Nat. Volatiles & Essent. Oils, 2021; 8(4): 9003-9012

NVEO
Natural Volatiles &
Essential Oils

Evaluation Of Glucagon Like Peptide-1 Receptor And Insulin-Like Growth Factor-1 Genes Expression In Type 2 Diabetic Patients Depending On Their Body Max Index

MurtadhaHadi Al-Qushawi* and Mahdi Saber Al-deresawi**

*MSC. Student /Department of Biology /College of Science/Wasit University/ Kut-Iraq

**Assist.prof. Dr.Department of pathological analysis/College of Science/Wasit University/Kut- Iraq

Abstract:

Type 2 diabetes mellitus (T2DM) is a complicated metabolic condition caused by a combination of many factors .

Methodology: A total 90 specimens of blood samples have been collected, including (75) samples from patients with T2DM from Al-Shaheed Fairooz Hospital and Al-Kut Hospital in the governorate of Wasit /Iraq, and (15) samples from healthy control individual without any history of T2DM during January to March 2021. The patients' ages were ranged between 18 and 70 years old.HbA1C test was done for a 75 sample of patients to determine glucose level . Body mass index was estimated for all Patients .The results showed that smoker had higher total HbA1c values than no smoker . As well, It was shown that patients with family history had higher total HbA1C values than patients with not any Family history with T2DM, and BMI showed significant increase in T2DM .

To evaluate the IGF-1and GLP genes expression, performed by RT-PCR technique .The results revealed that there was (0.764 ±0.28) in GLP-1Rand (0.847 ±0.32) in IGF-1 expression in all when compared to a healthy control group of patients (1.00 ±0.00) . The gene expression according to BMI was showed (overweight =-0.361 , obesity= -0.410 ,P value<0.05) in GLP-1R gene and (overweight =0.72, obesity= 0.75,P value <0.05) in IGF-1 gene .

KEY WORDS : Insulin like growth factor,Type 2 diabetes mellitus, glucan peptide 1receptor, body mass index

Introduction

Diabetes mellitus is a dangerous and complex condition characterized by high blood glucose levels (Petersmann and et al 2018) . An increased blood glucose level may not cause any symptoms in and of itself,however,With time, It may result in the development of micro- and macro-vascular consequences such as cardiovascular comorbidities, renal disease, limb amputation, and blindness (Dahlstrom, and et al 2017).

Diabetes mellitus is a common lifestyle condition that is divided into two types: T1DM and T2DM. While they are both cause hyperglycemia and other chronic illnesses are caused by a lack of insulin activity, Each type's cause differs, necessitating a different preventative approach.. T1DM is triggered by the autoimmune reaction of the body, which results in the loss of pancreatic islet cell. Type 2 diabetes, is caused by either insulin resistance in target cells or a failure to generate insulin in accordance with physiological requirements. (Goenka and et al 2020) . Diabetes can be diagnosed using fasting blood glucose levels as well as long-term blood glucose levels (HbA1C proportion) (Weykamp, 2013). T2D affects almost all diabetics. Diabetes is becoming more common around the world, as well as the World Health Organization (WHO) in 2002 by which 2031, The number of adults with diabetes would have increased by more than a factor of two. Approximately 99% of DM have T2D. Diabetes is becoming more common all around the world & the WHO 2002 has referred that by 2031 the number of adults with diabetes would have doubled worldwide.Diabetes is a condition characterized by abnormally high blood glucose levels. The food is the main source of glucose and insulin hormone enables glucose to enter the cells and provide energy.T2DM is the more common type of diabetes, Insulin is not produced or used efficiently by the body. insulin deficiency, the glucose increases in blood (WHO,2014) .Glucagon like peptide-1 receptor gene (GLP-1R) codes a 7-transmembrane protein that function as a receptor for glucagon –like peptide 1 (GLP-1) hormone, which promotes glucose-stimulated insulin production.This receptor, which functions at the beta cell surface in the pancreas, Internalization of GLP-1 and GLP-1 analogs occurs, and it plays a key role in the signaling pathways that lead to insulin production.In animal models, it also has neuroprotective benefits. (Brubaker and Drucker, 2002). Diabetes is linked to polymorphisms in this gene.The protein is an important drug target for the treatment of T2DM and stroke. This gene has numerous transcript variants due to alternative splicing. (Doria et al., 2008). On the long arm of chromosomes, the IGF-1 gene is found 12q23-23 and consists of six exons, including two leader exons , and has two promoters (Rotwein,1991). This gene coding for insulin-like growth factor 1 protein (IGF-1) is a 70-amino-acid hormone that affects nearly every tissue and organ in the body , IGF-1 Peptide growth factor that is secreted and is involved in a range of physiological and pathological processes such as somatic growth, tissue repair, and glucose, protein, and lipid metabolism (Al-Deresawiet al., 2019 **:**Rotwein, 1999). **The aim of this study:** was toassessment the glucagon like peptide-1 receptor and insulin-like growth factor-1 genes expression in patients with diabetes type-2 & to determine the possible associations between glucagon like peptide-1 receptor and insulin-like growth factor-1 genes expression according the Body Mass Index (BMI).

