Estimation of Serum Creatinine, Aspartate Aminotransferase, Alanine Transaminase, and Hemoglobin A1c% Levels among Diabetic Patients using Metformin/Dipeptide Peptidase-4 Inhibitor Combination and Insulin – A Cross-Sectional Study

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Abstract

BACKGROUND: Diabetes is a leading cause of morbidity and mortality worldwide, imposing a considerable burden on health systems and societies as it affects both individuals and their families and has a large impact on the economic and social development of a country. Nowadays, to treat diabetes, patients are taking drugs such as metformin in combination with dipeptide peptidase-4 (DPP-4) inhibitors and insulin for their treatment. There are many physiological and pathological changes while taking drugs for treating diabetes. AIM: Through this research, we can have an idea about the health of the patient and help to detect the level in which different drugs are affecting the patient's kidney and liver.

METHODS: A cross-sectional study was done on diabetes type 1 and type 2 diabetic patients who are undergoing treatment with insulin and DPP-4 inhibitors/metformin combination drugs. The study continued for 3 months including preparation, practice, and data analysis.

RESULTS: In this study, our results show there is no significant change in all biochemical parameters: aspartate aminotransferase (AST), alanine transaminase, creatinine, and hemoglobin A1c (HbA1c) between diabetic patients treated with insulin and metformin/DPP-4 combination.

CONCLUSION: Metformin/DPP-4 combination gives a better glycemic control than other medications, whereas the levels of AST, creatinine, and HbA1c are unaffected using either insulin or metformin/DPP-4 inhibitors combination.

Introduction

Diabetes mellitus (DM) is a major global public health issue with a growing incidence and prevalence, particularly in developing and newly developed countries. The total number of people with diabetes will rise to almost 20 million in 2025. By 2050, this is projected at >29 million people—a 165% increase over the 2000 level [1]. Concern about this chronic disease focuses on serious complications related to DM that can affect many critical organ systems, leading to more extreme and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy, cardiovascular diseases (CVD), as well as hepatopathy [2]. Type 1 diabetes is due to insufficient insulin production caused by the destruction and loss of pancreatic islet β-cells containing insulin. Type 2 diabetes is due to the relative tolerance of insulin [3].

Type 1 DM (T1DM) is a chronic immune-mediated disease that is characterized in genetically susceptible individuals by the selective destruction of β-cells. The proportion of patients with DM diagnosed with T1DM is projected to be 5–10%, with an annual rise of 3.8%–5.6%, although this proportion may be underestimated [3]. More recently, recombinant techniques have developed human insulin. Since its first use in the 1930s, insulin has been the pillar of type 1 diabetes care. In patients with type 2 diabetes, insulin is also used and is refractory to lifestyle measures (diet, physical activity, and weight loss) and oral hypoglycemic agents. Insulin can be administered intravenously, intramuscularly, and subcutaneously, and the dosage and duration of administration differ according to the formulation and the person being treated. Insulin adverse incidents are due to hypoglycemia [4]. Therapy with insulin also results in weight gain. Local injection reactions (lipoatrophy) and hypersensitivity reactions,

particularly with newer forms of recombinant insulin, are rare. Analysis shows that a variety of liver disorders are associated with DM such as irregular deposition of glycogen, nonalcoholic liver fat disease, fibrosis, cirrhosis, hepatocellular carcinomas, unusually elevated hepatic enzymes, acute hepatic disease, and viral hepatitis [4]. In addition, excessive liver fat accumulation can exacerbate insulin resistance and result in severe metabolic dysfunction. Hepatocytes can be killed by fatty liver, and hyperglycemia can lead to increased morbidity and mortality among diabetic patients [5].

The most well-known diabetes is type 2 diabetes mellitus (T2DM). Protection from insulin and malfunction of the pancreas islets are two significant flaws in the pathophysiological foundation of T2DM. In the current administration of T2DM, numerous antidiabetic drugs play important roles. Recommended as the first-line oral antidiabetic drug, metformin enhances glycemic regulation primarily by attenuating hepatic insulin resistance. Inhibitors of dipeptide peptidase-4 (DPP-4) avoid degradation of incretin hormones and thus enhance pancreatic islet dysfunction.

