GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Role of glucagon-like peptide 1 (GLP-1) and its association with inflammatory markers in the pathogenesis of type 2 diabetes mellitus

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GSC Biological and Pharmaceutical Sciences, 2023, 22(03), 099–106

Publication history: Received on 25 January 2023; revised on 06 March 2023; accepted on 08 March 2023

Article DOI: https://doi.org/10.30574/gscbps.2023.22.3.0094

## Abstract

**Background:** Diabetes mellitus is a multifactorial disease associated with hyperglycemia and increased risk of progression of vascular complications. Stimulation of insulin secretion by the incretin hormone glucagon-like peptide 1 (GLP-1) has been found to be diminished in hyperglycemia. We hypothesized that this impairment is due to defect at the receptor level induced by the diabetic state. Inflammatory markers like TNF-α and IL-6 plays a potential role in the pathogenesis of T2DM. Therefore, the present study aims to evaluate whether GLP-1 plays a role in the development of T2DM by modulating the balance between pro and anti-inflammatory markers.

**Material and methods:** A total of 60 subjects were recruited in this study among them 30 were T2DM cases and 30 were healthy controls. m-RNA expression and protein level of GLP-1 receptor, TNF-α and IL-6 in peripheral blood lymphocytes were determined by real time PCR and ELISA respectively.

**Results:** We observed plasma level of GLP-1 was significantly lower in diabetic subjects while serum level of IL-6 and TNF-α were significantly higher level in diabetic subjects ($p < 0.05$). We found significant down regulation of GLP-1 receptor m-RNA expression in diabetic subjects while expression level of IL-6 and TNF-α were 5.8 and 4 folds respectively higher in diabetic subjects. We found significant negative correlation of m-RNA expression of GLP-1 with protein level while IL-6 and TNF-α showed significant positive correlation.

**Conclusion:** Inflammation plays an important role in the pathogenesis of diabetes mellitus and low GLP-1 levels may promote expression of inflammatory markers due to lack of anti-inflammatory effects of GLP-1

**Keywords:** Inflammation; Glucagon-like peptide 1; TNF-α; IL-6;Diabetes mellitus

## 1. Introduction

Diabetes mellitus (DM) is a multifactorial disease associated with hyperglycemia and increased threat of vascular complications, which are the major causes of morbidity and mortality [1]. The International Diabetes Federation (IDF) states that as numerous as 8.8% of the world's population of around 425 million people suffer from DM in 2017 [2]. According to data of IDF, by 2035, some 592 million people, or one adult in 10 will have diabetes, if these trends continues. Diabetes is highly prevalent in Indian subcontinent too. India ranks second amongst all countries, with

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respect to the number of people suffering from diabetes. In India alone, it is estimated that the total number of people with diabetes, will rise to 109 million by 2035 [3].

Glucagon-like peptide-1 (GLP-1), an incretin hormone, is produced by post-translational proteolytic cleavage of the proglucagon gene product and mainly secreted from the enteroendocrine L cells in the distal intestine in response to nutrient ingestion. GLP-1 increases glucose-stimulated insulin secretion [4, 5]. Dipeptidyl peptidase-4 (DPP-4) quickly degrades GLP-1, and inhibition of that proteolytic enzyme enhances its biological half-life [6]. GLP-1 has numerous valuable effects on the control of blood glucose levels like stimulation of insulin secretion and inhibition of glucagon secretion, expansion of the beta-cell mass by stimulating beta-cell proliferation and differentiation and inhibiting beta-cell apoptosis, delay of gastric emptying, and reduction of food intake [7-9].

Expression of the receptor of GLP-1 is broadly detected in various cells and organs such as kidney, lung, heart, hypothalamus, endothelial cellsastrocytes, neurons, and microglia as well as pancreatic beta-cells, which suggest that GLP-1 plays additional roles other than glucose-lowering effects [10-13]. It was reported previously that anti-inflammatory effects of GLP-1 on pancreatic islets and adipose tissue, contributing to lowering glucose levels in diabetes [14-16]. In addition to these tissues, emerging data suggest that GLP-1based therapies also has anti-inflammatory effects on the liver, vascular system including aorta and vein endothelial cells, brain, kidney, lung, testis, and skin. The anti-inflammatory effect of GLP-1is probably due to reduction of production of inflammatory cytokines and inhibition of infiltration of immune cells in the tissues [17-21].Therefore, GLP-1 has been extensively studied as a possible treatment of type 2 diabetes mellitus (T2DM), and GLP-1 analogues and DPP-4 inhibitors are now widely in clinical use in these patients [22-25].

