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A DESCRIPTIVE COMPARISON OF RESPONSE OF ORAL HYPOGLYCEMIC AGENTS AMONG T2DM IN A BACKDROP OF INSULIN RESISTANCE

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ABSTRACT

Introduction: Different homeostatic models for the assessment of beta cell function in patients with insulin resistance in type 2 diabetes mellitus suggest that Dipeptidyl Peptidase (DPP-4) inhibitors cause less beta cell stress. **Aims:** The present study aimed to compare and contrast insulin resistance in two groups of patients taking oral hypoglycemic agents, DPP-4 plus metformin and glimepiride plus metformin, on the basis of fasting and postprandial c-peptide and insulin resistance estimated by homeostatic model assessment of insulin resistance (HOMA-IR). **Methods:** This preliminary descriptive observational study was conducted from 2018 to 2019 in the service Laboratory of the Department of Biochemistry, in collaboration with the Endocrinology Department, Nil Ratan Sircar Medical College and Hospital, Kolkata. Serum C-peptide, serum insulin, and plasma glucose levels were measured in both fasting and post-prandial states along with glycated hemoglobin. **Result:** In the fasting and fed state, the secretagogue effect of glimepiride-metformin combination was significantly higher ($p = 0.017$) than that of the linagliptin-metformin combination. **Conclusion:** Patients treated with glimepiride showed high post prandial insulin levels and high post prandial glucose excursion. This finding can be explained by the probable increase in insulin resistance, which is reflected in their post-prandial C peptide level. However, in the case of linagliptin, one mechanism of decreased post-prandial glucose is believed to be the inhibition of α-cell glucagon release, thereby relieving β-cell stress.

Keywords: DPP-4, GLP-1, HOMA-IR, OHA, T2DM

INTRODUCTION

The impact of diabetes mellitus (DM) and its complications on public health is increasing globally. India is no exception to this, with 77 million diabetic people projected to rise to more than 134 million in 2045(Pradeepa& Mohan, 2021). Thus, so far, the prevalence of DM is concerning; India is second in the world in accordance with the International Diabetes Federation.

Metabolic markers of type 2 DM (T2DM) include insulin resistance, impaired secretion of ofinsulin, and excessive glucose production in hepatocytes alongwith dyslipidaemia(Galicia-Garcia et al., 2020).Thehyperinsulinemic state in T2DM occurs as the disease progresses, and this state of insulin resistance is followed by compensatory hyperinsulinemia, which ultimately leads to pancreatic β cell failure, and glucose intolerance is marked by an increase in postprandial blood glucose(PPBG). A rise in hepatic glucose production, along with declining insulin secretion, leads to overt diabetes mellitus, causing beta cell failure. (Galicia-Garcia et al., 2020)

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Pancreatic β cells synthesize and secrete insulin as a preproinsulin. In the beta cellsC peptide and mature insulin are stored and secreted simultaneously after proteolytic cleavage of preproinsulin(Fu et al., 2013).Glucose regulates insulin secretion from thebeta cell of endocrinepancreas.

Glucose enters betacells by a facilitative glucose transporter (GLUT2), which stimulates insulin secretion. The rate-limiting step that controls glucose-mediated insulin secretion is the phosphorylation of glucose by glucokinase, and ATP is generated by glycolysis; this ATP-sensitive K+ channel is inhibited by ATP generated during glycolysis. These K+ channels consist of two different proteins: one binds to sulfonyl-ureas, a meglitinidin group of oral hypoglycemics, and the second protein (Kir6.2) is a transmembrane K+ channel protein. Depolarization of the beta cell membrane caused by K+ channel inhibitionopens voltage-gatedCa^{2+}channels, resulting in calcium influx. This event triggers the secretion of insulin(Walczewska-Szewc& Nowak, 2021).

Incretins are secreted from gastrointestinal (GI) tract neuroendocrine cells following food intake. This event causes the glucose-mediated upregulation of insulin secretion and downregulation of glucagon secretion. Glucagon-like peptide 1 (GLP-1),which is secreted by small intestinal enteroendocrine cells,is a 30-31 amino acid peptide. This most potent incretin, GLP-1, stimulates the secretion of insulin, which is glucose-dependent, and occurs when fasting blood glucose is high(Nauck et al., 2021).Glucose-dependent insulinotropic peptide (GIP) alongwith GLP-1 decrease blood glucose by glucosedependent manner by stimulating insulinsecretion(Holst, 2019). Unlike GIP, it is preserved in T2DM patients. This is why common pharmacotherapy includes the use of incretin analogs to stimulate endogenous insulin secretion. In circulation, the C peptide circulates longer because of its slow clearance. Therefore, estimation of serum C-peptide can be used as a marker of endogenous insulin secretion. (Hardy et al., 2000)

Insulin secretion and sensitivity are interrelated events. They play a major role in T2DM pathophysiology(Park et al., 2021). In initial phase of T2DM there is hyperinsulinemia,followed by insulin resistance toregulate glucose homeostasis. At the initialstage of T2DM, the defect of insulin secretionis mild. This specifically involves glucose-mediated insulin secretion. The cause forthis decrease in insulin secretion in T2DMstill remains unanswered. This is assumed that insulin resistance complicated by a second genetic defect may lead tobeta cells failure(Kaufman, 2002). Insulin resistance is defined as the inability to deal with increased glucose uptake and utilization after the addition of insulin in an individual compared with the euglycemic population(Lebovitz, 2001).

