The influence of metformin and empagliflozin administration on gut microbiota in individuals with type 2 diabetes mellitus and hypothyroidism


Abstract. Background. There is a lack of studies focusing on the combined impact of metformin, empagliflozin, and levothyroxine on the gut microbiota of patients with type 2 diabetes mellitus (T2DM) and hypothyroidism. The purpose of the study was to examine how the combination of metformin and empagliflozin affects gut microbiota composition in patients with type 2 diabetes and hypothyroidism. Materials and methods. We enrolled 47 patients who have been receiving hormone replacement therapy with levothyroxine at a stable dose over the past 2 years and were newly diagnosed with T2DM. All participants were divided into two groups and received either metformin alone or metformin plus empagliflozin for 6 months. Metabolic and hormonal parameters were measured before and after treatment, and stool samples were analyzed using PCR sequencing. Results. The study found that in both groups, there was an improvement in carbohydrate metabolism, lipid profile, and liver transaminases after treatment. The group treated with metformin plus empagliflozin had a more significant reduction in glucose, glycated hemoglobin, and atherogenicity coefficient than the group treated with metformin alone. We also found that combination therapy resulted in lower levels of Firmicutes and an increase in the number of Actinobacteria, as well as a higher ratio of Bacteroides fragilis to Faecalibacterium prausnitzii. Conclusions. The study shows for the first time that the combination of metformin, empagliflozin, and levothyroxine can directly affect the gut microbiota composition in patients with T2DM and hypothyroidism. These changes may be necessary for treating this cohort of patients and require further investigation.

Keywords: diabetes mellitus; hypothyroidism; metformin; empagliflozin; gut microbiota

Introduction

Recent studies have highlighted the complex interplay between gut microbiota and various metabolic and cardiovascular diseases (CVDs) [1]. The gut microbiota has been implicated in the pathogenesis of type 1 and type 2 diabetes mellitus through mechanisms involving immune response modulation and metabolic regulation [2–4]. Furthermore, alterations in gut microbiota composition, known as dysbiosis, have been associated with CVDs, including atherosclerosis, hypertension, and heart failure [4–6]. Interestingly, while the relationship between gut microbiota and diabetes, as well as CVDs, is well-documented, the specific connection to hypothyroidism is not directly addressed.

However, given the systemic nature of gut microbiota interactions, it is plausible that dysbiosis could also impact thyroid function, as the microbiota influences wide-ranging aspects of host physiology, including immune and metabolic pathways that could intersect with thyroid hormone regulation, although there are a small number of such works. Recently, there has been research conducted in this field, and one particular study, published in 2022, examined the impact of metformin, pioglitazone, and levothyroxine on the gut microbiota of overweight patients with type 2 diabetes mellitus and hypothyroidism [7]. The effect of metformin on the gut microbiota of patients with type 2 diabetes mellitus (T2DM) has been documented, with studies indicating that metformin may alter the composition of the gut microbiota,
promoting the growth of specific beneficial bacteria and potentially contributing to its hypoglycemic effects [3].

The impact of metformin on the gut microbiota has been well-documented, with studies indicating that it may exert hypoglycemic effects by altering the microbial composition, such as increasing the abundance of Bacteroidetes and Verrucomicrobia and affecting the integrity of the intestinal barrier and bile acid metabolism [8]. Metformin, a widely used antidiabetic agent, has been shown to influence the gut microbiome, remarkably increasing the abundance of certain beneficial bacteria. For instance, Methanobrevibacter, a genus associated with methane production and involved in the gut’s energy metabolism, has been reported to increase in abundance with metformin treatment in individuals with T2DM [9]. This aligns with findings from other studies that metformin can enrich beneficial taxa, such as Lactobacillus and Akkermansia, which are known to affect gut barrier integrity and anti-inflammatory properties positively [10].

There is a lack of studies focusing on the combined impact of metformin, empagliflozin, and levotriglycerol on the gut microbiota of patients with T2DM and hypothyroidism. Interestingly, while metformin is associated with beneficial changes in the gut microbiota, it also has potential adverse effects, such as an increase in Escherichia species, which may mediate some of its intestinal side effects [11, 12].

