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Role Of Leucine Rich Alpha 2 Glycoprotein 1 In Patients With Type 2 Diabetes Mellitus

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Abstract
Background: *Elevated levels of plasma Leucine rich apha-2 glycoprotein1(LRG1) which is associated with development and progression of type 2 diabetes mellitus where it is increased in patients with (T2DM) and also increased during complications of (T2DM) such as: retinopathy, neuropathy and kidney diseases.*
Objective: *The aim of the present study is to find the relationship between leucine rich alpha -2 glycoprotein1 (LRG1) with occurrences and development of Type 2 diabetes mellitus (T2DM).*
Method: *The current study is a case-control study conducted on 90 Iraqi participants for average age (20-70) years during the period between (November-2024) to (March -2025), participants from (Imam Hassan Center for Endocrinology and Diabetes) in (Holy Karbala Governorate - Iraq). This study including 90 participants divided into two groups: 60 patients with type 2 diabetes mellitus (T2DM) and 30 healthy control. Serum levels of (LRG1) was measured by using ELISA Kit.*
Results: *(LRG1) levels was significantly elevated in patients group with type 2 diabetes mellitus (T2DM) compered to control group. (LRG1) demonstrated an AUC of (0.853) with (P < 0.001) and (sensitivity 78% , specificity 70%) at a cut off of (0.76) ug/ml in the current study.*
Conclusion: *(LRG1) is valuable biomarker in diagnosing and differentiation of patients with type 2 diabetes mellitus (T2DM) and also it is considered as a risk factor for type2 diabetes mellitus (T2DM) in this study.*
Key words: *Type 2 diabetes mellitus (T2DM) , LRG1, ROC curve*

INTRODUCTION

Type 2 diabetes mellitus is an array of dysfunctions characterized by hyperglycemia resulting from a combination of resistance to insulin action, inadequate insulin secretion, and excessive or inappropriate glucagon secretion **[1]**. The incidence of diabetes mellitus type 1 and type 2 has increased dramatically worldwide in the past 20 years. Type 2 diabetes is probable to increase further in the future owing to the increase in obesity and the lack of exercise. This may rise the danger of unindustrialized cardiovascular illness in patients with type 2 DM **[2]**. This disease usually starts with insulin resistance—a condition that happens when muscle, fat, and liver cells cannot use insulin to deliver glucose into the cells of the body for energy use **[3]**.Type 2 diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Two main forms of diabetes were identified; type 1 and type 2. shortage of or severe decreasing in insulin secretion due to autoimmune or vital destructions of cells is responsible for type 1 diabetes mellitus (T1DM) which accounts for 5-10% of diabetic patients. The more prevalent form, type 2 diabetes mellitus (T2DM), accounts for more than 90% of cases **[4]** T2DM usually begins as insulin resistance, a disturbance in which the cells do not utilize insulin properly. As the need for insulin elevation, the pancreas gradually loses its ability to produce it **[5]**. Both La Barre and Heller proposed that the tentative substance could be utilized in the treatment of diabetes, which was rather farsighted considering that it was only shown in the early 1990 that the main incretin hormone had glucose reducing

actions in T2DM **[6]**. Genetic factors and lifestyle play a critical role in the development of T2DM **[7]**. The Asian population has strong genetic susceptibility to T2DM, developing diabetes at younger ages and at a lower degree of obesity **[8]** Currently the epsode of T2DM has reached epidemic levels in Asia .Despite knowledge of the critical role of genetic factors; these have not been confederated into the clinical appraisement of T2DM risk **[9]**.Type 2 diabetes mellitus is one of the most prevalent chronic diseases **[10].** Accumulated studies proved that the incidence of T2DM is forecasted to rise over the next two decades, especially among those aged 45 to 64 **[11]** and **[12]**. Nearly 463 million people in the world suffer with diabetes according to International Diabetes Federation in 2020 **[13].**In Iraq two million residents, or 7.43 % of the total population, were expected to be affected **[14]**. In 2017 (1,411,500) cases diabetes were registered in Iraq, 425 million people have diabetes in the world and more than 39 million people in the MENA Region; by 2045 this will raise to 67 million, Iraq is among the 19 countries and territories of the MENA region of the IDF **[15].**

LRG1 was first isolated from human serum in 1977 **[16]** and its amino acid sequence was determined in 1985**[17]** of the family of leucine-rich repeat (LRR). this protein found in many organ cells such as kidney, heart, retina and lung.when glucose levels rise in these organs. This lead to release of the protein from organ cells into the blood **[18]** and **[19].** LRG1 is consist of a single polypeptide chain of 312 amino acid residues and contains 8 LRRs. LRRs are protein–ligand interaction motifs, Each LRR consists of 19–29 amino acids, comprising a well-conserved N termina stretch of 9–12 amino acids, which is rich in the hydrophobic amino acid leucine, and a C-terminal domain that varies in length, sequence, and structure. Multiple repeats are typically arranged together to form a horseshoe shaped solenoid protein domain with a concave surface providing aplatform for protein–protein interactions **[20]** and **[21]**.