Recent advances in the pharmacotherapeutics and management of T2DM have formulated oral hypoglycemic medication that modifies different disease pathogenesis in T2DM. Based on their pharmacodynamics and kinetics, oral hypoglycemic medications are subdivided into different groups; one that increase insulin secretionand sensitivity along with reduce glucose production and enhance Glucagon like peptide -1 (GLP-1) action. Metformin, member of Biguanides, lowers hepatic glucose production and slightly improves peripheral glucose utilization(Foretz et al., 2019). The insulin secretagogue glimepiride helps insulin secretion through its interaction with the ATP-sensitive Potassium channel on the beta cell. “Incretins" modify and increases glucose-sensored insulin secretion. GLP-1 agonists or drugs that enhance endogenous GLP-1 activity are used to manage T2DM. Dipeptidyl peptidase-4 (DPP-4) inhibitors act by inhibiting the degradation of native GLP-1 and enhancing the incretin effect(Deacon,

2020). DPP-4 inhibitors are believed to reduce postprandial glucagon release from alpha cells, along with their main actions on β-cell(Phillips &Prins, 2011).

C-peptide, a marker for beta-cell function, is widely used to assess the risk of complications, drug response, and glycemic control in T2DM. In contrast to insulin, C-peptide had a substantially longer t1/2 than insulin (35 minutes vs. 3-5 min). Furthermore, in individuals receiving subcutaneous insulin replacement, insulin immunoassay fails to distinguish between endo-and exogenous insulin, but the differential kinetics of plasma C-peptide accurately estimates endogenous insulin secretion. The long half-life (t1/2) of C-peptide is believed to be its unique ability to evade hepatic degradation and ultimately renal clearance(Galgani et al., 2010).

The aim of our study was to recognize beta cell stress by estimating serum C-peptide in fasting and post prandial states and HOMA–IR in the linagliptin- and metformin-treated groups compared to the glimepiride and metformin combination group.

The objective of our study was to compare fasting and Postprandial C-peptide, HOMA- IR%, and β cell activity among three groups: T2DM patients taking different combinations of oral hypoglycemic drugs and another age- and sex-matched control group.

METHOD

This preliminary descriptive observational study was conducted between 2018 and 2019. First, the Institutional Ethics Committee reviewed the study protocol (No/NMC/7500 dated 13/11/2017). Permission was obtained prior to the commencement of the study. Both the participant and control gave willful consent to take part in this project.The study was performed in the service Laboratory of the Department of Biochemistry in collaboration with Endocrinology Dept., Nil RatanSircar Medical College and Hospital, Kolkata. Individuals of both sexes with T2DM were incorporated into this project in accordance with the American Diabetes Association guidelines, 2020.

Total 207 individuals which includes both case and control groups participated in this project Total 207 individuals participated in this project. These individuals wereT2DM patients taking oral hypoglycemic agents. Patients who were taking insulin (n-30) were excluded. Another 37 patients with increased urinary albumin-to-creatinine ratio were also excluded. Those taking antihypertensive drugs (n = 16) were excluded. Another 24 individuals declined to participate in the study. A total of 100 participants, including 60 T2DM patients who were on an oral hypoglycemic drug combination (30 subjects were on metformin and glimepiride and rest were on metformin and linagliptin) were included, and 40 healthy non-diabetic subjects were chosen for comparison.

Under all aseptic and antiseptic conditions, 5 ml whole blood and spot urine samples were drawn from the subjects and divided separately into EDTA vials, Fluoride& Oxalate vials, and clotted vials in fasting and post-fed conditions.

Post prandial plasma glucose was drawn 2 hours after 75 gm oral glucose load. Fasting and PPBG were measured using the GOD-POD (glucose oxidase and peroxidase) method(Trinder, 1969), which oxidizes glucose to gluconic acid and peroxidase cleaves hydrogen peroxide, which further reacts with phenol and 4-aminoantipyrine to form a red-colored quinonemine dye complex. The amount of glucose present in sampleis directly proportional to intensity of the colour formed which is measured at 505 nm HbA1c level was estimated using HPLC, and(Sacks, 2012). blood HbA1c≥ 6.5% was considered to indicate diabetes. Glycated hemoglobin, referred to as Hemoglobin A1C (IFCC mmol/mol and NGSP %), was estimated in the blood by high performance

liquid chromatography(HPLC) with a cation-exchange column in the Hb-Vario kit on the Hb-VarioTMAnalyser.