The role of inflammation in T2DM development is gaining importance as effects of the pro and anti-inflammatory cytokines like tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) on insulin signaling pathways, cross-linking and insulin resistance in β-cells of pancreas are being reported in recent studies [26-28]. Balance among these pro and anti-inflammatory cytokines is necessary to make β-cells immune to any infection which may lead to T2DM [29, 30]. The expression levels of TNF-α and IL-6 are higher in activated macrophages and pro-inflammatory marker such as TNF-α activates insulin resistance by inhibiting phosphorylation of IRS-1 and Akt substrate 160 on insulin signaling cascade pathways [31].

Very few studies have been reported on the association of GLP-1 and inflammatory markers in patients of T2DM. To best of our knowledge there are no studies which have evaluated the role of GLP-1 and inflammatory markers in the pathogenesis of T2DM amongst Indian population. Therefore, this study aims to evaluate whether GLP-1 plays a role in the development of T2DM by modulating the balance between pro and anti-inflammatory markers.

## 2. Material and methods

### 2.1. Subjects

The present study was carried out at Multidisciplinary Research Unit (MRU) UCMS, Delhi.A total of 30 patients of T2DM with disease duration > 5 years who regularly attended medical outpatient department and/or diabetic clinic of University College of Medical Sciences (UCMS) and GTB hospital, Delhi, were enrolled in this study. 30 healthy controls were also enrolled in the study by voluntary participation. The controls were departmental staff members, community participants or unrelated attendants of the patients. The study was approved by Institutional Ethics Committee-Human Research of UCMS. Written informed consent was obtained from each subject prior to entering the study. Diagnosis of diabetes was based on current criteria of the American Diabetes Association, 2021.

### 2.2. Inclusion criteria

Criteria for the diagnosis of diabetes FPG ≥126 mg/dl (7.0 mmol/l). Or2-hour plasma glucose ≥ 200 mg/dl (11.1mmol/l), using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water by standard protocol. Or HbA1c ≥6.5%. In the absence of unequivocal hyperglycemia, the result was confirmed by repeat testing.

### 2.3. Exclusion criteria

All patients on lipid-lowering therapy, Insulin therapy, GLP -1 analogues, DPP-IV inhibitors, corticosteroids, retinoid drugs and steroid hormones were excluded from the study.Patients with recent bariatric surgery and those who smoke were also excluded.

### 2.4. Healthy controls Inclusion criteria

Individuals with no known history of Diabetes Mellitus/ Hypertension/ Coronary Artery Disease/ Smoking/ OCP usage. BMI < 23 kg/$m^2$. Waistline< 90 cm in males and < 80 cm in females were included as controls.

A detailed clinical history was obtained. Age, sex, family history and physical (weight and height) and systemic examination findings were recorded in predesigned proforma. Fasting and postprandial blood samples were collected and the following parameters were estimated in serum.

### 2.5. Estimation of blood glucose, serum Insulin and HbA1c

Fasting blood glucose and postprandial blood glucose were estimated in plasma by glucose oxidase/peroxidase method. Serum Insulin was done using commercially prepared kit based on ELISA. The insulin resistance was calculated using HOMA-IR model. Glycosylated haemoglobin was determined in whole blood by HPLC method

### 2.6. Estimation of lipid profile

Total cholesterol in plasma was estimated by the cholesterol-oxidase method. HDL-C was estimated by direct enzymatic method after precipitating VLDL and LDL. Plasma TAG was estimated by GPO-POD method as described by Werner and Gabrieulsen. VLDL-C was calculated by TAG/5. LDL-C was calculated by Freidwald's equation (for TG<400mg/dl): LDL-C = Total cholesterol – (HDL-C + VLDL-C).

