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Correlation between Neutrophil Gelatinase Associated with Lipocaline and Megalin in Type 2 Diabetic patients

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Abstract

Background: Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder defined via elevated blood glucose caused by insufficiency of insulin-sensitive tissues to respond to insulin or defective insulin production by pancreatic β-cells due to environmental and genetic factors. Megalin is a glycoprotein that is mostly found in the proximal tubular cells, major role is to reabsorb albumin and other proteins that the glomerulus has filtered in the proximal tubules.

Methods: The purpose of research measure the concentration of megalin and Neutrophil Gelatinase Associated Lipocalin (NGAL) in type 2 patients, in the period from December 2022 to March 2023 at the Baghdad Teaching Hospital120 participants aged(30-60) years, the measured concentration of Megalin, NGAL, and fasting blood glucose in serum and whole blood estimated for glycated hemoglobin (HbA1C).

Results: The result indicates blood glucose concentration in patients with T2DM (344.975±63.68mg/dl) was significantly higher than control(106.62±12.59mg/dl), HbA1C (13.40±10.35%) in T2DM patients was significantly higher than control (4.310±0.336%), Megalin levels revealed for T2DM patients (220.70±42.47pg/ml) significantly higher than control (120.42±29.33pg/ml), NGAL(447.68±62.76pg/ml) in T2DM patients higher than control(264.93±51.79 pg/ml).

Conclusion: According to the results NGAL and Megalin levels were significantly higher in T2DM patients as compared to controls play a role in diabetes pathogenesis and as biomarkers for the early identification of diabetic nephropathy.

Introduction

Diabetes Mellitus (DM) is a multifactorial disorder with a progressive metabolic disease caused by a combination of environmental and/or genetic risk factors [1]. Disorders characterized by elevated blood glucose construction caused by defects in insulin action or secretion [2]. Classified as DM diabetes type 1 (5%) is an autoimmune disorder, Gestational diabetes occurs in pregnancy, type 2 diabetes and diabetes induced by a single gene mutation are extremely uncommon [3]. The most prevalent type of diabetes (about 90%) is type 2 diabetes, which is marked by a systemic inflammatory condition along with insulin resistance (IR) or reduced metabolic response to insulin in a variety of tissues, including skeletal muscle, liver, and adipose tissue, additionally by decreased insulin synthesis by pancreatic β-cells [4]. Type 2 Diabetes Mellitus is a slowly progressing glucose metabolism condition that can develop for 8 to 10 years before a diagnosis is made [5]. In 2021, DM prevalence was 10.5% the prevalence may reach 12.2% in 2045, with 783.2 million individuals worldwide, according to predictions [6]. Diabetes complications involve diabetic retinopathy, cardiovascular disease, and diabetic nephropathy (DN) most frequent leading cause of chronic kidney failure[7]. Megalin originally known as gp330 is a large transmembrane glycoprotein known, as low-density lipoprotein receptor-related protein 2 (LRP2). Megalin participates in the endocytosis of several substances like proteins, lipoproteins, and drugs in the proximal tubular cells (PTCs) of the renal [8]. Megalin is expressed selectively within the brush border of PTCs at the apical membrane where it functions as a multi-ligand receptor that is necessary for the reabsorption of a great volume of filtered substances [9]. Type 2 diabetic patients have elevated construction of protein metabolic and kidney metabolism of these proteins are potentially overloaded. A few of these proteins are themselves nephrotoxic, while others are carriers of nephrotoxic molecules. Megalin is participates in the proximal tubular uptake and reabsorption of these proteins may cause kidney hypoxia and result in a relative overload of protein metabolism in the renal. This megalin-mediated metabolic pathway might lead to the development of modern techniques for the treatment of diabetic nephropathies [10]. Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a 25-kDa member of the lipocalin family of proteins also known as siderocalin, lipocalin 2. In addition, small amounts of NGAL are present in a variety of cell types, such as kidney, stomach, lung, uterus, prostate, and colon. Its production increases with age [11]. NGAL is a lipoprotein involved in ligand transport, inflammation, preservation, and iron transport. As a marker of early renal tubular damage, NGAL in blood and urine rises within two hours following acute kidney injury. It is more detected earlier and more sensitive than creatinine [12].