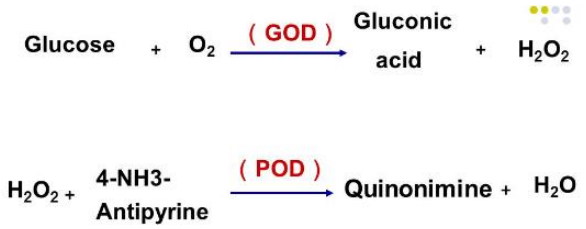

Figure 1. Showing basic principle of plasma glucose estimation by GOD-POD method

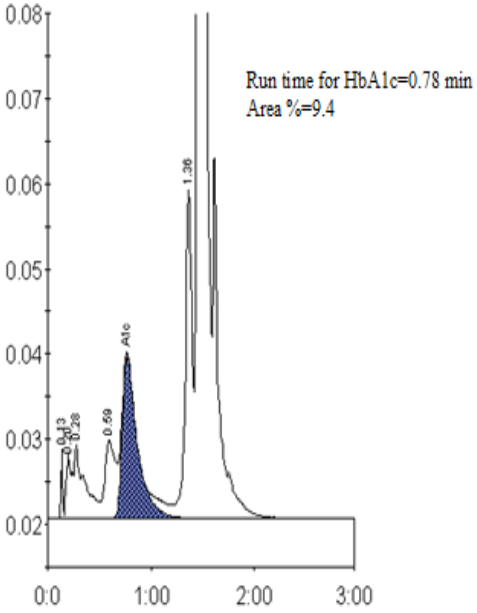

Figure 2. Showing standard chromatogram of HbA1C of a particular sample tested in our laboratory

Fasting plasma insulin was measured by chemiluminescence immunoassay using siemens immulite 1000 automated analyser(Tanaka & Matsunaga, 2000).It is a solid-phase, two-site chemiluminescent immunometricassay(Clark, 1999). The HOMA2 model was used to estimate insulin resistance, insulin sensitivity, and β-cell function from fasting plasma glucose and fasting plasma insulin concentrations using the HOMA 2 Calculator (www.OCDEM.ox.ac.uk)(Wallace,2004). Insulin resistance was defined as a HOMA2-IR more than 1.8(Praveen et al., 2012). Fasting and post-prandial C-peptide levels were assayed by chemiluminescence using a Siemens Immulite 1000 automated analyzer. This is a solid-phase competitive chemiluminescent enzyme immunoassay(Hardy et al., 2000). Urinary albumin: creatinine ratio(ACR) was measuredusingimmunoturbidometric method to rule out nephropathic changes(Bargnoux et al., 2014).

Suitable statistical methods and techniques were applied with the help of a software-based computer program (SPSS version17) to analyze the results, and the significance of differences among different groups was calculated using analysis of variance (ANOVA), with a p-value <0.05.

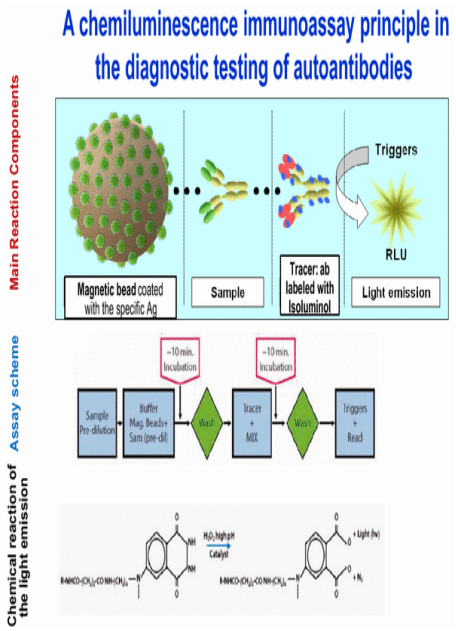

Figure 3. Showing basic principle of chemiluminescence of C-peptide in serum as mentioned Siemens Immulite1000 kit insert

RESULTS

Most of the study population belongs to 30-60 years old (average 43.7 years) group. A total of 100 patients were enrolled in this study from 2018 to 2019 and were randomized into three groups. Among them, 40, 30, and 30 patients were included in the control, metformin + glimepiride group(group1) and metformin + linagliptin(group2)add-on groups, respectively. In our study, FPG (mg/dl) was significantly increased in both case groups (group 1-167.9±53.1, group2-171.6±21.6) compared to the control group (92±7.2). HbA_{1C}values were raised in the case group than in the controls (Table1). T2DM cases were confirmed by estimating fasting blood glucose (FPG) and HBA1C levels. Fasting and post-prandial C-peptide levels were higher in the case group than in the control group.