Like all medicines, metformin can cause side effects, although not everyone gets them.

Metformin is contraindicated in patients with renal or hepatic insufficiency, very elderly patients, and in patients with conditions of circulatory dysfunction such as congestive heart failure due to increased risk of lactic acidosis. Although metformin-associated lactic acidosis is an extremely rare condition (most estimates are ≤10 events per 100,000 patient-years of exposure), cases continue to be reported and are associated with mortality rates of 30%–50%. Metformin plasma levels >5 μg/mL are generally found when metformin is implicated to cause lactic acidosis.

Such sustained very high elevations in plasma metformin concentrations (therapeutic range <2 μg/mL) usually are observed in individuals with poor renal function (i.e., reduced metformin clearance), impaired hepatic metabolism (i.e., reduced lactate clearance), and/or in the presence of increased production (i.e., sepsis, CHF, reduced tissue perfusion, or anoxia) [5].

There are also side effects that a person might experience depending on the type of insulin they are taking.

- Initial weight gain as the cells start to take in glucose
- Blood sugar that drops too low, or hypoglycemia
- Rashes, bumps, or swelling at an injection site
- Anxiety or depression
- A cough when taking inhaled insulin.

Insulin shots cause the cells in the body to absorb more glucose from the bloodstream. As a result, taking too much or administering injection at the wrong time may cause an excessive drop in blood sugar leading to hypoglycemia.

There is also the possibility that taking insulin

will cause more severe side effects, although these are less common.

Fat necrosis may develop in people who regularly inject insulin. This condition causes a painful lump to grow in the subcutaneous tissue, which is just below the skin's surface.

A 2018 review compared insulin therapy with metformin treatment. Metformin is another glucose-lowering treatment for people with type 2 diabetes [6].

These researchers found that the insulin therapy group in the study had an increased risk of several complications, including:

- Heart attack
- Stroke
- Eye complications
- Kidney problems.

Another review concluded that the risks of insulin therapy might outweigh the benefits for people with type 2 diabetes.

- The need to increase the dose and complexity of the treatment plan over time
- The increased risk of severe hypoglycemia
- A higher possible risk of death
- A potential increase in the risk of specific cancers, including pancreatic cancer.

Objectives

Primary objective

1. Estimation of serum levels of creatinine, aspartate aminotransferase (AST), alanine transaminase (ALT), and hemoglobin A1c (HbA1c) among diabetic patients using metformin/DPP-4 inhibitor combination and insulin.

Secondary objective

1. To evaluate the concentration of AST/ALT enzymes as liver markers of a diabetic patient
2. To measure the creatinine level in a diabetic patient's serum as a kidney marker
3. To estimate HbA1c level in diabetic patients using metformin/DPP-4 combination and insulin for the treatment
4. To correlate between the duration of the disease and AST, ALT, and creatinine with HbA1c levels.

Materials and Methods

Research design

This study was a cross-sectional study.

Study population

Diabetes type 1 and type 2 diabetic patients who are undergoing treatment

Inclusion criteria

Diabetic patients who are being treated with insulin and diabetic patients who are being treated with DPP-4 inhibitors/metformin combination were included in the study.

Exclusion criteria

Diabetic patients who are not undergoing treatment or using other types of treatment were excluded from the study.

Study settings

The study was conducted at the Thumbay Labs in Gulf Medical University/Thumbay university hospital, Ajman.

Duration of study

The duration of this study was 3 months including preparation, practice, and data analysis.

Study instrument and validation procedure

The validation procedure is done according to the CAP and CLIA for precision, accuracy, and linearity.

Ethical issues

The proposal of the study was submitted to the ethical committee for approval as per the GMU research policies. The IRB approval was obtained before the studies.

Procedure of study

Blood specimens were collected for HbA1c >6.5 (collect blood in ethylenediaminetetraacetic acid [EDTA]) test, serum creatinine, and liver enzyme (serum and plain container) test from the diabetic patients who are belonging to the inclusion criteria attending the Thumbay University Hospital, Ajman.

Measuring liver enzymes

ALT

The patient's serum sample which was in a yellow/plain vacationer tube for ALT was measured with DxC 700 AU Beckman Coulter chemistry analyzer.