METHODS, PATIENTS AND MATERIALS

Patients: The current study is a case-control study conducted on 90 Iraqi participants for average age (20-70) years during the period between (November-2024) to (March -2025) , participants from (Imam Hassan Center for Endocrinology and Diabetes) in (Holy Karbala Governorate-Iraq). The practical part was, conducted at laboratories of department of biochemistry at College of Medicine , Karbala University, Iraq.

The current study was carried out on 60 patients (30 males and 30 females) of average age (20-70) years with type 2 Diabetes and they were diagnosed by Consultant physician, according to their clinical signs, symptoms and laboratory tests (FBS, HbA1C).

The control group included 30 volunteers (15males and 15 females) of average age (20-70) years. The control group have neither symptoms and no signs of diabetes mellitus, so they were apparently healthy. The control group is tested for (FBS, HbA1C).

Inclusion Criteria: Patient group was selected with type 2 diabetes mellitus and then selected control group after show normal results of (FBS and HbA1C).

Exclusion Criteria: Diabetes Mellitus type-1, Chronic Liver diseases, Chronic Renal diseases , Chronic Heart diseases, Chronic Joints diseases, Cancers ,Obesity.

Collection of The Blood Samples: After a least 12 hours of fasting, blood was collected by the vein puncture with plastic disposable syringes took up to 5mL of venous blood from both control and patient group. 2 ml were added to EDTA tube for detection of (HbA1C %) by (Latex turbidity technique) by using device (Lifotronic H8). while the remaining (3 mL) of the blood, is distributed into, gel tube, which, was then left at room, temperature for 30 minutes, in order to initiate the, clotting process. The sample was then centrifuged to separate the serum at 4,000 ×g for, (15) minutes. The sera or serum were subdivided into aliquot for immediate glucose measurement by Ethical Considerations: The ethical approvals were obtained from the ethical committee team, The college of medicine, The university of Karbala, and The Karbala Health Directorates / Karbala-Iraq. The sera or serum were subdivided into aliquot for immediate glucose

measurement by (Enzymatic colorimetric technique), by using (Auto-analyzer) device. Serum (IGFBP7) levels are measured by (Competitive immunoassay technique), by using (ELIZA) device.

Statistical analysis: They were performed using IBM SPSS Statistics for Windows, Version 26.0. Descriptive statistics were applied to summarize the data, with continuous variables expressed as mean ± standard deviation and categorical variables reported as frequencies (n) and percentages (%). The Kolmogorov-Smirnov test was used to assess data normality .For inferential analysis, the two-independent-samples *t*-test was employed to compare continuous variables between groups. ANOVA test used to compare between more than two groups. Correlations between biomarkers were evaluated using Pearson's correlation coefficient. Associations between variables were quantified using odds ratios (ORs) with 95% confidence intervals (CIs), derived from unconditional logistic regression. (ROC) curve analysis was conducted to determine the optimal threshold for critical Diabetics, balancing sensitivity and specificity.

Sex			
Biomarker	**Male** **Mean ± SD(n=30)**	**Female** **Mean ± SD(n=30)**	**P-value**
LRG1(ug/ml)	(0.789±0.580)	(0.896±0.551)	0.432

RESULTS

Comparison of LRG1 Mean Values between The study Groups

The current study for biomarker (LRG1) in the comparison between two groups are diabetic and non diabetic. The diabetic group had leucine rich alpha-2-glycoprotein-1 (LRG1) mean of (1.01±0.36) ug/ml, while non diabetic group had leucine rich alpha-2 glycoprotein-1 (LRG1) mean of (0.56 ± 0.26) ug/ml with p. value (<0.001), which was significant. The proportions are found in the following table.

Table (1) Comparison of LRG1 Mean Values between The study Groups

Biomarker	**Diabetic** **Mean ± SD (n=60)**	**Non-Diabetic** **Mean ± SD (n=30)**	**P-value**
LRG1(ug/ml)	(1.01±0.36)	(0.56±0.26)	<0.001

n: Number of Patients, SD: Standard Deviation, Test: Tow independent samples T.test, Differences are significant at P-value< 0.05.

Comparison of LRG1 MeanValues According to Sex in Patients with Diabetes Mellitus

The table (2) shown that the mean of (LRG1) for male is (0.789±0.580) ug/ml and female (0.896±0.551) ug/ml with p value (0.432), which was no significant as shown in the following table.

Comparison of LRG1 Mean Values Accoding to Age in Patients with Diabetes Mellitus

The table (3) shown that the mean of (LRG1) for ages from (20- 40) year, the proportion was (0.323±0.078) ug/ml, from (41-60) year, the proportion was (0.897±0.286) ug/ml , and (> 60) year, the proportion was (1.272±0.324) ug/ml with p value (<0.001), which was significant. The proportions are found in the following table.

Table (3) Comparison of LRG1 Mean Values Accoding to Age in Patients with Diabetes Mellitus.