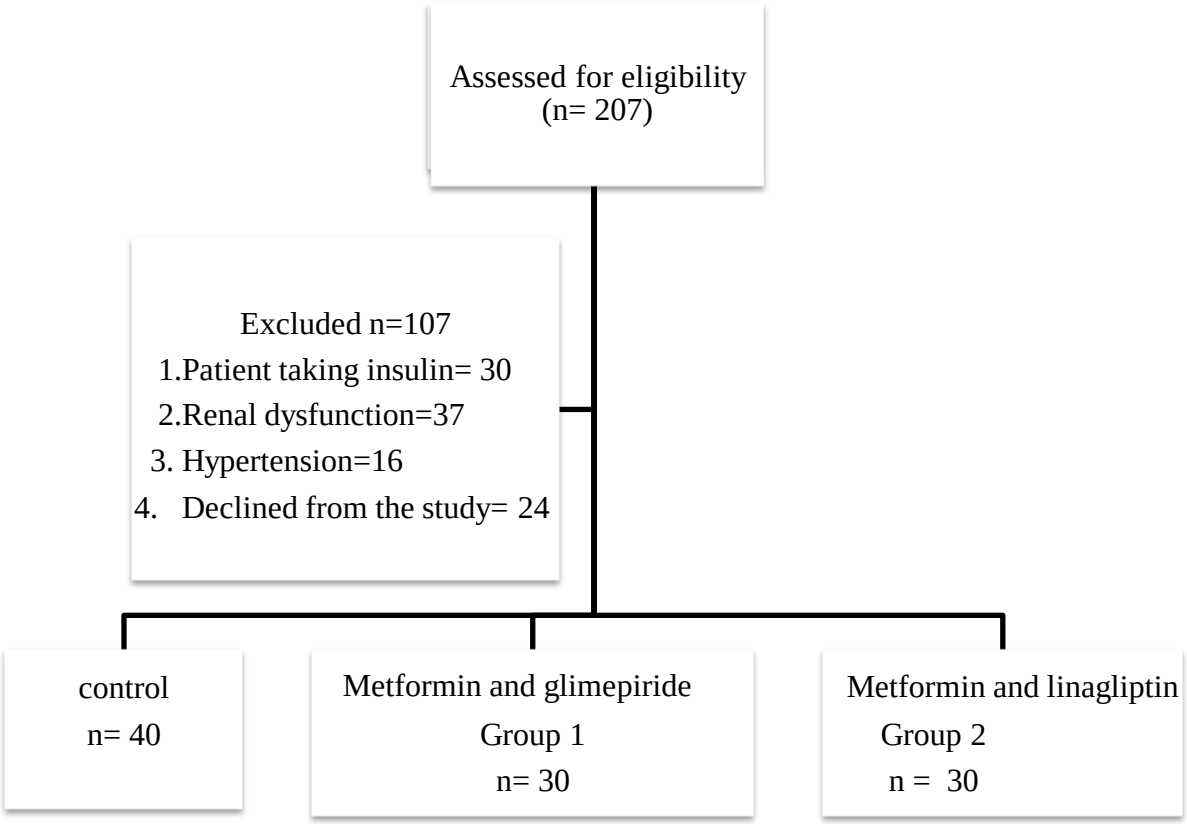

Diagram 1. STROBE Flow Chart

Table 1. Showing all the measured and calculated parameters in this study along with mean±S.D.

Biochemical parameters	**Group1(Mean±S.D.)** Metformin+ Glimiperide N=30	**Group2(Mean±S.D.)** Metformin +Linagliptin N=30	**Control** (Mean±S.D.) N=40
FPG[mg/dl]	167.9±53.1	171.6±21.6	92.0±7.2
PPPG[mg/dl]	252.7±75.1	246.3±57.2	121.0±29.7

Biochemical parameters	**Group1(Mean±S.D.)** Metformin+ Glimiperide N=30	**Group2(Mean±S.D.)** Metformin +Linagliptin N=30	**Control** (Mean±S.D.) N=40
HbA1C%	8.0±1.7	8.4±2.2	5.4±0.5
Peptide (fasting) [ng/ml]	3.1±1.5	2.4±0.6	1.7±0.7
CPeptide(p.p.)[ng/ml]	8.9±4.5	5.2±2.7	4.1±1.4
Insulin [mU/L]	24.2±19.3	9.9±6.1	6.4±2.8
HOMA IR	2.7±1.1	2.1±0.5	1.1±0.2
% Betacell activity	82.8±68.6	44.4±14.2	117.9±37.8
% Sensitivity	42.9±17.6	51.0±17.7	73.0±21.4
Urinary ACR(mg/gm)	19±3.05	18±4.02	12±2.1