### 2.7. Estimation of GLP-1 level in plasma

GLP-1 levels in plasma was estimated using commercially available ELISA kit as per manufacturer`s protocol.

### 2.8. Estimation of serum TNF-α and serum IL-6 levels

Serum TNF-α and serum IL-6 levels were estimated using commercially available ELISA kit as per manufacturer`s protocol.

### 2.9. GLP-1 receptor, TNF-α and IL-6 mRNA expression in peripheral blood lymphocytes

Total RNA was extracted from peripheral whole blood with Trizol (Invitrogen, Carlsbad, CA). The extracted RNA had an OD280/260 ratio between 1.9 and 2.0.c-DNA was synthesized taking 1µg of total RNA with Maxima first strand synthesis kit (Fermentas, Germany). Quantitative Real time PCR (LC480, Roche) was performed to measure expression of mRNA for GLP-1R, IL-6 and TNF-α using SYBER Green chemistry. The reaction mixture contains 4 µl of template c-DNA, 10 µl of SYBER Green Maxima PCR master mix (Fermentas, Germany) and 10 pmol of each primer of both target gene and housekeeping gene. The sequences of each primer are shown in Table 1. Amplification was performed with 40 cycles of denaturation for 18 s, annealing at 60ºC for 35 s and final extension for 35 s.The housekeeping gene β-actin was used for internal normalization. The expression level was quantified by Δ Ct value, lower the Δ Ct value, higher the expression level. Δ Ct value was calculated by Ct value (target gene) – Ct value (housekeeping gene). The relative expression level or fold change was calculated by $2^{-(\Delta\Delta Ct)}$.

**Table 1** Primer sequence for target genes GLP-1R, IL-6, TNF-α and β-actin housekeeping gene

| Gene | Sequence |
|---|---|
| GLP1R | 5' GAGGGGAAAGAAGCTCCCCG 3' |
| GLP1R | 5'GGACAATGCTCGCAGGATGA 3' |
| IL-6 | 5'ATGAACTC CTTCTCCACAAGC 3' |
| IL-6 | 5'GTTTTCTGCCAGTGC CTCTTTG 3' |
| TNF-α | 5'CCG AGG CAG TCA GAT CAT CTT 3' |
| TNF-α | 5'AGC TGC CCC TCA GCT TGA 3' |
| β-actin | 5'TAA TGT CAC GCA CGA TTT CCC 3' |
| β-actin | 5'TCA CCG AGC GCG GCT 3' |

### 2.10. Statistical analysis

Statistical analysis was carried out using standard statistical methods (SPSS software version 16.0). Data were expressed as mean ± standard deviation.Pearson's or Spearman's correlation analysis was done in diabetic subjects to determine the relation of m-RNA expression with protein level for GLP-1, IL-6 and TNF-α target gene. For all statistical tests, $P < 0.05$ was considered as the level of significance.

## 3. Results

### 3.1. Clinical characteristics of study subjects

The demographic and clinical profile of the study groups are shown in Table 2. There was no significant difference in the age or sex distribution between the study groups. Fasting blood glucose, post prandial blood glucose and $HbA_{1C}$ were significantly higher in diabetic subjects compared to healthy individuals. Among the lipid profile parameters, the serum level of triglyceride, LDL and cholesterol level were higher and level of HDL was significantly lower in diabetic patients compared to healthy individuals.

**Table 2** Demographic and clinical data for diabetic subjects and healthy controls. a $P < 0.05$

| Parameter | DM | Healthy subjects |
|---|---|---|
| Subjects | 30 | 30 |
| Age (years) | 51.9±8.6 | 50.0±7.6 |
| Sex distribution (Male/Female) | 14/16 | 17/13 |
| Duration of diabetes (in years) | 7.2±1.8 | 0 |
| BMI (kg/m2) | 25.1±2.6 | 24±2.1 |
| Fasting sugar (mg/dl) | 143.4±44.8a | 86.6±7.2 |
| Post prandial sugar (mg/dl) | 216.8±65.4a | 103.6±13.1 |
| HbA1C (%) | 7.5±1.0a | 5.4±0.48 |
| Total serum Cholesterol (mg/dl) | 172.1±32.1a | 142.5±28.4 |
| LDL Cholesterol (mg/dl) | 131.2±21.7a | 115.5±18.8 |
| HDL Cholesterol (mg/dl) | 42.6±6.1a | 52.3±8.8 |
| Triglycerides (mg/dl) | 152.1±45.5a | 118.4±40.0 |

