Preliminary biochemical study on metformin monotherapy response in Malaysian type 2 diabetic patients

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Abstract

Background and objective: Metformin is widely prescribed for the treatment of type 2 diabetes (T2D). Despite its extensive use, there are reports on patients who do not respond well even with good clinical compliance. The investigation on metformin monotherapy effectiveness in T2D patients is translated as responders or non-responders presented through patients biochemical analyses following a 3-month treatment.

Methods: Sixty-five (65) patients, newly diagnosed with diabetes on metformin monotherapy from various community clinics in Selangor, Kuala Lumpur and Putrajaya, Malaysia were recruited with consent for this study. The patients were classified into two groups following 3-month of metformin monotherapy. Responders were those who showed more than 0.5% HbA1C reduction from baseline and non-responders were those who did not show more than 0.5% HbA1C reduction from baseline or were prescribed additional medication within the 3 months medical follow-up. Other biochemical parameters measured were lipid profile, C-peptide and fasting plasma glucose (FPG)concentrations.

Results: Twenty-eight (28) of the 65 patients who initially participated in the study had withdrawn. Of the remaining 37 patients, 27 (18 Malays, three Chinese, and six Indians) were responders while 10 (five Malays, three Chinese, and two Indians) were non-responders. After 3-month of metformin monotherapy, both responders and non-responders had displayed significantly decreased HbA1c % ($p < 0.05$). However, the FPG level was significantly reduced ($p < 0.05$) in responders only.

Conclusions: Despite medication adherence, balanced diet, and regular exercise, patients are unable to control their fasting blood glucose levels with metformin. Genetic and proteomic studies are recommended to shed more light on the mechanisms driving metformin responses, thereby was hoped to explain metformin pharmacotherapy efficacy and efficiency.

Keywords:

diabetes; metformin; glucose level; responder; type 2 diabetes mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is the most prevalent non-communicable disease worldwide. It is characterized by chronic elevated blood glucose levels that are higher than the typical baseline, which subsequently with time encourages the state of insulin resistance (Courtney et al., 2023). Symptoms of diabetes, such as weight loss, heavy thirst, blurry vision, fatigue, numb or tingling feet, can be manageable that they go unnoticed. However, when diabetes is poorly controlled, the complications may worsen resulting in diabetic coma, heart attack and stroke. Diabetes is projected to affect over 50% of the populations in many countries without borders. This in turn will impose a significant economic burden on those nations. HbA1c is known as glycated haemoglobin where the glucose molecule is chemically linked to hemoglobin of the red blood cells. The HbA1c test is widely considered as the gold standard for diabetes diagnosis and monitoring since it provides an average blood glucose level of a person over the previous three months in the form of a percentage and fairly more friendly comparing to Oral glucose tolerance test (OGTT). The fraction of glycated hemoglobin increases when the average amount of plasma glucose increases, thus indicating a patient's poor glycemic control over the last 3 months. Comorbidity risks of liver disease, cardiovascular disease, renal disease and other microvascular disorders can be substantially reduced when patients possess optimal glycemic control (Qaseem et al., 2018).

Metformin is a biguanide oral anti-diabetic class of drug widely used as the first-line treatment in newly diagnosed type 2 diabetic patients (CPG, 2020). Metformin works in such a way that it decreases hepatic gluconeogenesis, glycogenolysis as well as intestinal glucose absorption (Lamoia et al., 2021). It is also known to contribute better lipid profile status by lowering cholesterol, triglycerides and LDL levels as well as raising HDL level (Szu Han Lin et al., 2018). Previous reports also delineated that metformin was able to reduce body weight in obese patients. Pu Ruiyang et al. (2020) reported their finding from 21 randomized controlled trial studies with a total population sample size of n=1004, that, patients on metformin therapy experienced a modest reduction (about a one-unit) in BMI at the end of the treatment (Ryu Puiyang et al., 2020). Metformin monotherapy is reported to be able to sensitize the cells in the insulin signalling pathway by working on targeting towards insulin receptor tyrosine kinase activity (Xin Yang et al., 2017). This in turn activates post-receptor insulin signalling pathways and thus cater the gluconeogenesis.