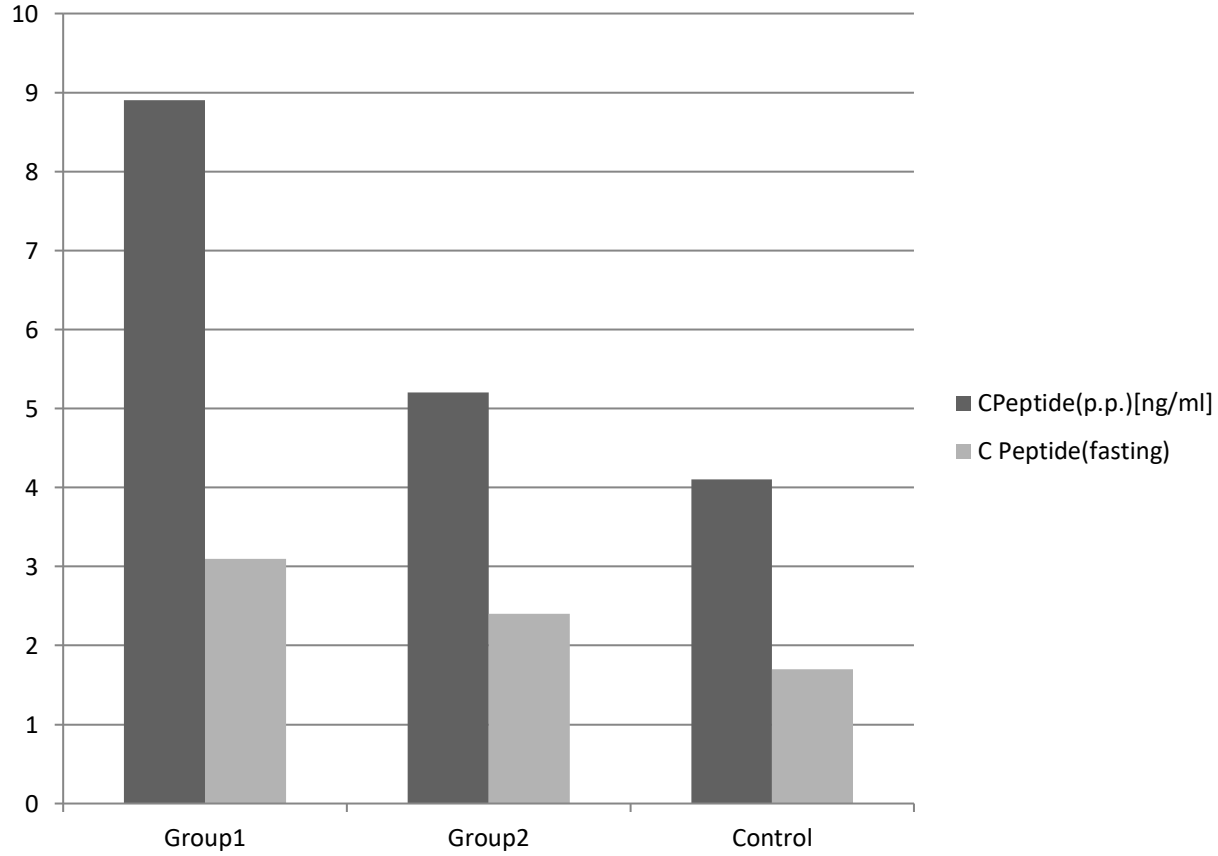

Figure 4. shows a comparison of the mean C-peptide levels in the fasting and Post prandial states among the three different groups.

