer in Malaysia. **Method:** A qualitative study was conducted at the University Malaya Medical Centre, Kuala Lumpur, Malaysia, from February to September 2020. Convenient sampling was used to recruit 20 healthcare professionals (doctors=6, pharmacists=8, nurses=6) and eight patients. In-depth interviews were conducted till data saturation was achieved and analyzed using Inductive Thematic Analysis. **Results and Discussion:** Three main themes emerged: (1) efficacy and side effects of biosimilar to reference product, (2) cost of biosimilar, and (3) decision-making on trastuzumab biosimilar use. While participants generally had a good understanding of trastuzumab biosimilar, some expressed concerns about its safety, efficacy, and side effects. Patients prioritized the product that offered the highest chance of "curing" their cancer, but factors such as cost-effectiveness, affordability, and accessibility also influenced their decision. Most doctors preferred to make the treatment decision for the patients, citing the patients' lack of knowledge and their own expertise. However, some participants advocated for shared decision-making between doctors and patients. **Conclusion:** Healthcare professionals and patients are willing to use trastuzumab biosimilars, but they require further reassurance regarding safety, efficacy, and side effects. Cost was identified as a significant concern. The findings provide valuable insights for healthcare professionals, policymakers, and stakeholders in addressing challenges and promoting the adoption of biosimilars to improve patient outcomes and healthcare delivery.

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An Overview of the Antimicrobial Stewardship Programme in Penang Adventist Hospital and Measures Taken to Overcome Challenges in Year 2022

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Objectives: Identify challenges in the implementation of Antimicrobial Stewardship (AMS) Programme in Penang Adventist Hospital (PAH) and review the effectiveness of measures undertaken to overcome the challenges. **Method**: From January to December 2022, AMS pharmacists collect data for 12 AMS-regulated antimicrobial agents, which were analysed monthly to determine compliance to hospital policy and the National Antibiotic Guideline (NAG). Where the compliance rate is unsatisfactory, challenges were identified followed by the proposal for temporary and long-term solutions, for which the outcomes were monitored continuously to determine effectiveness. **Results and Discussion:** Since the start of using the new Health Information System (HIS) in PAH, four main challenges were identified, namely the drastic drop in completion rates of AMS request forms, inability to track antimicrobial treatment day, lack of effective ways to communicate pharmacist's recommendation to prescribers, and also duplication of antimicrobial therapy. After setting up pop-up reminder and reinforcing AMS workflow, completion rates of the AMS request forms have increased but there is still room for improvement. Tracking of antimicrobial treatment day is still a challenge but with more AMS pharmacists, tracking has become better. Besides, pharmacists can effectively provide suggestions to prescribers pertaining to antimicrobial therapy through telephone calls and remarks in patients' electronic medication administration record (e-

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MAR). Pharmacy Intervention Note is later incorporated into HIS as supplementary communication platform. Lastly, the proposed measure to include 'antimicrobial icon' for all antimicrobial agents for easy identification and prevent antimicrobial therapy duplication is still pending improvement from HIS. **Conclusion:** Continuous education and reinforcement of workflow is required for an effective AMS Programme.

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RESEARCH PRESENTATIONS

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P01

Exploring Supplement Usage Among Older Adults in Primary Healthcare Settings in Penang: A Qualitative Interview

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Background: Supplementation can potentially improve the overall health and well-being of older adults. However, the utilization of supplements remains questionable in developing countries. **Objective:** To explore the reasons and awareness of older adults on supplementations. **Methods:** A semi-structured interview was conducted in Penang, Malaysia, involving 15 participants targeting older adults aged above 60 who visited public health clinics, private clinics, and community pharmacies. Transcription was conducted by one author and was checked for validity by another author, an academician with experience in qualitative data analysis. Categories were then derived from the codes corresponding to emerging themes. NVivo 12 aided the analysis. **Results:** The median age of participants was 66.48±5.74. Over 70% of the older adults had secondary-level education and stayed with their families. The common healthcare facilities visited were public health clinics and community pharmacies. The majority of the participants reported the consumption of Fish oil, Vitamin C, multivitamins, and Coenyme-Q10 supplements. Three themes emerged from these qualitative findings (i) Reasons for supplementation; (ii) Source of information; and (iii) Integration of supplements with prescription medication. These supplements were primarily taken to promote general health, boost the immune system, enhance physical activity, improve memory, reduce numbness, and replenish essential minerals. Participants expressed that supplements and prescription medication should be utilized to maintain their health. Friends, social media, and recommendations from community pharmacists regarding supplementation often influenced the decision to purchase supplements. However, some participants noted a lack of awareness regarding supplements' indications and potential side effects. Participants wanted community pharmacists to provide sufficient supplementation information and advice on any interaction with prescription medications. **Conclusion:** Older adults can understand supplementation usage; nonetheless, it is necessary to provide comprehensive information and recommendations to ensure the safe and high-quality use of supplements.