Materials and procedures:

Selection of Subject:

Patients whom chosen for this study were men and women with diabetes mellitus type 2 disease. A total 90 specimens of blood samples have been collected, including (75) samples from patients from Al-Shaheed Fairooz Hospital and Al-Kut Hospital in the governorate of Wasit /Iraq, and (15) samples from healthy control individual without any history of DT2M or other chronic disease, from January to March 2021. The patients' ages were ranged between 18 and 70 years old**.**

Measurement of Body Mass Index (BMI)

Body Mass Index was measured by weight in kilos divided by height in meters squared (kg/m^2). (**Flegalet al., 2005**) .

Blood Specimens Collection :

Each patient and a control group were drawn about 4 ml of blood and putting into two EDTA Vacuum tubes (2 ml)and then labeled, one tube for HbA1C test and another freezing until to be used for RNA Extraction.

HbA1C Determination :

Tosoh apparatus automated glycohemoglobin analyzer HLC used in this study to determine the HbA1C level, by using Tosoh HbA1C kit . Operation the Tosoh automated glycohemoglobin Analyzer System according to the user manual.

Expression of Genes

All of the samples' total RNA was extracted using (Genome) General RNA Extraction Kits by manufacturer's directions. Total RNA was conversely translated to cDNA utilizing AccuPower® RT PreMix Kit. The system was completed in a response volume of 20 µl. Three main steps were applied to conversion by thermocycler (step 1: 42°C for 60 min, step 2: 94°C for 5 min, and 4°C for 5 min: one step). Levels of GLP-1R expressionand IGF-I genes were assessment by real-time quantitative polymerase chain reaction (qRT-PCR) Analytikjena . To ensure that the test gene is expressed, Go Taq® qPCR Master Mix (SYBR) was used. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA levels were amplified and utilized to normalize the mRNA levels of the IGF and GLP-1R genes.Prime design and preparation were created using the Primer3 web version (online at website http://primer3.ut.ee) for the qRT-PCR process. IGF primers were supplied by genes were synthesized by macrogenComspany, Korea, and GLP-1R primers were supplied by genes syntheized by Scientific Researcher company, Iraq .were synthesized by as a lyophilized product of different picomole concentrations. Primers were lyophilized and dissolved in a free DNase/RNase water to make a stock solution

with a concentration of 100 pmol/µl, and a work solution with a concentration of 10µM suspended (10 pmol/µl in 90 µl) of deionized water to arrive at a concentration in the end of 10 µMas a workplace solution, the program of the IGFreaction was:(Initial denaturation: 95°C for 5 min/one cycle, denaturation: 95°C for 40 s, annealing: 59°C for 40 s, and extension:72°C for 1 min), and the program of the GLP-1R reaction was:(Initial denaturation: 95°C for 5 min/one cycle, denaturation: 95°C for 40 s, annealing: 53°C for 40 s, and extension:72°C for 1 min), The run consisted of 35 cycles followed by one cycle at 4°C, for every reaction . Primer sequences for GAPDHwere F: 5'-TCGGAGTCAACGGATTT-3' , R: 5'-CCACGACGTACTCAGC-3' ,IGF-1 gene was F: 5'-CTTTGCGGGGCTGAGCTGGT-3' , R: 5'-CTTCAGCGAGCAGTACA-3' , and GLP-1R gene was F: 5'-GTTCCCCTGCTGTTTGTTGT-3' , R: 5'-TGGCCTTCAGTTTGGATACC-3' .