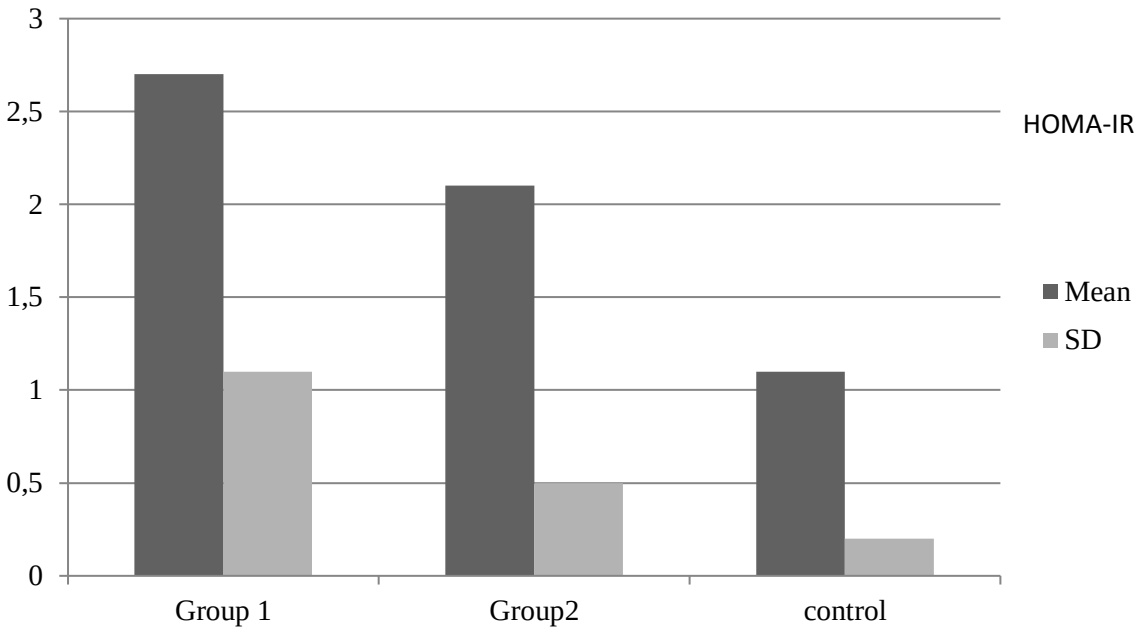

Figure 5. shows a comparison of the mean and SD of HOMA-IR among the three different groups.

This is a descriptive study. ANOVA is used to compare the significance of the parameters among 3 different study groups. Statistical significance was set at $P < 0.05$. There was no significant difference in FPG values among the case groups ($p = 0.908$), but FPG values among both case groups were significantly higher than those of the control group ($p < 0.001$). Similarly, HbA1C also increased significantly among both case groups compared to the control ($p < 0.001$) (table 1), but no significant difference was found between the two case groups(p-value - 0.719). In both fasting and fed states, the C-peptide value was significantly higher in group 1 than in group 2(p-value-0.017), and in both case groups, C peptide in the fasting state was significantly raised in comparison to the control ($p < 0.05$). HOMA-IR was significantly higher in both groups than in the control group ($p < 0.05$). As shown in table 1all urinary ACR values were below30 mg/g.

DISCUSSION

Hyperinsulinemia is a characteristic marker of insulin resistance and contributes to the pathology of T2DM. In this study, fasting insulin concentration was 24.2±19.3 in group 1 and 9.9±6.1 in group 2, whereas HOMA-IR in both groups was found to have more insulin resistance (group 1- 2.7±1.1; group 2- 2.1±0.5) in comparison to the control population, that is, euglycemic healthy individuals (1.1±0.2). This variation in insulin concentration was in accordance with the observations obtained by Praveen et al. HOMA IR > 1.8 was considered insulin resistance according to Bruno Gelonze et al(Geloneze et al., 2009).

The pathogenesis of T2DM is critical and obscure, and in most cases manifests defects in both β-cell dysfunction and insulin sensitivity. Postprandial pathogenesis in T2DM is characterized by insulin resistance and subsequent changes in GLP-1, insulin, and glucagon secretion(Jalleh et al., 2022).

Mechanism of action of DPP-4 inhibitors are believed to lower plasma glucose levels by increasing the physiological level of GLP-1. GLP-1 modifies and improves glucose-dependent insulin secretion from β-cells; the process is energy dependent, where ATP is converted to cAMP. In

contrast, the sulfonylurea group of the drug showed a beta cell-exhausting effect(Solis-Herrera et al., 2013). In this study, the post-prandial C peptide value was (8.9±4.5 in group 1 and 5.2±2.7 in group 2) which was significantly higher in group 1 than in group 2(p-value-0.017). In 2014, Thomas Forst et al. showed that treatment with glimepiride and linagliptin significantly improved HbA1c levels and PPBG control. In patients treated with glimepiride, a sharp increase in postprandial(PP) insulin levels was accompanied by an improvement in PPBG control, whereas it was not observed in the group treated with agliptin(Forst et al., 2014). This finding corroborates the results of the present study.

Patients treated with glimepiride showed high post prandial insulin levels and high post prandial glucose excursion. This finding can be explained by probable increased insulin resistance level which is reflected in their post prandial c peptide level.But in case of linagliptin, one mechanism of decreased post-prandial glucose is believed to be inhibition of the plasma glucagon release from the alpha cell of pancreas, and resulting in relief of beta cell (Forst et al., 2014) Carolina Solis-Herrera in 2013 showed that DPP-4 inhibitor alone or along with metformin combined produce decrease plasma glucagon(Solis-Herrera et al., 2013). In 2011, Del Prato showed that single-drug therapy with linagliptin achieved a clinically significant and observable improvement in glycemic control, accompanied by enhanced parameters of β-cell function(del Prato et al., 2011).