As aging process is influenced by a multitude of factors, identifying, and dealing with various indicators of degenerative-related diseases is a vital step to preserve and improve patients' quality of life. Metformin is pharmacological therapy with proven safety track record and is a commonly acknowledged treatment for diabetes. Unlike phenformin and buphormin which had been withdrawn from general use by Food and Drug Association (FDA) in 1977 due to lactic acidosis and reports of mortality cases in patients using them, metformin rarely results in any adverse side effects (Yerevanian & Soukas, 2019). However, adverse reactions of all oral anti-diabetic drugs, like all drugs are still subjected to wide inter-individual variability (Semiz et al., 2013). A drug regimen may be effective for the majority of individuals, whilst on the other hand, may pose some risks of therapeutic failure or drug toxicity to a minority of individuals (Mohammed et al., 2021). Metformin, however, has been associated with positive side effects. There are global reports from physicians suggesting the beneficial effects of metformin use in obese patients with prediabetes condition or insulin resistance specifically in patients' glucose control as well as weight management. Metformin is the possible alternative given to patients with the above-mentioned criteria to cope with weight issues especially when they do not respond well to lifestyle modifications or other anti-obesity medications (Ryu Puiyang et al., 2020, Yerevanian & Soukas, 2019, Graff et al., 2016).

The American Diabetes Association recommends metformin monotherapy as the first-line treatment for T2DM since 1994. Currently, there are many options for second-line T2DM therapy after metformin. Second-line treatment agents such as sulfonylureas, pioglitazone, dipeptidyl peptidase-4 inhibitors (DPP4i) and sodium glucose transporter-2 inhibitors were disclosed to make the insulin work efficiently in cells (Feingold, 2022). However, some of these second line agents may possess a limited advantage due to patients' poor systemic tolerance. Thus, precludes these medications from being considered as desirable second-line therapies. On top of this reason, second-line oral antidiabetic drugs may also induce adverse effects such as severe hypoglycemia, abrupt weight change, and myocardial infarction, to name a few. However, in order to reconcile efficacy, cost, and undesirable individual reactions to specific medications, clinicians may still need to consider the use of a second-line agent as alternative and/or complementary therapy.

Although insulin therapy has traditionally been recommended as the last resort for T2DM management, insulin sensitizers (metformin, thiazolidinediones), secretagogues (sulphonylureas), incretin-based therapies (dipeptidyl peptidase-4 inhibitors, glucagon-like peptide-1 receptor agonist), and insulin sparing agents (alpha-glucosidase inhibitor) have been implemented in clinical practice for quite

some time (Linong et al., 2021). However, metformin is still the most widely used antidiabetic medicine. Metformin for one, has pleiotropic effects, which could positively influence lipid metabolism, diabetic cardiomyopathy, and vascular function (Salvatore et al., 2021). On the other hand, the main challenge with metformin use is the variability in response.