There is evidence that SGLT2i reduces cardiovascular morbidity and mortality, probably by increasing ketogenesis, which may influence the gut microbiome. While the papers do not explicitly discuss the relationship between SGLT2i-induced ketogenesis and the gut microbiome, it is known that SGLT2i can increase ketone body production as a result of shifting energy substrate utilization from glucose to fat [13]. This shift could theoretically affect the gut microbiome composition and function, which has been implicated in cardiovascular health. Some studies show increased Lactobacilli and decreased phylum Bacteroidetes in patients with established coronary artery disease. However, the specific interactions between empagliflozin, levotriglycerol, and the gut microbiota are not detailed in the provided context. In summary, while metformin has been shown to influence the gut microbiota in ways that may contribute to its therapeutic effects in T2DM, the combined impact of metformin, empagliflozin, and levotriglycerol on the gut microbiota of patients with T2DM and hypothyroidism remains unclear.

The purpose of the study was to examine how the combination of metformin and empagliflozin affects gut microbiota composition in patients with type 2 diabetes and hypothyroidism.

Materials and methods

We conducted a study with 47 individuals recently diagnosed with T2DM. Within the study group were 26 women and 21 men, averaging 54.0 ± 6.8 years. Additionally, these individuals had previously been diagnosed with hypothyroidism and had been undergoing treatment for at least 2 years. After an initial examination, the individuals were divided into two groups and prescribed distinct treatment protocols.

The first group (Mtf), which included 23 patients, received a daily dose of 2000 mg of metformin. The second group (Mtf + Empa), consisting of 24 individuals, was given a combination treatment of 25 mg of empagliflozin and a daily dosage of 2000 mg of metformin. Both groups received treatment with levotriglycerol from 50 to 175 mcg daily to maintain a euthyroid state. It should be noted that the initiation and gradual increase of the metformin dose for both groups began with a daily dose of 500 mg and increased every 2 weeks until the total daily dose of 2 g was reached.

After six months of consistent treatment, the patients were scheduled for a follow-up evaluation. This assessment involved analyzing various factors such as fasting glucose, HbA1c, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), TSH, fT3, aspartate transaminase (AST), and alanine transaminase (ALT) levels. In addition to these measures, stool samples were collected to examine gut microbiota composition.

It was analyzed using amplicon sequencing to examine and determine the gut microbiota composition in each stool sample. Stool samples are an excellent source for studying the gut microbiome, representing the gastrointestinal tract’s luminal bacteria. Amplicon, mainly targeting the 16S rRNA gene, is widely used for characterizing microbial species in such samples [14]. This technique identifies and quantifies different taxa within a sample by using sequence differences in the 16S rRNA gene as a microbial fingerprint [14]. However, it is essential to note that stool samples may not adequately reflect the mucosally adherent bacteria, and there can be substantial variability in the assessment of the gut microbiotal community according to the type of sample used [15]. We could identify and examine the different microbial species in the patient’s gut through the amplicon sequencing analysis of stool samples. This data provided valuable insights into how different treatment plans could potentially affect the composition of the gut microbiota and how it may impact the management of diabetes.

We collected stool samples from all patients to evaluate the gut’s microbiotal composition. From these samples, we extracted DNA and focused on specific genetic markers to determine the levels of various gut bacteria species, including Firmicutes, Bacteroidetes, and Actinobacteria. We also calculated ratios such as Firmicutes/Bacteroidetes and Bacteroides fragilis group/ Faecalibacterium prausnitzii, as these ratios have been linked to metabolic disorders like diabetes.

Biochemical blood serum parameters were analyzed using an automatic analyzer, Cobas Pro (using test systems from Roche Diagnostics, Germany), with c 501 modules. This analyzer detected the levels of total cholesterol, triglycerides, HDL, LDL, glucose, HbA1c, TSH, fT3, AST, and ALT.

Additionally, the atherogenicity coefficient (AC) was calculated according to the formula:

\[
AC = \frac{\text{Total cholesterol, mmol/L} - \text{HDL, mmol/L}}{\text{HDL, mmol/L}}.
\]

The atherogenicity coefficient greater than 3 was considered to be elevated.