However, another meta-analysis of the homeostatic model cited by Wu et al. on the comparison of incretin-based therapy with sulfonylurias and placebo showed no significant β cell-preserving effect between the two groups(Wu et al., 2019). Therefore, the homeostatic model of insulin resistance (HOMA-IR) and β-cell function should be interpreted with caution. From this study, it can be concluded that more uses of DPP-4 analogs will elicit lesser beta cell stress in comparison to sulfonuria in this study, with little difference in glycemic control. This observation can be further reinforced by increasing the sample size and properly designing therapeutic trials for longer durations.

CONCLUSION

It can be concluded that both regimes of oral hypoglycemic drugs improve fasting and post prandial blood glucose levels along with HbA1C levels in T2DM patients included in this study. However, serum C-peptide in fasting and post-prandial state and HOMA –IR, β cell defect suggest lower β cell stress in the linagliptin- and metformin-treated groups than in the glimepiride and metformin combination group.

LIMITATIONS

This study has a few limitations, including the small sample size and discrepancy in the dose and duration of oral hypoglycemic agents and other confounding variables that were not considered, such as renal clearance. The insulin-insulin ratio could not be estimated. The findings of this study can be better evaluated by properly designed therapeutic trials of longer durations.

REFERENCES

Bargnoux, A.-S., Barrot, A., Fesler, P., Kuster, N., Badiou, S., Dupuy, A.-M., Ribstein, J., &Cristol, J.-P. (2014). Evaluation of five immunoturbidimetric assays for urinary albumin quantification and their impact on albuminuria categorization. *Clinical Biochemistry*, *47*(16–17), 250–253.

https://doi.org/10.1016/j.clinbiochem.2014.07.014

Clark, P. M. (1999). Assays for Insulin, Proinsulin(S) and C-Peptide. *Annals of Clinical Biochemistry: International Journal of Laboratory Medicine*, *36*(5), 541–564. https://doi.org/10.1177/000456329903600501

Deacon, C. F. (2020). Dipeptidyl peptidase 4 inhibitors in the treatment of type 2 diabetes mellitus. *Nature Reviews Endocrinology*, *16*(11), 642–653. https://doi.org/10.1038/s41574-020-0399-8

Del Prato, S., Barnett, A. H., Huisman, H., Neubacher, D., Woerle, H.-J., & Dugi, K. A. (2011). Effect of linagliptin monotherapy on glycaemic control and markers of β-cell function in patients with inadequately controlled type 2 diabetes: a randomized controlled trial. *Diabetes, Obesity and Metabolism*, *13*(3), 258–267. https://doi.org/10.1111/j.1463-1326.2010.01350.x

Foretz, M., Guigas, B., & Viollet, B. (2019). Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus. *Nature Reviews Endocrinology*, *15*(10), 569–589. https://doi.org/10.1038/s41574-019-0242-2

Forst, T., Anastassiadis, E., Diessel, S., Löffler, A., &Pfützner, A. (2014). Effect of linagliptin compared with glimepiride on postprandial glucose metabolism, islet cell function and vascular function parameters in patients with type 2 diabetes mellitus receiving ongoing metformin treatment. *Diabetes/Metabolism Research and Reviews*, *30*(7), 582–589. https://doi.org/10.1002/dmrr.2525

Fu, Z., Gilbert, E. R., & Liu, D. (2013). Regulation of insulin synthesis and secretion and pancreatic Beta-cell dysfunction in diabetes. *Current Diabetes Reviews*, *9*(1), 25–53. https://doi.org/10.2174/1573399811309010025

Galgani, J. E., de Jonge, L., Rood, J. C., Smith, S. R., Young, A. A., &Ravussin, E. (2010). Urinary C-Peptide Excretion: A Novel Alternate Measure of Insulin Sensitivity in Physiological Conditions. *Obesity*, *18*(9), 1852–1857. https://doi.org/10.1038/oby.2010.70

Galicia-Garcia, U., Benito-Vicente, A., Jebari, S., Larrea-Sebal, A., Siddiqi, H., Uribe, K. B., Ostolaza, H., & Martín, C. (2020). Pathophysiology of Type 2 Diabetes Mellitus. *International Journal of Molecular Sciences*, *21*(17), 6275. https://doi.org/10.3390/ijms21176275

Geloneze, B., Vasques, A. C. J., Stabe, C. F. C., Pareja, J. C., Rosado, L. E. F. P. de L., Queiroz, E. C. de, &Tambascia, M. A. (2009). HOMA1-IR and HOMA2-IR indexes in identifying insulin resistance and metabolic syndrome: Brazilian Metabolic Syndrome Study (BRAMS). *ArquivosBrasileiros de Endocrinologia&Metabologia*, *53*(2), 281–287. https://doi.org/10.1590/S0004-27302009000200020

Hardy, R. W., Cohn, M., & Konrad, R. J. (2000). Automated chemiluminescent assay for C-peptide. *Journal of Clinical Laboratory Analysis*, *14*(1), 17–19. https://doi.org/10.1002/(SICI)1098-2825(2000)14:1<17::AID-JCLA4>3.0.CO;2-5