P02

Evaluating Pharmacists' Responses Towards Enquiries on Symptoms of Drug-induced Gastroesophageal Reflux Disease (GORD): A Simulated Client Telephone Call Study in Community

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Background: A comprehensive and systematic assessment of patients presenting with symptoms of common ailments in community pharmacy setting is imperative. Negligence in doing so often results in failures to identify underlying drug-related problems as the primary causative factor of any illness. Little is known about the performance of community pharmacists in providing remote consultation services via telephone during the pandemic period. **Objectives:** This study primarily explored how pharmacists addressed the enquiries regarding symptoms of non-steroidal anti-inflammatory drugs (NSAID)-induced GORD presented by a client remotely via phone and their readiness in adopting telepharmacy interventions. **Methods:** A cross-sectional simulated client telephone call study was conducted among community pharmacists in Malaysia between September - October 2020. A simulated client was trained to present a standardised case scenario featuring a patient with symptoms of heartburn to the respondents via telephone. Out of a total of 339 attempts made, 295 telephone encounters were included in this study for analysis. The approaches taken by the pharmacists in response to the enquiries were examined. **Results:** The simulated client managed to consult a pharmacist in 87% of the telephone encounters (n=295). The call durations ranged between 1 to 8 minutes (median=3.5 minutes). Most pharmacists asked

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questions to investigate the duration of symptoms (96%, n=284) and co-morbidities (85%, n=252). A minor fraction of them explored and enquired about medication history (37%, n=109), potential triggering factors (14%, n=40), as well as any action taken to relieve symptoms (10%, n=30). Up to 37% pharmacists (n=109) were able to detect the underlying NSAID-induced GORD in the case scenario spontaneously. **Conclusion:** A positive response rate in this phone-based survey implies favourable readiness for tele-interventions in community pharmacy. Appropriate guidelines and training resources may be instituted to optimise providers' competency in the delivery of minor ailments services on telehealth platforms.

P03

Knowledge, Awareness and Practice of Proper Medication Disposal among the General Public in Malaysia

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Background: Inappropriate medication disposal could negatively impact the environment, healthcare, and community. However, knowledge on proper medication disposal among the general public remains inadequate. Despite ongoing initiatives to encourage the use of the Medication Return Programme (MRP), awareness and utilisation of the service remain unsatisfactory. **Objectives:** The aim of this research was to analyse the respondents' knowledge towards proper medication disposal and their medication disposal practice. The study also examined the awareness and utilisation of the MRP. **Methods:** A questionnaire was disseminated to the public between September to November 2022. The target population was the public above 18 years old throughout Malaysia. The collected data was analysed using the Statistical Package for Social Sciences (SPSS) version 28, applying both descriptive and inferential statistics. **Results:** The study involved 341 respondents. The median score for knowledge towards proper medication disposal was 58.3%. The respondents had basic knowledge on the subject but misunderstood that wastewater treatment plants are able to remove medicinal residues (n = 63, 18.5%). The majority of the respondents disposed their medications in the rubbish as it was convenient (n = 215, 63.0%). Less than 50% of the respondents were aware of MRP, and only 31.1% (n = 106) of the respondents have utilised the service. The mean satisfaction score for health facilities offering MRP was average (3.21/5.00). The lack of awareness towards MRP was identified as a primary barrier, and respondents urged for better accessibility to MRP. A correlation was established between the level of knowledge and the respondents' disposal practices. **Conclusion:** The findings highlighted the mediocre knowledge towards proper medication disposal and the lack of awareness towards MRP. Proactive strategies by the Ministry of Health and stakeholders are required to enforce safe and convenient medication disposal practice.