Age				
Biomarker	**20-40 y N=18 Mean ± SD**	**41-60 y N=23 Mean ± SD**	**>60 y N=19 Mean ± SD**	**P-value**
LRG1 (ug/ml)	0.323±0.078	0.897±0.286	1.272±0.324	<0.001

n: Number of Patients, SD: Standard Deviation,Test: One way ANOVA Differences are significant at P-value< 0.05

Comparison of LRG1 Mean Values Accoding to BMI in Patients with Diabetes Mellitus

The table (4) shown that the mean of biomarker (LRG1) .The normal weight for LRG1was (0.307±0.129) ug/ml and the overweight for LRG1 was (1.132±0.407) ug/ml with p value (<0.001) which was significant. The proportions are found in the following table.

Table (4) Comparison of LRG1 Mean Values Accoding to BMI in Patients with Diabetes Mellitus.

BMI			
Biomarker	**Normal weight Mean ±SD (n=21)**	**Overweight Mean ±SD(n=39)**	**P-value**
LRG1 (ug/ml)	(0.307±0.129)	(1.132±0.407)	<0.001

n: Number of Patients, SD: Standard Deviation, Test: Tow independent samples T.test, Differences are significant at Pvalue<0.05

Roc Test of LRG1 in Diabetic Patients Group

ROC analysis demonstrated strong diagnostic performance for Biomarker (LRG1) that had an AUC of 0.853 with (p value <0.001) and Sensitivity was (78%) and specificity was (70%) at a cut off of 0.76 ug/ml in this study. The proportions are elucidated in the table (5) and the figure (1)

Table (5) Roc Test of LRG1 in Diabetic Patients Group

Biomarker	AUC	P-value	Cut off	Sensitivity	Specificity
LRG1 (ug/ml)	0.853	<0.001	0.76	0.78	0.70

Roc: Receiver operating charateristic; significant at p ≤ 0.05; Area under curve.

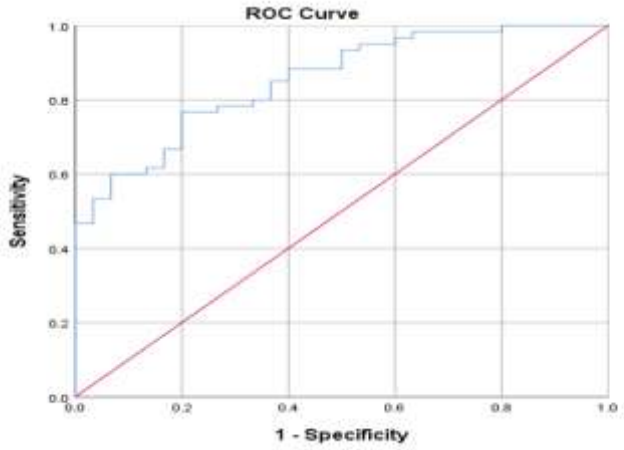

Figure (1) Receiver Operation Characteristics (ROC) curve of LRG1 in Studied Groups

DISCUSSION

In the current study, it was observed that the mean of diabetic group was significantly higher in (T2DM) patients more than control group as shown in table (1). In previous study which is showed increased (LRG1) levels have been found in the plasma of (T2DM) patients and described as statistically significant predictors of type 2 diabetes mellitus (T2DM) due to elevated of (LRG1) gene in islets of the pancreas for diabetic group more than control group as well as (LRG1) reduced of insulin secretion through impaired P21- activated kinase1(PAK1) function which is participated in insulin secretion from pancreas cells. Serum (LRG1) levels are increased with increased of insulin resistance for (T2DM) patients **[22]**. In current study, observed there was no statistically significant difference in the mean of (LRG1) between male and female (T2DM) patients as indicated in the table (2). In previous study unlike with present study, it was found elevated serum (LRG1) levels in men more than women due to (LRG1) DNA methylation levels were associated with (T2DM) in men but not in women **[23]**. In the present study, observed there was statistically significant differences in the mean of (LRG1) between (T2DM) patients as elucidated in the table (3). In previous study, observed that (LRG1) is correlated with age. B. cell of the pancreas with advanced age of (T2DM) patients which is lead to release of (LRG1) from (β. cell) to the blood. (LRG1) is reduced insulin secretion and increased insulin resistance through impaired of p 21- activated kinase1(PAK1) responsible about insulin secretion and reduced insulin resistance in the body and this is lead to excess of blood glucose and (LRG1) levels **[24]**. In the current study, observed there was statistically significant differences in mean of (LRG1) for body mass index (BMI) between (T2DM) patients as shown in the table (4). In previous study, observed that (LRG1) is correlated with body mass index (BMI). B.cell of pancreas with increased of body mass index (BMI) which is increased of insulin resistance and this lead to release of (LRG1) from (β.cell) to the blood **[25]**. In the present study, observed that (LRG1) is showed a strong diagnostic performance with an AUC of (0.853) and p value (< 0.001), sensitivity was (78%) and specificity was (70%) at a cut off of (0.76) ng/ml as indicated in table (5). In previous study which is showed that (LRG1) had excellent descriptive power with an area under ROC curve (AUC) **[26]**.

CONCLUSION: LRG1: is valuable biomarker in diagnosing and differentiation of patients with type 2 diabetes mellitus (T2DM) and also it is considered as a risk factor for type2 diabetes mellitus (T2DM) in this study.

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