Individuals may undergo treatment intensification or drug addition to metformin or transition to another antihyperglycemic drug (AHA) if metformin is not tolerated or not working as expected. For example, in some cases where metformin monotherapy fails to exert glycemic control at the highest tolerated dose over 3 to 6 months despite good compliancy, clinicians may suggest additional treatment or changing of drugs. Adverse effects of metformin monotherapy studies based on a meta-analysis research, had revealed mixed results with undetermined significance in regards to gastrointestinal (GI) distress and lactic acidosis. Kalantar-Zadeh et al. reported that although there are 20% to 30% patients on metformin monotherapy complaining of GI disturbances, this rarely necessitates the discontinuation of metformin (Kalantar-Zadeh et al., 2016). Although there are several case reports of lactic acidosis in people receiving metformin were also published (Schousboe, 2012). Taking the fact that there is an estimation of 50% mortality due to lactic acidosis (Huang et al., 2016), clinicians would be cautious to continue the metformin therapy when patients are showing any metabolic conditions related to lactic acidosis. However, this meta-analysis involving 18 randomized controlled trial studies (RCTs) with multiple study arms concluded that there is no clear evidence whether metformin monotherapy has any real benefit or harm to patients' important outcomes listed as all-cause mortality, serious adverse events, health-related quality of life, macro- and micro-vascular complications (Huang et al., 2016). Nonetheless, other researches on *in-vivo* model as well as clinical investigations have revealed that metformin has many other benefits apart from just glucose control that has favourable effects as part of diabetes treatment and in the patients' overall health management. Metformin, for instance, reduces inflammatory cytokines such as TNFa, interleukin (IL-6) and IL-1, as well as other proinflammatory cytokines including macrophages, IL-4, and IL-10 (Hyun et al., 2013).

Therefore, this current research was carried out to identify and gather data on the responses of metformin in patients from parts of Klang Valley, Putrajaya and Selangor, Malaysia. The patients were selected on the basis of two criteria; a. T2D patients who has been consuming only metformin for a period of time and b. Patients who are newly diagnosed with T2D who where started on metformin by their respective general practitioners (GPs) to control their blood glucose levels. In

this research, patients' diabetes status were identified from the biochemical parameters gathered following a three-month of metformin monotherapy. The biochemical data were gathered at baseline and at the end of a 3-months of study. The main objective of the study is to investigate the preliminary biochemical responses of type 2 diabetes patients who had been prescribed with metformin monotherapy.

Material & Methods

Study site and study population

This cross-sectional study was conducted at 13 different community clinics in Selangor, district of Petaling Jaya, Kuala Lumpur and Putrajaya. Blood biochemistry profiles were analyzed at the Chemical Pathology Laboratory, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM) and Molecular Biomedicine Laboratory, Institute of Bioscience, UPM from December 2009 to December 2010.

The study involved the recruitment of Malay, Chinese, and Indian patients aged 20 to 65 who had just been diagnosed with type 2 diabetes from the designated clinics involved and were prescribed with metformin or existing patients in those clinics who have been on metformin monotherapy. The diagnosis was made by a medical officer at each participating community clinic. After the nature of the study was explained to the patients and consent was obtained, the first blood withdrawal for the study was carried out. Based on Clinical Practice Guidelines (CPG), healthcare professionals followed the procedure according to the standard practice given. According to CPG, Malaysia (2020), it was stated that from Diabetes Clinical Audit National Diabetes Registry (2019), 83% of diabetic patients who sought treatment in government clinics, were prescribed metformin (CPG, 2020). Furthermore, based on the Pharmaceutical Service Division, Ministry of Health Facilities (2013), metformin was reported to be the highest anti-diabetic drug being prescribed in health clinics (17.7%), other than glibenclamide (7.5%), gliclazide (6.2%) and insulin recombinant synthetic (Pharmaceutical Service Division, MOH, 2013). Patients from this study were also on metformin monotherapy prescribed by the medical officer following the CPG guidelines. Patients with chronic GI disease, chronic pancreatitis, history of malignant disease, known alcohol and drug abuse, liver and kidney failure as well as patients with other clinical problems potentially causing hyperglycemia were not recruited in this study. Patients with

mixed inter-race marriage were also excluded from this study. This is because, the researchers were aiming to further the study into genes expression profiling using the same samples.

The division of subjects into two groups namely responders versus non-responders were decided based on biochemical data, interview sessions and consultations with the GPs. Responders were defined as the T2D patients who during and after 3 months follow up, had a HbA1c reading reduction more than 0.5%, and were prescribed only metformin as a treatment either for the first-line medication or for follow up treatment continuation. Non-responders could be of three categories. They could be T2D patients who did not manage to reduce their HbA1c reading during their 3 months follow up even at a minimum at 0.5% from the baseline or they were able to reduce the HbA1c reading at that specific figure but only when they were prescribed with other oral antidiabetic drugs (OADs) or insulin injection on top of metformin treatment or they were prescribed with other OAD than metformin entirely during or after the 3 months medical follow up.