The results were presented with the format M ± m, where M represents the arithmetic mean, m indicates the mean square deviation, and n corresponds to the number of patients examined in each group. A p-value of less than 0.05 was considered statistically significant to determine differen-
Table 1. Laboratory parameters of the examined patients before and after the treatment

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before treatment</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mtf n = 23</td>
<td>Mtf + Empa n = 24</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>11.89 ± 0.44</td>
<td>12.34 ± 0.51</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>8.39 ± 0.25</td>
<td>8.42 ± 0.32</td>
</tr>
<tr>
<td>TSH, mIU/mL</td>
<td>3.83 ± 0.12</td>
<td>3.79 ± 0.14</td>
</tr>
<tr>
<td>fT3, pg/ml</td>
<td>2.60 ± 0.09</td>
<td>2.54 ± 0.08</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.42 ± 0.26</td>
<td>6.55 ± 0.34</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.31 ± 0.24</td>
<td>2.26 ± 0.29</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>5.39 ± 0.47</td>
<td>5.65 ± 0.44</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.02 ± 0.20</td>
<td>0.97 ± 0.12</td>
</tr>
<tr>
<td>AC, units</td>
<td>5.23 ± 0.28</td>
<td>5.68 ± 0.32</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>51.74 ± 3.39</td>
<td>53.84 ± 3.56</td>
</tr>
<tr>
<td>AST, U/l</td>
<td>48.20 ± 3.27</td>
<td>46.40 ± 3.44</td>
</tr>
</tbody>
</table>

Notes: * — p < 0.05 compared to values before treatment; ** — p < 0.05 compared to values of the Mtf group.

Results

Both study groups had abnormalities in carbohydrate metabolism, lipid profile levels, and liver transaminases at the start of the study, as shown in Table 1. The levels of thyroid hormones of both groups did not exceed the reference norms since all patients were on stable replacement therapy at the start of the study, as shown in Table 1. The levels of thyroid hormones of both groups did not exceed the reference norms since all patients were on stable replacement therapy. After treatment, the indicators of the thyroid panel of both groups did not change significantly.

The carbohydrate metabolism levels improved, with a significant decrease in glucose and HbA1c observed in both groups after treatment. However, when comparing the two groups, the Mtf + Empa group exhibited a significantly lower reduction in glucose and HbA1c compared to the Mtf group. In the analysis of the atherogenicity coefficient, similar findings were observed. Additionally, it is worth noting that treatment for 6 months led to significant improvements in the lipid profile. In the Mtf + Empa group, there were improvements in the levels of the entire lipid profile, while the Mtf group showed improvements in total cholesterol and LDL levels. Significantly improved levels of transaminases were observed in both groups after treatment, as indicated by a return to reference norms. This finding was statistically significant when compared to pre-treatment indicators. All of the above-noticed changes are reflected in Table 1.

The composition of the gut microbiota underwent significant changes in the two groups, as shown in Fig. 1. Specifically, the Mtf group exhibited a decrease in the number of Firmicutes at 18.08 ± 0.64 %, which was lower than the Mtf + Empa group with 21.82 ± 0.86 % (p < 0.05). Likewise, the levels of Actinobacteria were found to be significantly higher (p < 0.05) in the Mtf + Empa group (4.52 ± 0.26 %) compared to the Mtf group (2.02 ± 0.17 %). The abundance of Bacteroidetes in both the Mtf group (66.27 ± 1.02 %) and the Mtf + Empa group (60.68 ± 1.12 %) was high, and no significant difference was observed between these two treatment approaches. After treatment, the Firmicutes/Bacteroidetes ratio significantly decreased from 1.32 ± 0.11 to 2.02 ± 0.17 in the Mtf group and 0.270 ± 0.021 in the Mtf + Empa group and 0.360 ± 0.028 in the Mtf + Empa group. In contrast, the patients in the Mtf + Empa group exhibited a significantly higher Bacteroides fragilis group to Faecalibacterium prausnitzii ratio 66.70 ± 1.36 compared to the Mtf group 0.130 ± 0.018 (p < 0.05).

Discussion

Hypothyroidism is a condition characterized by an underactive thyroid gland, which can lead to various metabolic abnormalities, including insulin resistance and impaired
glucose control. T2DM is also characterized by insulin resistance and impaired glucose regulation [7, 14]. The combination of metformin and empagliflozin, two commonly prescribed medications for type 2 diabetes, has been found to affect the composition of gut microbiota in patients with co-existing hypothyroidism. Several studies have suggested that alterations in gut microbiota composition may contribute to the development and progression of both hypothyroidism and type 2 diabetes [15, 16]. Therefore, investigating the impact of medications used for these conditions on gut microbiota is very important.