### 3.2. GLP-1 level in plasma

Plasma level of GLP-1 determined by ELISA was significantly lower in diabetic subjects compared with healthy controls shown in Table 3. ($p < 0.05$).

### 3.3. IL-6 and TNF-α level in Serum

We observed significant higher serum IL-6 and TNF-α level in diabetic subjects compared to healthy controls(Table 3)($p < 0.05$).

**Table 3** Plasma level of GLP-1 and serum level of IL-6 and TNF-α measured by ELISA. *P<0.05

| | Healthy Control | Diabetic Subjects |
|---|---|---|
| GLP-1 (pg/ml) | 31.5±21.5 | 16.8±10.4* |
| IL-6 (pg/ml) | 0.37±2.4 | 8.15±19.4* |
| TNF-α (pg/ml) | 5.22±2.7 | 11.96±6.8* |

### 3.4. m-RNA Expression of GLP-1 receptor, TNF-α and IL-6 in peripheral blood lymphocytes

We observed significant down regulation of GLP-1 receptor expression in diabetic subjects compared to healthy individuals while m-RNA expression level of IL-6 and TNF-α was 5.8 and 4 folds respectively higher in diabetic subjects in peripheral blood lymphocytes assayed by quantitative real time PCR (Figure 1).

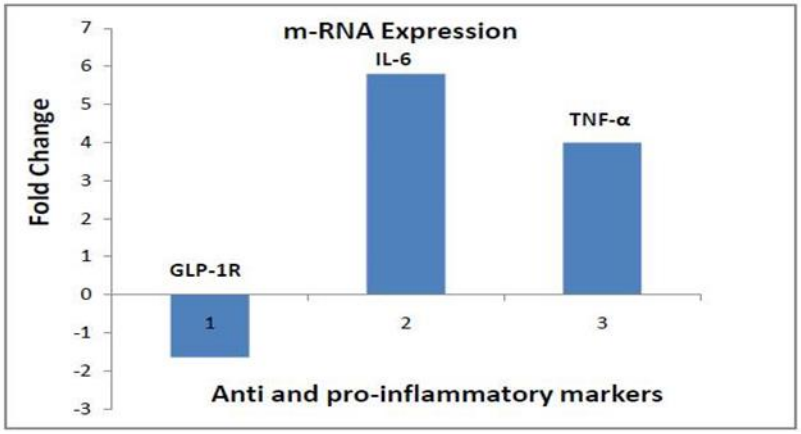


**Figure 1** m-RNA expression level in fold change of GLP-1R, IL-6 and TNF-α measured by real time PCR $2^{-(\Delta\Delta Ct)}$ method

### 3.5. Correlation of m-RNA Expression of GLP-1 receptor, TNF-α and IL-6 with their protein levels among diabetic subjects

Pearson Correlation analysis was carried out to evaluate the association of m-RNA expression with protein levels of GLP-1, IL-6 and TNF-α target gene among diabetic patients. We found significant negative correlation of m-RNA expression of GLP-1 with protein level ($r = -0.385$, $P= 0.018$) while IL-6 ($r = 0.438$, $P= 0.004$) and TNF-α ($r = 0.728$, $P= 0.0001$) showed significant positive correlation.