All patients were given respondent information sheet to explain the nature of the study and inform consent was obtained prior to blood withdrawal. Each patient had a designated diary where daily activities, such as exercises, diets and medication compliancy were documented. The researcher would contact the patients every biweekly to document the information. Questions were modeled based on Morisky Medication Adherence Scale Score. This experimental protocol was reviewed and approved by the Medical Research and Ethics Committee (MREC), Ministry of Health, Malaysia (National Medical Research Register reference number NMRR-08-1603-2724) and the Research Ethics (Medical) Committee, Faculty of Medicine and Health Sciences, UPM.

Sample Size Determination

According to Cochran 1977 formula, sample size is calculated as (Cochrane, 1977):

$$n = z^2 [1 - \alpha/2] X [(P)(1-P) / d^2]$$

P = prevalence at the clinic which is 0.12

$z^2 [1 - \alpha/2] = 1.96$

d = 10%

$n = 26.7$

A similar study done in Japan had recruited 33 patients, of which resulted in 24 were responders and nine non-responders (Shikata et al., 2007).

Biochemical analysis

For the first blood withdrawal, 10 ml of blood was aliquoted into three different tubes; EDTA tube, sodium fluoride tube and plain tube for glycated hemoglobin (HbA1c) percentage, fasting plasma glucose concentration and serum lipid profile test respectively. After three months, another 10 ml of blood withdrawal was performed for similar purpose. Once again HbA1c percentage, fasting plasma glucose concentration and serum lipid profile were measured. In addition to that, C-peptide concentration was also measured from the second blood withdrawal in order to compare insulin reservoir between responders and non-responders. HbA1c was determined using Vitalab Selectra XL (Vital Scientific B. V., The Netherlands) with dedicated reagents (ELITech Group, France). Fasting plasma glucose (FPG) concentration and serum lipid [High density lipoprotein (HDL), low density lipoprotein (LDL) and total cholesterol (TC)] were measured using Roche Hitachi 902 analyzer instrument (Diamond Diagnostic, USA) while fasting C-peptide concentration was evaluated using enzyme-linked immunosorbent assay (ELISA) kit. T2DM is diagnosed clinically based on the American Diabetes Association criteria which are fasting glucose ≥126 mg/dl (≥7 mmol/L or two hours post-load glucose ≥200 mg/dl (≥11.1mmol/L) or any symptoms of diabetes plus casual fasting plasma glucose ≥200 mg/dl (≥11.1mmol/L) (American Diabetes Association, 2022).

After 3 months on follow up, patients were interviewed and their blood biochemical data were analyzed. The patients were divided into two groups: responders and non-responders based on their HbA1c results, a similar research approach carried out by Shikata et al. (Shikata et al., 2007). The responders were those whose HbA1c percentage (%) had decreased by more than 0.5% (>0.5%) from the baseline within three months of metformin monotherapy. On the other hand, non-responders were those either HbA1c percentage decreased not more than 0.5% (<0.5%) or whom metformin therapy had been discontinued and/or another hypoglycemic drug had been added to the therapy because of adverse effect of diabetes symptoms or insufficient improvement in HbA1c percentage an/ or fasting plasma glucose levels.

Statistical analysis

Analyses were performed with SPSS 16.0 for Windows (SPSS, USA). Changes of HbA1c, fasting plasma glucose concentration, total cholesterol, triglycerides, HDL, LDL and C-peptide concentration were analyzed using one-way analysis of variance (ANOVA) to compare between pre and post-treatment in each group of responders and non-responders. Data are presented as means ± standard deviation (SD). *P*-value was expressed in <0.05 (*), <0.001 (***) or <0.0001 (****) to indicate the significant difference.