Analysis of the Data

1. To determine the impact of various factors on study parameters, the Statistical Analysis System (SAS 2012) application was used. To detect considerable compression between means, a t-test was used. If the P value for all tests was less than 0.05, it was declared statistically significant.

2. The means of folding for genes (target and control), as well as Ct values and gene expression levels, were recorded; the values of the housekeeping gene were also included in this investigation. The (Livak and Schmittgen, 2001) equation was used to calculate ΔCT and ΔΔCT.

RESULTS AND DISCUSS

Correlation coefficient between HbA1C level and Folding genes in patient

Table (1) Shows the mean genes folding among HbA1C levels for GLP and IGF genes in patients. to confirm the current study's findings, the relationships between the genes folding that occurs in parameters of GLP and IGF genes and its effect on (T2DM) has been studied . The levels of GLP and IGF genes expression reported a high significant increase ($p \leq 0.05$) in patients with (T2DM)in the current study. Although, The results demonstrated increase in parameters levels in (T2DM) patients, but no statistically significant differences .

Table 1: Correlation coefficient between HbA1C and Folding genes

Parameters	Correlation coefficient-r wit **HbA1C levels**	P-value
Folding GLP gene	-0.13 NS	0.251

Folding IGF gene	0.02 NS	0.897
NS: Non-Significant.		

The results of Correlation coefficient with HbA1Cdemonstrated decrease in Folding GLP gene in patients with (T2DM), the result in table 1 were in accordance with (Tella , and et al 2015) The most therapeutic promise has been demonstrated for GLP. DPP-4 inhibitors have been shown to reduce HbA1c by preventing the endogenous GLP-1 breakdown. The GLP-1, with HbA1C decrease as the major requirement. However, there are a variety of medications that improve HbA1C in a similar way. The high interest in this class of drugs originates from the weight reduction seen in the approval studies, rather than the effect on HbA1C. Patients with diabetes and those with obesity without diabetes both lose weight when given GlP-1 agonists. (Vilsboll, T.,and et al 2012) .

The results of Correlation coefficient with HbA1C demonstrated increase in Folding IGF gene in patients with (T2DM), the result were in accordance with (NeamTu, et al, 2017)When compared to the control group, diabetes individuals showed higher IGF-I levels. The findings of research into the relationship between IGF levels and diabetes . Other research has found that persons with obesity or type 2 diabetes had normal or higher IGF levels. (Rajpathak SN, and et al 2008) .

Relationships between GLP-1R gene and BMI

Results in table (2) appeared the values of GLP-1R expression with BMI in different patients in weight, patients are normal weight, overweight and obesity . There was a link between GLP-1R expression and BMI in different patients in weight; (Normal weight = -0.131, Overweight= -0.361 and obesity = -0.410) . Regarding , GLP-1R expression was shown to be inversely associated to BMI, meaning patients with a greater BMI had lower GLP-1R expression.

BMI	Correlation coefficient-r wit **GLP-1R**	P-value
Normal weight	-0.131 NS	0.251
Overweight	-0.361	0.039*
obesity	-0.410	0.018*
P<0.05 Significant		

The results in table (2) were in accordance with (Ejarque, **and et al 2019**) . Both lean and insulin-resistant morbidly obese patients have GLP-1R expression, with higher expression in lean participants In insulin-resistant people, expression is reduced grossly obese subjects(Ejarque, **and et al 2019**).GLP-1 is a powerful activator insulin secretion triggered by glucose that also has beneficial effects on stomach regulation of appetite and emptying. GLP-1 works primarily through interacting GLP-1R, a G protein-coupled receptor, which is found in the pancreas, heart, kidney, gastrointestinal tract, and fat tissue in humans (Cantini, **and et al 2016**) . GLP-1R expression was found to be higher in morbid obese people with a high insulin-resistant profile when compared to insulin-sensitive lean, overweight, and low insulin-resistant obese subjects in humans. Furthermore, higher GLP-1R expression was associated with a better outcome (Vendrell, **and et al 2011**). Our findings led us to believe that our patient group may have disturbed GLP-1/GLP-1R signaling, resulting in a reduction in GLP-1 insulin sensitivity. This might explain why those patients with greater BMI , who would be expected to have the lowest expression of GLP-1R in receptor . However, the extra pancreatic mechanisms and actions of GLP-1, especially in adipose tissue , remain insufficiently understood .