## 4. Discussion

As we know, the worldwide frequency of diabetes, especially T2DM, is escalating in every country, and major economic, social and health care impacts will be seen in developing countries, as these countries are home to as much as 80% of people with diabetes. In the recent years, the incretin system has become an important target in the treatment of T2DM and GLP-1 is of particular interest for its glucose-lowering effects. GLP-1 is an incretin hormone, which increases glucose-stimulated insulin secretion [4, 5].GLP-1 has several helpful effects on the control of blood glucose levels such as stimulation of insulin secretion and inhibition of glucagon secretion, expansion of the beta-cell mass by enhancing beta-cell proliferation and differentiation and inhibiting beta-cell apoptosis, delay of gastric emptying, and reduction of food intake [7, 8]. GLP-1 receptor expression is widely detected in various cells and organs including the kidney, lung, hypothalamus,heart, neurons, astrocytes,endothelial cells, and microglia as well as pancreatic beta-cells, which suggests that GLP-1 may play an additional roles other than glucose-lowering effects [10-13]. Previously, it was reported that GLP-1 showed anti-inflammatory effects on pancreatic islets and adipose tissue, which leads to lowering glucose levels in diabetes [14-17].

In the present study we observed that under hyperglycemic condition GLP-1 receptor expression was found to be downregulated both at the gene expression and protein levels. We hypothesized that under hyperglycemic condition; the downregulation of the GLP-1 receptor expression is largely responsible for the impaired incretin effects and thus in part explains the β-cell dysfunction. As we know, under hyperglycemic condition, insulin secretion stimulation by the incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) has been found to be diminished. These findings suggest that the design of new therapies based on activation of the GLP-1 receptor. An earlier study found a modest reduction of GLP-1 gene expression in rat islets cultured in high glucose concentrations [32].

Administration of GLP-1 receptor agonists enhances GLP-1 receptors, leads to increasing insulin secretion in response to oral and intravenous glucose to similar extents; this suggest that the extent of the incretin effect should remain unchanged [25]. Various agonists of GLP-1 receptor are now approved in the United States for the treatment of T2DM.

Inflammation plays an important role in pathogenesis of T2DM. The pro and anti-inflammatory cytokines TNF-α, IL-6 has been reported to affect insulin signaling pathways, cross-linking and increases the risk of developing insulin resistance in β-cells of pancreas which leads to development of T2DM. In the present study, we observed significant higher m-RNA and protein level of TNF-α and IL-6 levels in T2DM patients compared to controls. In agreement to our study, Phosat et al. reported that there was greater risk of T2DM with higher levels of inflammatory mediators [33]. In this study, in both male and female sexes the importance of inflammatory mediators in the pathogenesis of T2DM has been highlighted. The protein and m-RNA levels of TNF-α and IL-6 were significantly higher in both sexes, male and female compared to the control group, this confirms an independent predictor of the risk of developing T2DM. Our findings are in agreement with the results of Samuel et al. which showed serum expression of candidate mediators TNF-α and IL-6 are elevated in T2DM cases which are independent of physical activity and other risk factors [34]. It is suggested that TNF-α is an important predictor for the development of T2DM. These results which support the hypothesis that systemic inflammation is a common precursor for T2DM [34-36].We found significant negative correlation of m-RNA expression of GLP-1 with protein level ($r = -0.385$, $P= 0.018$) while IL-6 ($r = 0.438$, $P= 0.004$) and TNF-α ($r = 0.728$, $P= 0.0001$) showed significant positive correlation.

## 5. Conclusion

As expected, GLP-1 level is reduced in diabetes. The reduction in GLP-1 receptor m-RNA expression in response to reduced GLP-1 protein in circulation. Inflammatory markers TNF-α and IL-6 are increased and positively correlated with its m-RNA expression. This indicates that inflammation plays an important role in the pathogenesis of DM and low GLP-1 levels may promote expression of inflammatory markers due to lack of anti-inflammatory effects of GLP-1.

## Compliance with ethical standards

### *Acknowledgments*

This work is supported through funds provided by the Department of Health Research - Indian Council of Medical Research, Government of India, and New Delhi under Multi-disciplinary research scheme.

### *Disclosure of conflict of interest*

The authors declare that there is no conflict of interest.

### *Statement of ethical approval*

The study was approved by Institutional Ethics Committee-Human Research of University College of Medical Sciences and GTB hospital, Delhi, India

### *Statement of informed consent*

Written informed consent was obtained from each subject prior to entering the study.

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