Results

A total of 65 patients were initially recruited for this study. During the course of the study, 28 patients withdrew due to various reasons. These patients were not included in the study because they were unable to fully commit to the research procedure which required them to provide information regarding their compliancy towards medication, exercise and diet routine every two weeks.

Out of the remaining 37 patients, it was found that 27 (73%) were responders and 10 (27%) were non-responders. The demographic and clinical characteristics of the patients are summarized in Table 1. There were notable significant differences detected in the responder group's average BMI which portrayed a significant decreased after three months of metformin monotherapy. On the other hand, a small increase of average BMI in the non-responde groups was observed.

Table 1 also indicates a significant reduction (p<0.05) of HbA1c average (approximately 17.3%) and FPG (approximately 37%) relative to the baseline after three months of metformin monotherapy in responders' group. On the other hand, lipid profiles such as LDL and HDL showed significant differences (p<0.05) between pre and post metformin monotherapy in responders and non-responders. However, total cholesterol and TG did not show significant differences (p<0.05) between pre and post-treatment in responders and non-responders group.

Current study shows that the non-responders had displayed higher C-peptide average concentrations than the responders. However, no significant difference (p<0.05) in the readings was observed when comparison was made between responders and non-responders.

Table 1. Demographic and clinical characteristic in the two groups.

Variables	Responders (n=27)		P-value	Non-responders (n=10)		P-value
	Pre-treatment	Post-treatment		Pre-treatment	Post-treatment	
N (Male/Female)	27 (13/14)			10 (7/3)		
Age	46.40 ± 2.70			46.60 ± 2.53		
BMI (kg/m^2)	29.91± 0.873	28.79 ± 0.672	***<0.0001**	27.02 ± 0.882	27.43 ± 0.311	0.40
HbA1c (%) Normal HbA1c (4 – 6.5%)	8.91 ± 0.08	7.37 ± 0.28	**<0.0001***	11.5 ± 0.15	7.6 ± 0.4	**<0.0001***
Fasting Plasma Glucose (mmol/L) Normal FPG (<6.0)	12.25 ± 0.24	7.72 ± 0.90	**<0.0001***	9.69 ± 0.30	9.13 ± 0.15	0.07
C-peptide (ng/mL) Normal of C-Peptide (0.5 – 2.7)	3.44 ± 0.42			3.95 ± 0.29		
Total Cholesterol (mmol/L) Normal TC (<5.2)	4.82 ± 2.34	4.78 ± 0.59	0.99	4.43 ± 0.12	4.38 ± 0.13	1.00
TG (mmol/L) Normal TG (<1.7)	2.15 ± 0.16	2.07 ± 0.11	0.09	2.08 ± 0.19	2.16 ± 0.12	0.39
HDL (mmol/L) Normal HDL (> 1.0)	0.99 ± 0.13	1.17 ± 0.07	**<0.0001***	0.8 ± 0.12	1.09 ± 0.13	**<0.0001***
LDL (mmol/L) Normal LDL (<3.4)	2.71 ± 0.26	2.47 ± 0.24	**<0.001***	2.95 ± 0.13	2.54 ± 0.06	**<0.001***

Values are means ± SD. *P*-value was expressed in <0.05 (*), <0.001 (***) or <0.0001 (****) when tested using two-way ANOVA.

For better understandings, each parameter was described as pre- and post-treatment of metformin monotherapy for the responders and non-responders. It was observed that average HbA1c baseline level in responders group is 8.91% (± 0.08) and was reduced significantly (p<0.0001) to 7.37% (± 0.28) after metformin monotherapy. Whereby, HbA1c in the non-responders showed higher concentration at the beginning of the study which was 11.5% (± 0.15). Following metformin monotherapy for three months, HbA1c in the non-responders was also significantly reduced (p<0.0001) to 7.6% (±0.4). Meanwhile, the findings revealed that the responders had higher average concentration of FPG at baseline relative to the non-responders. It shows that the responders managed to secure a significant reduction (p <0.0001) in FPG concentration, from (12.25 ± 0.24) mmol/L to (7.72 ± 0.90) mmol/L after three months of metformin monotherapy. However, although slight reduction of FPG concentration was observed in the non-responders, no significant difference was indicated.