P04

Lyophilised Rice Starch Sponge as a Potential Buccal Delivery System: Impact of Plasticisers on the Physicochemical

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Background: Rice starch is a promising mucoadhesive biopolymer for formulation development in buccal drug delivery. Lyophilisation of starch mixture can improve formulation stability by reducing starch retrogradation. **Objectives:** This study aims to compare the effects of plasticisers (sorbitol and Tween® 80, T80) on the physiochemical properties and drug release profiles of lyophilised rice sponges (RS) incorporated with propranolol hydrochloride (PPN). **Methods:** RS were prepared by lyophilising rice starch mixture (5%w/v) added with sorbitol or T80 (0.2 and 0.3 g/g rice starch) and PPN (10, 20, 30%w/w). The RS were characterised for mechanical, water absorption capacity (WAC), and drug release profiles. **Results:** Control RS exhibited a rough wrinkled surface with loose layers. Sorbitol resulted in a compact structure with a higher puncture strength (PS) but a lower WAC while T80 loosened the flakes which reduced PS and increased WAC. The modified PS and WAC properties were evidenced in the disturbance of plasticiser-starch interactions in ATR-FTIR and DSC analyses. PPN incorporation increased PS and decreased WAC in plasticised RS but T80-RS has a lower PS than sorbitol-RS. Particularly, T80-RS achieved outstandingly high PS and the lowest WAC with 30%w/w PPN due to PPN-starch interaction via hydrogen bonding. Drug dissolution of control RS reduced with drug loading due to drug

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crystallinity. Control RS with 10%w/w PPN achieved complete drug release owing to drug amorphicity but plasticiser reduced drug release because of a compact structure. Complete drug dissolution achieved at lower sorbitol or higher T80 contents for 20%w/w PPN may be contributed by partial drug crystallinity and high WAC. Nevertheless, the negative effects of drug crystallinity and poor WAC reduced drug release at 30%w/w PPN despite the inclusion of plasticiser. **Conclusion:** In conclusion, this study highlights the influence of plasticisers on physicochemical properties and drug release profiles of RS at different drug loadings.

P05

Identification of Potential Antibiotic-Producing Microorganism

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Background: To explore the soil microbial diversity that has the greatest potential to secrete clinically important antibiotics, this research focuses on the isolation, cultivation and identification of the microorganisms in the samples. **Objective:** To analyze the antibiotic producing capabilities of microbes obtained from various soil samples. **Methods:** Soil was collected from AIMST University. The liquid nutrient agar medium was autoclaved before preparation. After that, a four-fold soil sample serial dilution was initiated. The soil solution was incubated for 24 hours. Colonies of microbes with the inhibitory zone were isolated into the nutrient broth. Subsequently, the nutrient broths with the desired microorganisms had been incubated for 24 hours. The cultivated unknown microbes were spread at the center against the test microorganisms, Klebsiella Pneumonia (ATCC 10031), Escherichia coli (ATCC 8739), Staphylococcus Aureus (ATCC 29737) and Bacillus Subtilis (ATCC 6633). Next, gram staining and soft agar stabbing techniques were carried out. **Results:** The microorganisms in sample A, B and C appeared in pink or red color under the microscope after gram staining. The soil isolates in sample A were comprised of the coccobacillus and diplobacillus. The soil in sample B consists of cocci and streptococci bacteria while sample C were comprised of vibrio, cocci, diplococci, and streptococci bacteria. Diffusion edges were observed in the motility agar of sample A and sample B. Sharp edge was also detected in the motility agar of sample C. **Conclusion:** Gram staining proved that the soil isolates of sample A, B and C were gram negative. Lastly, with the soft agar stabbing test, the unknown microorganisms in sample A and B were concluded to be motile while the movement of microorganisms in sample C was deduced as non-motile.

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