The main principle is that ALT transfers the amino group from alanine to 2-oxoglutarate to form pyruvate and glutamate. The addition of pyridoxal phosphate to the reaction mixture ensures the maximum catalytic activity of ALT. The pyruvate enters a lactate dehydrogenase catalyzed reaction with NADH to produce lactate and NAD+. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the ALT activity in the sample. Endogenous pyruvate is removed during the incubation period.

AST

The patient's serum sample which was in a yellow/plain vacationer tube for AST was measured in DxC 700 AU Beckman coulter chemistry analyzer.

The main principle is that AST transfers the amino group from aspartate to 2-oxoglutarate to form oxaloacetate and glutamate. The addition of pyridoxal phosphate to the reaction mixture ensures the maximum catalytic activity of AST. The oxaloacetate is reduced to L-malate by malate dehydrogenase catalyzed reaction while NADH is simultaneously converted to NAD+. The decrease in absorbance due to the consumption of NADH is measured at 340 nm and is proportional to the AST activity in the sample.

Measuring creatinine in serum

The patient's serum sample which was in a yellow/plain vacationer tube for serum creatinine was measured in DxC 700 AU Beckman coulter chemistry analyzer.

It is a colorimeter/modified Jaffe's kinetic method.

The main principle is that the creatinine forms a yellow-orange colored compound with picric acid in an alkaline medium. The rate of change in absorbance at 520 is proportional to is proportional to the creatinine concentration in the sample

Measurement of HbA1c

The patient's whole blood sample which was in EDTA/lavender vacationer tube for HbA1c was measured in DxC 700 AU Beckman coulter chemistry analyzer.

It's a turbid metric/turbid metric immunoinhibition method.

The main principle is that the HbA1c advanced assay B (30009) involves the use of three reagents: total hemoglobin (T-Hb) (R1), HbA1c (R1 and R2), and hemolyzing reagent (R1). The DxC 700 AU chemistry analyzer automatically performs the whole blood hemolysis using 200ul of hemolyzing agent (R1) and 2ul of whole blood in the cuvette.

Tetradecyltrimethylammonium bromide in the hemolyzing reagent eliminates interference from the leukocytes. The hemolyzed whole blood is then added to both the T-Hb reagent (R1) is used to measure T-Hb concentration by a colorimetric method. Change in absorbance is measured at 570/660 nm. HbA1c reagent (R1 and R2) is used to measure HbA1c concentration by turbid metric immunoinhibition method. In the reaction, HbA1c antibodies in the reagent combine with HbA1c from the sample to form soluble antigen–antibody complexes. Polyhaptens from the reagent then bind with the excess antibodies, and the resulting agglutinated complex is measured turbid metrically. Change in absorbance is measured at 340/700 nm.

Results

A total of 61 diabetic patients were included in this study. Out of 61 patients, 15 (9%) are treated with insulin and 46 (28%) patients are treated with metformin/DPP-4 combination. The population consisted of both male and female patients.

The demographic characteristics of the population included in this study are listed in Table 1 and Pie Chart 1. Our result shows a weak negative correlation between the duration of treatment of diabetes with insulin and metformin/DPP-4 inhibitor combination versus ALT ($p = 0.0468$, $R = -0.260$; Figure 1 and Pie Chart 2).

Table 1: Demographics and clinical characteristics among diabetic patients using metformin/DPP-4 combination and insulin drug at baseline

Variable	Frequency (%)
Gender	
Male	36 (22)
Female	23 (14)
Age (years)	
>50	34 (56)
<50	22 (36)
BMI	
>25	33 (54)
<25	14 (22)
Duration	
>10	32 (52)
<10	20 (32)
Hypertension	
Present	21 (34)
Absent	38 (62)
Kidney disease	
Present	0
Absent	61 (100)
Heart disease	
Present	5 (8)
Absent	56 (92)
Liver disease	
Present	3 (5)
Absent	58 (95)

BMI: Body mass index.