To assess insulin production during the metformin monotherapy treatment, C-peptide was included and analyzed. The results indicated that C-peptide level in responders was lower compared to the non-responders. However, there was no significant difference in those readings when compared between responders (3.44 ± 0.42) ng/mL and non-responders (3.95 ± 0.29) ng/mL.

On the other hand, average total cholesterol concentration in the responders and the non-responders showed no significant difference between baseline and the three months of metformin monotherapy (Table 1). The responders had shown no significant difference in average total cholesterol levels between baseline (4.82 ± 2.34) mmol/L and three months after metformin monotherapy (4.78 ± 0.59) mmol/L. Meanwhile, total cholesterol in non-responders also exhibited similar results as responders elicited no significant reduction between baseline (4.43 ± 0.12) mmol/L and three months of metformin monotherapy (4.38 ± 0.13) mmol/L.

Generally, both the responders (2.15 ± 0.16) mmol/L and the non-responders (2.08 ± 0.19) mmol/L had almost the same level of TG concentration at the beginning of the study. The responders (2.07 ± 0.11) mmol/L showed minute drop in triglycerides concentration following three months of metformin monotherapy and no significant difference was detected. On the other hand, slight peculiar increment in TG concentration was observed in the non-responders (2.16 ± 0.12) mmol/L after three months. However, it was found to be statistically indifferent.

On the other hand, it was observed that the responders (0.99 ± 0.13 vs 1.17 ± 0.07) mmol/L and non-responders (0.8 ± 0.12 vs 1.09 ± 0.13) mmol/L had significantly higher (p<0.0001) in HDL concentration after three month of metformin monotherapy. As a result, both groups had showed improvement in HDL concentration after three months of metformin monotherapy.

Furthermore, it was observed that responders had significantly reduced (p<0.001) LDL level from the baseline (2.71 ± 0.26) mmol/L to (2.47 ± 0.24) mmol/L after three months of metformin monotherapy. similarly to non-responders which also had significantly reduced (p<0.001) LDL level from the baseline (2.95 ± 0.13) mmol/L to (2.54 ± 0.06) mmol/L after three months of metformin monotherapy.

Discussion

Metformin is a biguanide prescribed as the first-line oral anti-diabetes medication. Patients with HbA1c levels higher than 6.5% or FPG levels higher than 6.0 mmol/L are prescribed metformin as part of their lifestyle management plan. The current study found that, non-responders exhibited no significant changes in FPG before and after treatment despite significantly reduced HbA1c levels at the end of the 3-month treatment. This means that although the non-responders had just as high FPG level prior and post metformin or other glucose lowering agents' medication, having a lower HBA1c after 3 months of treatment explains that they have generally managed to control their blood glucose level. Furthermore, non-responders who were using medications other than metformin or the combination with metformin had experienced a significant decrease in blood glucose levels during or after three-month period. Nevertheless, 73% of patients with metformin monotherapy were subcategoried into the responder group, due to a significant reduction in HbA1c. This study also exhibited that the responders had reduced their FPG levels significantly.

Fasting C-peptide concentration was measured to identify insulin production of the patients. C-peptide is a by-product of insulin synthesis in beta-islet Langerhans cells. It's cleaved from proinsulin, the precursor to insulin, in the pancreas. After transcription and translation of the insulin gene, preproinsulin is formed, which includes C-peptide. As preproinsulin is processed in the endoplasmic reticulum and Golgi apparatus, C-peptide is cleaved off, resulting in the production of insulin and C-peptide as separate molecules. C-peptide improves blood flow, enhances