Methods

Participants 60 patients diagnosed by physicians depended on ADA (American diabetes association,2021) and 60 healthy two groups contains 30 Males, and 30 Females, aged (30-60) years in the Baghdad Teaching Hospital, Between the period from December 2022 to March 2023. Approximately 8 mL of blood sample was drawn. Three milliliters of blood were dispensed into EDTA tubes to HbA1C estimation on the same day for each (patient &control), emptying the residual blood into a gel tube, letting it coagulate, and then centrifuging it were the next steps for 10 minutes at 3000 rpm to get serum. stored the serum in Eppendorf tubes at -20 °C until it was used to estimate glucose by using the colorimetric method and estimate the concentration of NGAL and megalin by ELISA techniques with the sandwich method.

Ethical approval: for research obtained from the Iraqi Ministry of Health on November 24, 2022, at Medical City.

Statistical analysis

Used to revise, code, and analyze the data Statistical Package for Social Sciences version 26 (SPSS-26). The difference between the two means' significance was determined using an independent sample t-test. were taken into account Statistical significance (P≤0.05), (P≤0.01)Highly significant (HS), and (P>0.05) there is no significance.

Results

Distribution of the age and gender in study groups

The gender and age distributions that appear in Table 1 of the research group differed significantly. The most frequently observed age and gender group within T2DM was (50-60) female 32.5%, male 35%, For the control group, the most common age and gender group was (50-60) female 25%, male 25%.

Study groups	Age groups (year)	Gender		Study groups	Age groups (year)	Gender	
		Male	Female	T2DM		Male	Female
Control	(30-39)	6.7%	8.3%		(30-39)	2.5%	7.5%
	(40-49)	18.3%	16.7%		(40-49)	12.5%	10%
	(50-60)	25%	25%		(50-60)	35%	32.5%

Table 1: Distribution of the age and gender in Control and T2DM Patients.

Estimation of Glucose (mg/dL) and HbA1C% in the Patients and Control groups

As shown in Table 2, there were highly significant (P≤0.01), changes of glucose (mg/dL) and HbA1C% when comparing between the control and patient groups. The mean in glucose (344.97563±68) vs.

Control (106.62±12.59) and mean HbA1C% (13.40±10.35) vs. Control (4.310±0.336) between control and patient groups were extremely significant.

Study groups	Mean ±Std.		
	Glucose (mg/dL)	HbA1C%	*P*-value
Control	106.62±12.59	4.310±0.336	*P*=.000 (<0.01 HS)
Diabetes Mellitus	344.975±63.68	13.40±10.35	

HS= Highly Significant ($p < 0.01$).
Table 2: Comparison of Glucose (mg/dL) and HbA1C% between Control and T2DM Patients.

Estimation of megalin (pg/ml) concentrations in the patient and control groups

Table 3 indicates a highly significant (P≤0.01) rise of megalin levels in the T2DM groups (220.70±42.47pg/ml) as compared to the control group (120.42±29.33pg/ml).

Megalin (pg/ml)	Total (Mean± SD.)	t-test	P-Value
Control	120.42±29.33	13.976	P=.000 (<0.01 HS)
Diabetes Mellitus	220.70±42.47		

HS= Highly Significant ($p < 0.01$).
Table 3: Comparison of megalin (pg/ml) concentration between Control and T2DM Patients.

Estimation of NGAL (pg/ml) concentrations in the patient and control groups.

Table 4 shows a highly significant (P≤0.01) increase in NGAL consternation in T2DM patients (447.68±62.76pg/ml) as compared to the control(264.93±51.79 pg/ml).

NGAL (pg/ml)	Total (Mean± SD.)	t-test	P-Value
Control	120.42±29.33	15.870	P=.000 (<0.01 HS)
Diabetes Mellitus	220.70±42.47		

HS= Highly Significant ($p < 0.01$).
Table 4: Comparison of NGAL (pg/ml) concentration between Control and T2DM Patients.