In addition to that there is no correlation between the duration of treatment and AST ($p = 0.128$ $R = -0.193$; Figure 2), creatinine ($p = 0.866$, $R = 0.019$; Figure 3), and HbA1c ($p = 0.328$, $R = 0.009$; Figure 4). The p-value for ALT ($p = 0.0468$) is significant but not very highly significant and as per the other biochemical parameters that are AST, creatinine, and HbA1c, there
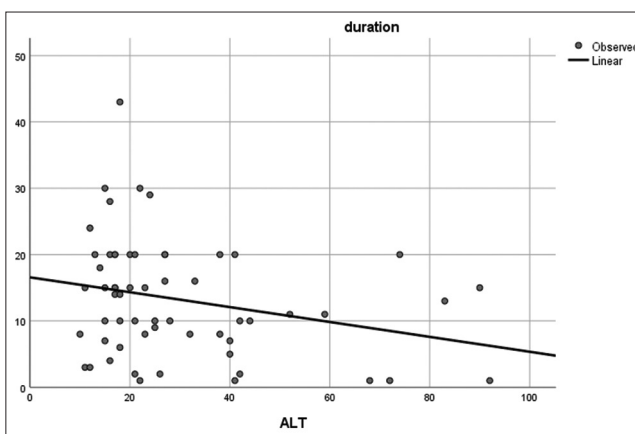

Figure 1: Correlation between alanine transaminase levels and duration of treatment with insulin or metformin/dipeptide peptidase-4 combination in diabetic patients ($p = 0.0468$, $R = -0.260$)

is no statistical evidence suggesting that they have any significant value with the duration of treatment. In this study, our results show there is no significant change in all biochemical parameters AST, ALT, creatinine, and HbA1c between diabetic patients treated with insulin and metformin/DPP-4 combination, as shown in Table 2.
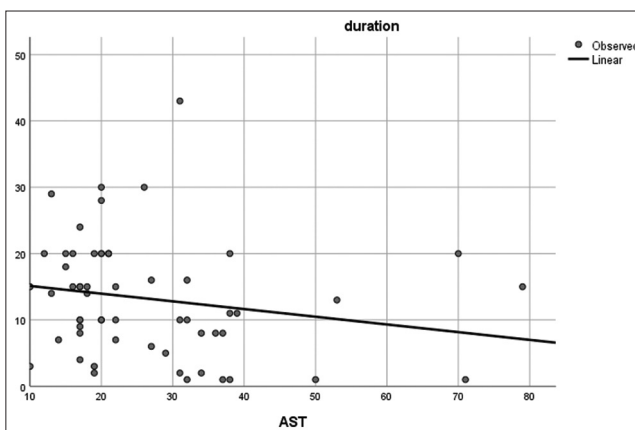

Figure 2: Correlation between aspartate aminotransferase levels and duration of treatment with insulin or metformin/dipeptide peptidase-4 combination in diabetic patients ($p = 0.128$, $R = -0.193$)

Discussion

DM is a major global public health issue with a growing incidence and prevalence, particularly in developing and newly developed countries. The total number of people with diabetes will rise to almost 20 million in 2025. By 2050, this is projected at >29 million people—a 165% increase over the 2000 level [1]. Concern about this chronic disease focuses on serious complications related to DM that can affect many critical organ systems, leading to more extreme and irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy, CVD, as well as hepatopathy [2]. Type 1 diabetes is due to insufficient insulin production caused by the

destruction and loss of pancreatic islet β-cells containing insulin. Type 2 diabetes is due to the relative tolerance to insulin [3].
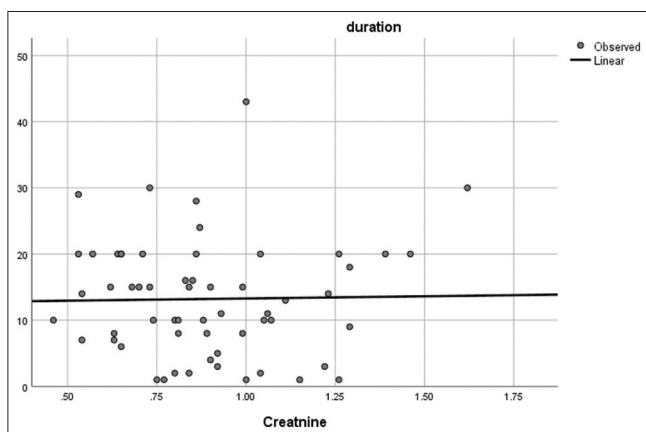