Holst, J. J. (2019). The incretin system in healthy humans: The role of GIP and GLP-1. *Metabolism*, *96*, 46–55.

https://doi.org/10.1016/j.metabol.2019.04.014

Jalleh, R. J., Wu, T., Jones, K. L., Rayner, C. K., Horowitz, M., & Marathe, C. S. (2022). Relationships of Glucose, GLP-1, and Insulin Secretion With Gastric Emptying After a 75-g Glucose Load in Type 2 Diabetes. *The Journal of Clinical Endocrinology & Metabolism*, *107*(9), e3850–e3856. https://doi.org/10.1210/clinem/dgac330

Kaufman, F. R. (2002). Type 2 Diabetes Mellitus in Children and Youth: A New Epidemic. *Journal of Pediatric Endocrinology and Metabolism*, *15*(Supplement). https://doi.org/10.1515/JPEM.2002.15.S2.737

Lebovitz, H. (2001). Insulin resistance: definition and consequences. *Experimental and Clinical Endocrinology & Diabetes*, *109*(Suppl 2), S135–S148. https://doi.org/10.1055/s-2001-18576

Nauck, M. A., Quast, D. R., Wefers, J., & Pfeiffer, A. F. H. (2021). The evolving story of incretins (<scp>GIP</scp> and <scp>GLP</scp> -1) in metabolic and cardiovascular disease: A pathophysiological update. *Diabetes, Obesity and Metabolism*, *23*(S3), 5–29. https://doi.org/10.1111/dom.14496

Park, S. Y., Gautier, J.-F., & Chon, S. (2021). Assessment of Insulin Secretion and Insulin Resistance in Human. *Diabetes & Metabolism Journal*, *45*(5), 641–654. https://doi.org/10.4093/dmj.2021.0220

Phillips, L. K., &Prins, J. B. (2011). Update on incretin hormones. *Annals of the New York Academy of Sciences*, *1243*(1), E55–E74. https://doi.org/10.1111/j.1749-6632.2012.06491.x

Pradeepa, R., & Mohan, V. (2021). Epidemiology of type 2 diabetes in India. *Indian Journal of Ophthalmology*, *69*(11), 2932. https://doi.org/10.4103/ijo.IJO_1627_21

Praveen, E., Sahoo, J., Khurana, M., Kulshreshtha, B., Khadgawat, R., Gupta, N., Dwivedi, S., Kumar, G., Prabhakaran, D., &Ammini, A. (2012). Insulin sensitivity and β-cell function in normoglycemic offspring of individuals with type 2 diabetes mellitus: Impact of line of inheritance. *Indian Journal of Endocrinology and Metabolism*, *16*(1), 105. https://doi.org/10.4103/2230-8210.91204

Sacks, D. B. (2012). Measurement of Hemoglobin A1c. *Diabetes Care*, *35*(12), 2674–2680. https://doi.org/10.2337/dc12-1348

Solis-Herrera, C., Triplitt, C., Garduno-Garcia, J. de J., Adams, J., DeFronzo, R. A., &Cersosimo, E. (2013). Mechanisms of Glucose Lowering of Dipeptidyl Peptidase-4 Inhibitor Sitagliptin When Used Alone or With Metformin in Type 2 Diabetes. *Diabetes Care*, *36*(9), 2756–2762. https://doi.org/10.2337/dc12-2072

Tanaka, T., & Matsunaga, T. (2000). Fully Automated Chemiluminescence Immunoassay of Insulin Using Antibody−Protein A−Bacterial Magnetic Particle Complexes. *Analytical Chemistry*, *72*(15), 3518–3522. https://doi.org/10.1021/ac9912505

Trinder, P. (1969). Determination of blood glucose using 4-amino phenazone as oxygen acceptor. *Journal of Clinical Pathology*, *22*(2), 246–246. https://doi.org/10.1136/jcp.22.2.246-b

Walczewska-Szewc, K., & Nowak, W. (2021). Photo-Switchable Sulfonylureas Binding to ATP-Sensitive Potassium Channel Reveal the Mechanism of Light-Controlled Insulin Release. *The Journal of Physical Chemistry B*, *125*(48), 13111–13121. https://doi.org/10.1021/acs.jpcb.1c07292

Wu, S., Gao, L., Cipriani, A., Huang, Y., Yang, Z., Yang, J., Yu, S., Zhang, Y., Chai, S., Zhang, Z., Sun, F., & Zhan, S. (2019). The effects of incretin-based therapies on β-cell function and insulin resistance in type 2 diabetes: A systematic review and network meta-analysis combining 360 trials. *Diabetes, Obesity and Metabolism*, *21*(4), 975–983. https://doi.org/10.1111/dom.13613