microvascular circulation, and protects against diabetic complications like neuropathy and nephropathy (Venugopal et al., 2023). From this study, there is no significant difference in C-peptide concentration between the responders and non-responders. Thus, the patients were diagnosed as Type 2 Diabetes Mellitus (T2DM) instead of Type 1 Diabetes Mellitus. A high level of C-peptide indicates beta cells are producing more than the required amounts of insulin suggesting a type 2 diabetes condition. Instead of having a deficiency of insulin, these patients thus may suffer from impaired insulin sensitivity in which case the cells do not respond as they should. Besides, this suggests that C-peptide levels may not be a distinguishing factor in predicting treatment response or outcomes. Other factors such as physiological markers, genetic make-up, or clinical parameters may be more influential in determining treatment response between responders and non-responders (Mahrooz et al., 2015). Furthermore, the similarity in C-peptide levels between responders and non-responders in this study could reflect the natural biological variability in C-peptide production among individuals. They may not be directly related to treatment outcomes and the complexity of the diabetic condition. The progression of diabetes involves multiple pathways and mechanisms that may not be translated in the variability of C-peptide production (Chen et al., 2023). This data may also suggest that as patients were treated with metformin which or with other OADs, both groups were having similar beta cells capacity which in turn reflected as similar C-Peptide production levels. Regardless of metformin therapy or other OADs, both groups were displaying C-Peptide levels which higher than its normal range (0.5 – 2.7 ng/mL), 3 months of OADs treatments did not improve the sensitivity of cells involve in insulin signalling pathways.

On the other hand, there are few possible reasons why 10 out of 37 respondents were considered as non-responders to metformin monotherapy. Morisky Medication Scale Score which was implemented in this study implemented self-recall during follow-up (García-Muñoz 2023). Based on the follow-up reports given by the non-responders, metformin was discontinued its prescription in the patients or they were introduced to the alternative antidiabetic medicines other than metformin such as sulfonylureas or other combinations treatments. These patients were either suffering the side effects of metformin, sucah as GI disturbances and nausea or did not show significant improvement despite considerable amount of duration taking metformin. Noncompliancy towards medications was also one of the reasons why these patients could not manage a good HbA1C status (Koski et al., 2004).

According to CPG, the optimal concentration of TGs should be less than 1.7 mmol/L. However, in this study, responders and non-responders exceeded this

range by 0.47 and 0.56 mmol/L, respectively. Responder' HDL concentrations are higher than the CPG's suggested HDL concentration; yet, non-responders also met the optimal concentration. Despite HbA1c in non-responders being reduced significantly however, the same positive results was not captured for the FPG levels. Nevertheless, lipid profile status in non-responders as well was improved particularly in HDL and LDL. This could in part be explained by varied response to the medications which they had taken based on two-week follow-up assessment over three months of metformin monotherapy treatment. Metformin has been established as lipid lowering by inhibiting the lipogenesis. Goldberg et al. reported the benefits of metformin by showing the improvement status in HDL and LDL levels in alleviating atherogenic risks on diabetic patients (Goldberg et al., 2022). For the LDL concentration, both responders and non-responders achieved an ideal target of < 3.4 mmol/L after three months of metformin monotherapy (CPG, 2009). Nevertheless another OADs such as sulfonylureas and pioglitazone also exhibited the lipid lowering drugs. According to CPG 2020, lifestyle modification must be maintained at every juncture of T2DM treatment.

Metformin is associated with possibility of slight to modest weight loss and it has been proven to reduce the BMI (Rashid et al., 2019). In present study, the average BMI of the responder's group is significantly decreased after the study treatment in 3 months. In this study, metformin significantly improves BMI in responders compared to the non-responders. Nevertheless, patients BMI did not show any significance changes in non-responders eventhough the HbA1c levels exhibited significance reduction. Previous study showcased that sodium glucose cotransporters-2 (SGLT-2) inhibitors and sulfonylureas (DPP-4i) significantly lowered subjects HbA1c however this is not the case in BMI (Bidulka et al., 2024). The study focused on the rescue phase for non-responders who did not demonstrate improvement in their clinical indications. The study found that there were no significant changes in BMI and other clinical indicators when the antidiabetic drugs were switched. We hypothesised that transitioning patients from metformin to other oral antidiabetic drugs (OADs) would cause fluctuations in their weight.