Relationships between IGF-1 gene expression and BMI

Results in table (3) appeared the values of IGF-1 gene expression with BMI in different patients in weight, patients are normal weight, overweight and obesity . association was found between IGF-1 expression with BMI in different patients in weight; (Normal weight = 0.59, Overweight= 0.72 and obesity = 0.75) . Regarding , IGF-1 expression was Positively correlated with BMI, indicating that patients with higher BMI had higher IGF-1 expression.

Table (3) correlation between IGF-1 gene expression and BMI

BMI	Correlation coefficient-r wit **IGF-1**	P-value
Normal weight	0.59 NS	0.07
Overweight	0.72	0.02*
obesity	0.75	0.01*
P<0.05 Significant		

The results in table (3) were in accordance with (Yan, **and et al 2017**) To study the potential mechanistic relationship between diabetes and obesity, researchers analyzed IGF-1R expression in overweight people with and without diabetes.In a study of overweight patients with T2DM , the net effect was an increase in the

ratio of IGF-I expression. Obesity and plasma IGF concentrations have been linked before, however the evidence is mixed. The role of growth hormone in IGF control may also make data from adolescents more difficult to interpret. According to one study, obese people had a higher percentage of bioactive IGF-I than lean or overweight people (Haywood, **and et al 2019**) . Our research demonstrates that any increase in body mass index leads to an increase in IGF gene expression in patients with T2DM, where it is directly proportional.

CONCLUSIONS

Gene expression of IGF-1 expression was Positively correlated with BMI, indicating that patients with higher BMI had higher IGF-1 expression , while gene expression of GLP-1 decreases and is inversely proportional to BMIin patients with T2DM.

REFERENCES

Al-Deresawi, M. S; Al-Rekaabi, A.R; and Al Edee,A.A;. "Evaluation of hormonal profile and insulin growth factor-1 gene expression before and after metformin treatment in women with polycystic ovary syndrome." Drug Invention Today 12.11 (2019).

Brubaker, P. L., and D. J. Drucker. "Structure-function of the glucagon receptor family of G protein-coupled receptors: the glucagon, GIP, GLP-1, and GLP-2 receptors." Receptors and Channels 8.3-4 (2002): 179-188.

Cantini, G., Mannucci, E. &Luconi, M. Perspectives in GLP-1 research: new targets, new receptors. Trends EndocrinolMetab 27, 427–438 (2016).

Chang, S.A. Smoking and type 2 diabetes mellitus. Diabetes Metab. J. 2012, 36, 399–403.

Chen, Vivian Yi-Ju, and Tse-Chuan Yang. "SAS macro programs for geographically weighted generalized linear modeling with spatial point data: Applications to health research." Computer Methods and Programs in Biomedicine 107.2 (2012): 262-273.

Choi, Dong-Woo, et al. "Association between smoking behavior patterns and glycated hemoglobin levels in a general population." International journal of environmental research and public health 15.10 (2018): 2260.

Dahlstrom, E. and N. Sandholm, Progress in Defining the Genetic Basis of Diabetic Complications. CurrDiab Rep, 2017. 17(9): p. 80.

Davidson MB and Schriger DL: Effect of age and race/ethnicity on HbA1c levels in people without known diabetes mellitus: Implications for the diagnosis of diabetes. Diabetes Res ClinPract. 87:415–421. 2010.

Doria, Alessandro, Mary-Elizabeth Patti, and C. Ronald Kahn. "The emerging genetic architecture of type 2 diabetes." Cell metabolism 8.3 (2008): 186-200.

Ejarque, Miriam, et al. "Role of adipose tissue GLP-1R expression in metabolic improvement after bariatric surgery in patients with type 2 diabetes." Scientific reports 9.1 (2019): 1-9.

Flegal,K.M.,Graubard,B.I.,Williamson,D.F., and GAIL,M.H.(2005). Excess deaths associated with underweight , overweight, and Obesity. American Med Assoc ;293(15).

Goenka, V., Borkar, T., Desai, A., & Das, R. K. (2020). Therapeutic potential of mesenchymal stem cells in treating both types of diabetes mellitus and associated diseases.

Haywood, Natalie J., et al. "The insulin like growth factor and binding protein family: Novel therapeutic targets in obesity & diabetes." Molecular metabolism 19 (2019): 86-96.