Figure 3: Correlation between creatinine levels and duration of treatment with insulin or metformin/dipeptide peptidase-4 combination in diabetic patients (p = 0.866, R = 0.019)

The complementary mechanism of action of DPP-4 inhibitors and metformin is the reason for combining the two drugs. The primary effect of metformin is the reduction of hepatic output and increased insulin sensitivity in muscle and liver. GLP-1 levels are increased by DPP-4 inhibitors and hence stimulate insulin secretion and inhibit glucagon secretion [7].
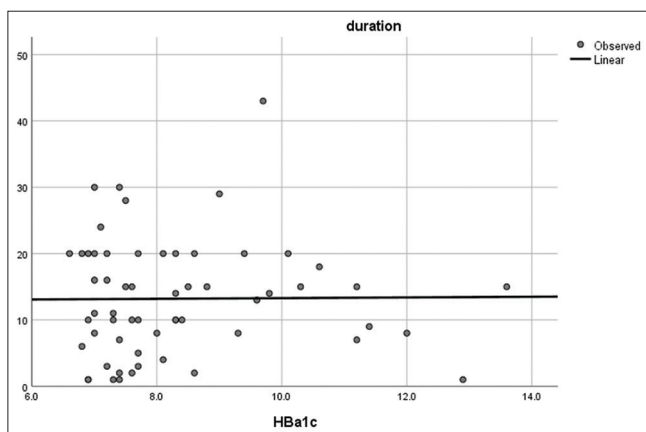

Figure 4: Correlation between hemoglobin A1c% levels and duration of treatment with insulin or metformin/dipeptide peptidase-4 combination in diabetic patients (p = 0.328, R = 0.009)

Our study shows that there is no significant change between diabetic patients using insulin and metformin/DPP-4 inhibitor combination in all biochemical parameters AST (0.128), ALT (0.468), creatinine (0.866), and HbA1c (0.328) as listed in Table 2. Our results are the same as in this study that was performed on patients who had type 1 diabetes who took insulin as treatment and was diagnosed with advanced kidney disease. They were younger and had a higher affinity for CVD. The patients receiving metformin alone or in combination had a significantly higher estimated glomerular filtration rate (eGFR). The lowest value of urinary albumin–creatinine ratio was observed in patients taking DPP-4 inhibitors alone or in combination [8].

Table 2: Comparison of biochemical parameters among diabetics using metformin/DPP-4 combination and others using insulin drugs

Parameters	Mean ± SD	p
Creatinine		
Insulin	0.9020 ± 0.35815	0.866
Metformin/DPP-4	0.8852 ± 0.21128	
AST		
Insulin	24.13 ± 14.999	0.128
Metformin/DPP-4	27.32 ± 14.432	
ALT		
Insulin	26.00 ± 16.886	0.468
Metformin/DPP-4	31.39 ± 21.294	
HbA1c		
Insulin	8.887 ± 1.9813	0.328
Metformin/DPP-4	8.152 ± 1.4407	

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine transaminase, HbA1c: Hemoglobin A1c.

In a study to compare the effects of DPP-4 inhibitors and sulfonylureas on albuminuria in type 2 diabetic patients, it was found that there was no significant difference in the albumin–creatinine ratio. However, in patients subjected to DPP-4 inhibitors, the albumin–creatinine ratio was significantly low, suggesting that DPP-4 inhibitors prevent albuminuria, which indicates kidney disease. In the same study, it was found that there was a significant change in serum creatinine, eGFR, and systolic blood pressure [9]. The results of both the studies are in agree with each other because serum creatinine values were insignificant (p = 0.866), and no correlation was found between the duration of disease and levels of creatinine as displayed in Figure 3.