Discussion

Diabetes incidence increased with age according to the findings. similarly, El Omri et al., observed that T2DM occurred elevated in the fifth decade of age [13]. Aging is linked to impaired pancreatic islet function and an elevated risk of type 2 diabetes [14]. It showed that a 1-year age increase in the diagnosis of diabetes was related to a high risk of complications. Our age finding is consistent with the findings of research by [15]. The age of diabetic patients was planned to vary from before to more than 45 years in order to research and detect the presence of diabetes problems in an attempt to treat or prevent their development which is compatible with some of the prior studies described by [16]. About the mechanism of diabetes type 2, it is well recognized that aging causes a decrease in insulin sensitivity in addition to an alteration or deficient compensation of beta-cell functional mass in the face of increased insulin resistance. Aging is associated with decreased β-cell proliferation and elevated sensitivity to apoptosis [17]. In this study males were shown to be more affected by type 2 diabetes than females. These results are similar to [18]. however, this disagrees with the findings of the research by [19]. There are a variety of screening methods and instruments available to detect disease in a population. The most often used biochemical assays are fasting plasma glucose (FPG) and glycated hemoglobin A1C (HbA1c), however, urine glucose tests and random blood glucose tests are also available, a fasting plasma glucose construction of 126mg/dL or higher is consistent with a type 2 diabetes diagnosis according to documentation by [20]. According to a study by Ahmed et al., Glycated hemoglobin was shown to be highly significant in diabetic type 2 Patients when compared with control [21]. HbA1c concentrations have long been used to monitor diabetes glycemic control in people who have previously been diagnosed. The WHO recommends an HbA1c of 6.5% as the cut-off point for diabetes diagnosis [22]. who established that HbA1c had great specificity and sensitivity for diabetes diagnosis concur with other research such [23].

This study indicates that raised kidney level of megalin (220.70±42.47) expression in patients with T2DM because complex reabsorbs albumin and other proteins filtered by the glomerulus in the proximal tubules. and this is in agreement with his study by Bryniarski et al., [24]. Megalin ligands like insulin, leptin, albumin, PTH, and angiotensin II have been implicated in diseases such as hypertension, diabetes, and obesity [25]. Also been demonstrated that there was a significantly higher expression of megalin protein detected in early type 2 diabetes [26]. In this model, DM caused kidney damage with significantly increased glucose concentrations, tubular injury, and proteinuria. These results associated with Megalin upregulation may indicate a tubular injury and apoptosis as progresses diabetic Nephritis(DN).

Examine urinary megalin forms and concentrations, as well as their clinical importance as new biomarkers for diabetic Nephritis and associated disorders in type 2 diabetic patients[27]. Disagree with De et al., there show a decrease in megalin expression in PTCs of patients with developed diabetes related to atrophy of brush borders. Extracellular vesicles might assist as a mediator for megalin excretion in the urine [28].

In this study we examined at T2DM affects circulating levels of increase in NGAL in diabetic patients compared to control group results agrees with Najafi evaluation of NGAL involvement in diabetic patients [29]. Starting 2-4 hours after a kidney injury, elevated NGAL levels can be seen in plasma and urine, due to changes in glomerular filtration, tubular reabsorption, and also via increased secretion in tubular epithelial

cells [30]. When the proximal tubule is injured by tubular necrosis filtered NGAL can leave tubular reabsorption and secrete in urine, high serum NGAL and high filtered saturated tubular capacity lead to increased urinary and serum NGAL [31]. NGAL is a good biomarker for detection of acute kidney failure because of a small molecule in size and resistance degradation, NGAL excretion in serum and urine was found to be an early predictive biomarker of renal injury [32]. NGAL is one of the most tubular markers use in the diagnostic domain of acute and chronic kidney disorders [33].

Increasing the concentration of NGAL and megalin in diabetic patients compared to control leads to using biomarkers for early diagnosis of Diabetic Nephropathy.

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Author Contributions

Duaa Abdul-Jabbar Dawood: the research article proposal, experiment design, explaining the findings, article writing and editing.
Emad Mohamed Rasheed: Preparing materials, Funding acquisition, and data curation.
Ahmad Saadi Hassan: Statistical analysis and review.

Competing Interest

The authors declare that there is no conflict of interest.

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