Joan Vendrell, Rajaa El Bekay,BelénPeral, Eduardo García-Fuentes, Anna Megia, Manuel Macias-Gonzalez, José Fernández Real, Yolanda Jimenez-Gomez, Xavier Escoté, Gisela Pachón, Rafael Simó, David M. Selva, María M. Malagón, Francisco J. Tinahones, Study of the Potential Association of Adipose Tissue GLP-1 Receptor with Obesity and Insulin Resistance, Endocrinology, Volume 152, Issue 11, 1 November 2011, Pages 4072–4079

Lee, Young-Hoon, et al. "Effect of family history of diabetes on hemoglobin A1c levels among individuals with and without diabetes: the dong-gu study." Yonsei medical journal 59.1 (2018): 92-100.

NeamȚu, Marius Cristian, et al. "The correlation between insulin-like growth factor with glycemic control, glomerular filtration rate, blood pressure, hematological changes or body mass index in patients with type 2 diabetes mellitus." Rom J MorpholEmbryol 58.3 (2017): 857-861.discussion

Petersmann, A., et al., Definition, Classification and Diagnosis of Diabetes Mellitus. ExpClinEndocrinol Diabetes, 2018. 126(7): p. 406-410.

Qinglin Ma, Q., Liu, H., Xiang, G., Shan, W., Xing, W."Association between glycated hemoglobin A1c levels with age and gender in Chinese adults with no prior diagnosis of diabetes mellitus". Biomedical Reports 4.6 (2016): 737-740

Rajpathak SN, McGinn AP, Strickler HD, Rohan TE, Pollak M, Cappola AR, Kuller L, Xue X, Newman AB, Strotmeyer ES, Psaty BM, Kaplan RC. Insulin-like growth factor-(IGF)-axis, inflammation, and glucose intolerance among older adults. Growth Horm IGF Res, 2008, 18(2):166–173.

Sliwinska-Mosson,Mariola, and Halina Milnerowicz. "The impact of smoking on the development of diabetes and its complications." Diabetes and Vascular Disease Research 14.4 (2017): 265-276.

Tella, Sri Harsha, and Marc S. Rendell. "Glucagon-like polypeptide agonists in type 2 diabetes mellitus: efficacy and tolerability, a balance." Therapeutic advances in endocrinology and metabolism 6.3 (2015): 109-134.

Umayahara, Yutaka, et al. "CCAAT/enhancer-binding protein δ is a critical regulator of insulin-like growth factor-I gene transcription in osteoblasts." Journal of Biological Chemistry 274.15 (1999): 10609-10617.

Vendrell, J. et al. Study of the potential association of adipose tissue GLP-1 receptor with obesity and insulin resistance. Endocrinology 152, 4072–4079 (2011).

Vilsbøll, T., Christensen, M., Junker, A., Knop, F., Gluud, L. (2012) Effects of glucagon-like peptide-1 receptor agonists on weight loss: systematic review and meta-analyses of randomised controlled trials. Br Med J 344: d7771.

Weykamp, C., HbA1c: a review of analytical and clinical aspects. Ann Lab Med, 2013. 33(6): p. 393-400.

World Health Organization. Global status report on noncommunicable diseases 2014. No. WHO/NMH/NVI/15.1. World Health Organization, 2014.

Wu, Ming, et al. "Familial history of diabetes is associated with poor glycaemic control in type 2 diabetics: a cross-sectional study." Scientific reports 7.1 (2017): 1-10.

Xi, Chen, et al. "Association of GLUT4 gene variants with HbA1c level in Japanese men." Endocrine journal (2012): EJ11-0409.Discussion

Yan, Yuerong, et al. "Expression characteristics of proteins of IGF-1R, p-Akt, and survivin in papillary thyroid carcinoma patients with type 2 diabetes mellitus." Medicine 96.12 (2017).

Yang L, Shen X, Yan S, Xu F and Wu P: The effectiveness of age on HbA1c as a criterion for the diagnosis of diabetes in Chinese different age subjects. ClinEndocrinol (Oxf). 82:205–212. 2015.

Younes, Nada, et al. "HbA1c and Blood Pressure Measurements: Relation with Gender, Body Mass Index, Study Field, and Lifestyle in Lebanese Students." Endocrine Practice 25.11 (2019): 1101-1108.

Zhang J, Yang Z, Xiao J, Xing X, Lu J, Weng J, et al. Association between family history risk categories and prevalence of diabetes in Chinese population. PLoS One 2015;10:e0117044