Table 3: Comparison of biochemical parameters among diabetics using metformin/DPP-4 combination and others using insulin drugs according to gender

Parameters	Mean ± SD	p
Creatinine		
Male	0.9456 ± 0.21920	0.044
Female	0.8017 ± 0.8895	
AST		
Male	29.64 ± 14.245	0.037
Female	21.61 ± 13.836	
ALT		
Male	34.78 ± 21.193	0.016
Female	22.57 ± 16.517	
HbA1c		
Male	8.486 ± 1.7772	0.385
Female	8.109 ± 1.3090	

SD: Standard deviation, AST: Aspartate aminotransferase, ALT: Alanine transaminase, HbA1c: Hemoglobin A1c.

According to a narrative review, 60%–85% of each dose of the DPP-4 inhibitors is predominantly excreted through urine in the unchanged parent compound form. In hypertensive patients, sitagliptin (DPP-4) attenuates blood pressure. In addition to the above mentioned findings, numerous preclinical studies and meta-analyses imply that DPP-4 inhibitors have a direct renoprotective effect which possibly helps in acute and chronic failure [10].

In this study, we included patients with HbA1c levels >6.5% who were being treated with either insulin or metformin/DPP-4 combination therapy for at least 1 year. In another study, 99 patients with newly diagnosed type 2 diabetes were treated with a combination of metformin and OHA (100 mg sitagliptin, 15 mg of pioglitazone, and either gliclazide-MR or glimepiride) over a study period of 24 weeks. On the completion of the study, the percentage of HbA1c level >7.5% had reduced by 2.5%–2.7% [11]. The
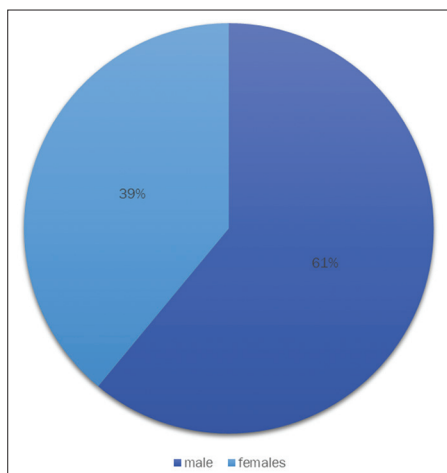
Pie Chart 1: Percentage distribution of study group according to gender

results of this study correspond with HbA1c level >6.5% in our study which we measured at baseline for patients being treated with metformin/DPP-4 combination for more than 1 year. In a cross-sectional study conducted in Sri Lanka, it was observed that the HbA1c levels (6.2%) in patients treated with DPP inhibitors were lower than other oral hypoglycemic drugs [12].
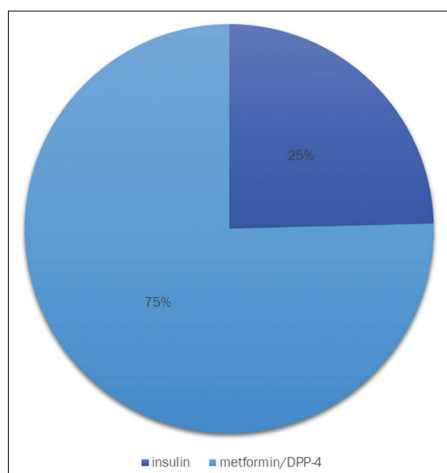
Pie Chart 2: Percentage distribution of study group according to medication

In our study, the duration of treatment with metformin/DPP-4 combination and insulin showed no significant change in the levels of AST, creatinine, and HbA1c. However, there was a mild significant difference in the levels of ALT. According to the J Am pharma association, metformin is usually advantageous for patients who suffer from nonalcoholic fatty liver. Although nonalcoholic fatty liver often results in increased transaminase levels, it should not be considered a contraindication to metformin [13]. Our study corresponds with these findings since there is no significant change in the levels of AST, creatinine, and HbA1c in patients treated with metformin/DPP-4 inhibitors and insulin. Nonetheless, a significant change in the level of ALT shown in our study could be because of pre-existing liver disease in some patients (5%) as presented in Tables 1 and 3.

Conclusion

Metformin/DPP-4 combination gives better glycemic control than other medications, whereas the levels of AST, creatinine, and HbA1c are unaffected using either insulin or metformin/DPP-4 inhibitors combination.

Acknowledgments

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