The literature suggests that metformin, as a first-line treatment for type 2 diabetes mellitus, influences the gut microbiota composition. However, the specific changes and their implications for glucose metabolism are complex and only partially consistent across studies. Metformin has been associated with increased proportions of specific microbiota, such as Bacteroidetes, Firmicutes, and Escherichia, which may contribute to its hypoglycemic effects [3, 17]. Additionally, metformin treatment has been linked to alterations in gut metabolomics, particularly an increased ability to produce butyrate and propionate, which are involved in glucose homeostasis [18]. Contradictions arise with the findings of a prospective cohort study that reported no significant overall change in microbiome composition structure after metformin treatment, although specific microbial species did show alterations [19, 20].

Furthermore, other studies have indicated that metformin can lead to a decrease in the Firmicutes to Bacteroidetes ratio and affect functional pathways in the gut microbiota, such as amino acid metabolism [16]. The list of studies examining or comparing the gut microbiota composition in patients taking empagliflozin in combination with metformin is minimal. Therefore, the combination of these two drugs can have an exciting effect on the composition of the microbiota.

In contrast, the patients who received both MtF and Empa treatments exhibited a significantly higher ratio of the Bacteroides fragilis group to Faecalibacterium prausnitzii than those who received only MtF treatment. This increase in ratio is a cause for concern as it is associated with diarrhea and abscesses in the intestine. Nevertheless, the study also showed improvements in the diversity and abundance of beneficial bacterial species, particularly an increase in Actinobacteria levels. This increase indicates an overall improvement in gut health as these bacteria have favorable anti-inflammatory, anti-allergic, and antitumor properties. Furthermore, the study revealed reductions in pro-inflammatory bacterial groups like Firmicutes and improved lipid and glucose homeostasis markers. These findings emphasize the potential of combination therapy in modulating gut microbiota and suggest its role in managing diabetes and hypothyroidism.

A study of the effect of metformin and the combination of metformin with empagliflozin on gut microbiota composition was conducted in two groups of patients. This made it possible to detect more pronounced changes in the microbiota in patients who received empagliflozin. However, it remains an open question whether the combination of metformin and empagliflozin leads to the potentiation of their effects on the gut microbiota or vice versa to the cross-destructive impacts. To get answers to these questions, further observations and a more significant number of studies in patient groups are needed.

Conclusions

The study found that both groups had improvements in carbohydrate metabolism, lipid profile, and liver transaminases after treatment. The group that received metformin plus empagliflozin had more significant reductions in glucose, glycated hemoglobin, and atherogenicity coefficient than the group that received metformin alone. We also found that the combination therapy led to a decrease in Firmicutes, an increase in Actinobacteria, and a higher ratio of Bacteroides fragilis group to Faecalibacterium prausnitzii. Our study shows for the first time that the SGLT2 inhibitor empagliflozin, in combination with metformin on the base of replacement therapy with levothyroxine, can directly affect the composition of intestinal microbiota in patients with type 2 diabetes and hypothyroidism. However, further research is needed to understand the underlying mechanisms better and to determine if these changes in gut microbiota contribute to the overall therapeutic effect of the combination therapy.

References

Вплив призначення метформіну та емпагліфлозину на мікробіоту кишечника в осіб із цукровим діабетом 2-го типу та гіпотиреозом

Резюме. Актуальність. На сьогодні недостатньо досліджень, присвячені комбінованому впливу метформіну, емпагліфлозину та левотироксину на мікробіоту кишечника в патієнтів із цукровим діабетом 2-го типу (ЦД2) та гіпотиреозом. Метою дослідження було визначити, як комбінація метформіну і емпагліфлозину впливає на склад кишкової мікробіоти в патієнтів із цукровим діабетом 2-го типу та гіпотиреозом. Матеріали та методи. Ми залучили 47 пацієнтів, які отримували метформін у поєднанні з емпагліфлозином та левотироксином в стабільній дозі впродовж року.

Висновки. Дослідження вперше показують, що комбінація метформіну та емпагліфлозину може бути важливою для лікування цієї когорти хворих та потребують подальших досліджень.

Ключові слова: цукровий діабет; гіпотиреоз; метформін; емпагліфлозин; кишкова мікробіота