Other variables, including genetics, organ function, and the nature of the disease, may influence drug response and disposition. Variability in metformin transporters, which is involved in the drug metabolism, may be one of the causes of a poor response to the medication. Additionally, the observed outcomes of metformin therapy among non-responders subjects may be explained by the interaction between various gene polymorphisms and non-genetic factors. Future research is recommended to examine how polymorphisms, particularly those in the genes of organic cation transporters (OCTs) with diminished functionality, impact

individuals reaction to various medications in particular, metformin as well as other combinations treatments, which are frequently used together with metformin.

In 4 – 10% of patients, the adverse effect of metformin presented in the form of GI intolerance has resulted in the premature termination of treatment. One of the explanations of why this happened is probably because of several genes that have been identified to affect the response of metformin such as *solute carrier family 22 member 4* (*SLC22A4)* and *peroxisome proliferator-activated receptor gamma coactivator 1-alpha* (*PPARGC1A)* (Nabrdalik et al., 2022). Vohra et al. has suggested that in the responders, the gene, *SLC22A4* which is involved in the transport and absorption of drugs in the intestines, has evoked improved response to metformin (Vohra et al., 2023). Meanwhile, in non-responders, there was a reduction in the number of copies of the *PPAR coactivator 1 alpha* (*PPARGC1A*) gene which in turn may lead to altered glucose processing (Vohra et al., 2023).

Diabetic patients with multiple symptoms are usually treated similarly despite the various complications of T2DM (Fang et al., 2022). Action of oral anti-diabetic drugs and their adverse drug reactions such as hypoglycemia are subjected to wide inter-individual variability (Baye et al., 2021). In most cases, a drug regimen may be effective for the majority. This, however, does not exempt the risk of adverse effects, therapeutic failures or drug toxicity in a minority of others. (Mohammed et al., 2021). Management of T2DM thus may involve considerable amount of time and cost due to the lengthy trial and error method (CPG, 2020).

There are cases that result in different drug responses due to genetic polymorphism. In spite of the fact that physiological factors such as age and concomitant drug therapy may also influence the effects of medication, genetic determinants which were inherited remains stable through a person's life (Zhang et al., 2017). In other words, genetic factors attributed to along-term impact on metformin response and other biochemical features, unless specific gene therapies are explored for diabetes control. It is critical to recognize the complexity of such approaches and the necessity for ongoing study and development in pharmacogenomics research.

In the current study, diet and physical activity are recommended for all patients during the metformin monotherapy. However, many factors could be seen as limitations to this study. Both groups showed that metformin improved lipid levels, specifically HDL and LDL. Better treatment options for non-responders to metformin which improve their cholesterol and glucose levels should be clearly

addressed. Additional and more extensive research with a longer treatment time as well as a larger sample size is needed to understand whether metformin response influences the therapeutic effects of lipid-altering medication in T2DM patients.

Conclusion

In conclusion, the responders group had shown positive clinical response to metformin monotherapy in accordance to HbA1c and FPG levels. However, some patients had stopped taking metformin monotherapy or were prescribed additional therapy due to multiple issues and thus was categorised as non-responders Despite metformin's ability to lower the atherogenic risk, it may be reflected as negligible alterations in lipid profiles. We suggest further investigation on the pharmacological interaction of metformin between responders and nonresponders.

Declaration of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authourship contribution statement

Zalinah Ahmad: Funding acquisition, Writing – review & editing. Suriani Sukor: Writing – Original draft, Project administration and Formal analysis. Nurliyana Najwa Md Razip: Writing – review & editing, Validation and Formal analysis. Dayang Sarah Yasmine Abang Yusuf: Writing – review & editing.

Data availability

The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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