CLEC4E is A New Biochemical Tool for Evaluation the Progression of Nephropathy Complications of Type 2 Diabetes Mellitus

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ABSTRACT

Background: Diabetes Mellitus is *a group of metabolic disorders represented by high levels of glucose in the blood as a result of a decrease or lack insulin secretion from β-cell in the pancreas or weakness in mechanical acting or both of them or defect in insulin receptors as a result of hereditary disturbance.* Sometimes in another situation of diabetes disease, it is ascribed to increase the secretion of antibodies to insulin act or antibodies to pancreas gland and destroy β-cells that secrete insulin, which is due to the occurrence of disorder in carbohydrate metabolism glucose specially. *Hyperglycemia is accompanied by a range of long-term diseases, dysfunction and failure of various organs, especially the heart, eyes, blood vessels, kidneys and nerves.* Subjects: From the beginning of December 2018 to the end of May 2019, 158 individuals residents of Najaf and Karbala Governorates were included to participate in the current study. Method: Sandwich-ELISA technique was applied to estimate levels of CLEC4E. Results: The study showed that the largest percentage of people with diabetes who suffered from kidney failure as a result of a large and uncontrolled increase in blood sugar levels, which required the use of hemodialysis mechanism to help the kidney to perform its vital functions, although at the lowest levels were in the middle age groups (between 50 -70 years). The statistical analysis using the *Student's t-test* showed a significant difference (p=0.001) when comparing the two study groups (renal failure patients undergoing hemodialysis and healthy individuals), where the recorded results showed an increase in the levels of measured lectin (CLEC4L) in the sera of the patients group comparison to the controls group. The present study found a statistically significant raise (p=0.000) of the measured lectin in sera samples of males with renal failure compared with their peers in the control group, this finding was consistent with what was observed when comparing the levels of lectin in infected female samples with those in the control group (p=0.000). Moreover, the present study found significant gender differences within the same group, it was observed that the levels of the measured lectin in males (in both studied groups) were higher than in females, (p=0.001 for comparison in the patients group, and p=0.043 for comparison in the controls group, respectively). In more detail, the highest level of lectin recorded in the present study (4.587 pg/ml)was found in a sample of a woman with renal failure and undergoing repeated hemodialysis for more than a year. This woman was diagnosed with type 2 diabetes 33 years ago. It was also found the lowest level of CLEC4L (0.026pg/ml) in a sample of a 57-year-old woman who was diabetic only 12 years and undergoing hemodialysis only two months. Results of the present study indicate a positive correlation in more than three-quarters (78.23% at p<0.005) of renal failure samples, when it had been recorded a simultaneous rise in the new lectin (CLEC4L) levels assessed in the current work with the age of patients

KEY WORDS: CLEC4E, DM, Renal Failure, Hemodialysis

INTRODUCTION

Diabetes Mellitus (DM) is *a group of metabolic disorders represented by high levels of glucose in the blood (hyperglycemia) as a result of a decrease or lack insulin secretion from β-cell in the pancreas or weakness in mechanical acting or both of them or defect in insulin receptors as a result of hereditary disturbance [Bob,2002]*. Sometimes in another situation of diabetes disease, it is ascribed to increase the secretion of antibodies to insulin act or antibodies to pancreas gland and destroy β-cells that secrete insulin, which is due to the occurrence of disorder in carbohydrate metabolism glucose specially [Aseel, 2019]. *Hyperglycemia is accompanied by a range of long-term diseases, dysfunction and failure of various organs, especially the heart, eyes, blood vessels, kidneys and nerves [Richarg,2010]*. Lectins simply are ubiquitous proteins or glycoproteins that are probably present in all eukaryotic cells and many bacterial species, as well as in some viruses

[Santos, 2014]. They are capable to bind mono - and oligosaccharides with high affinity, and usually agglutinate cells or precipitate polysaccharides and glycoconjugates specifically and reversibly [Renata, 2015], the binding involves hydrophobic interactions as well as hydrogen bonds. They possess at least one non-catalytic domain, i.e. are not enzymes. In contrast to antibodies, they are not products of an immune response [Dania, 2018]. Comprehensively, lectins constitute a superfamily of ubiquitously distributed proteins, which are described and characterized in a steadily increasing number of publications [Luana, 2014]. They are easily detected through agglutination assay which allows for easy viewing of the agglutinating erythrocyte property of lectins. Lectins consist of two major groups based on their metal dependence: the Calcium-Dependent (C-type lectins) and the Calcium-Independent (S-type lectins)**.** The present study aims for investigation of CLEC4E as a new marker for prediction, detection of renal failure caused by diabetic complications undergoing hemodialysis .

MATERIALS AND METHODS

Subjects: During the period from the beginning of December 2018 to the end of May 2019, 158 individuals residents of Najaf and Karbala Governorates were included to participate in the current study. The participator individuals in the current study were classified into two groups depending on their healthy to: patients with renal failure caused by diabetic complications undergoing hemodialysis and controls. The first included 108 patients between the ages of 20 and 80 (54.160±13.347). The second group included 50 healthy persons between the ages of 22 and 65 years (26.140±6.940). Total data about the study groups were summarized in Table 1.

Table 1: Levels (Mean±S.D.) of Age (Year) in The Study Individuals

Study Groups (n)	*Gender (n)*	*Age (Year) Mean ± S.D.*	*Min.-Max. Range*	*p-value*
Patients 108	Male 71	54.380 ± 12.827	24-80 56	*0.786 For 1 vs 2*
	Female 37	53.730 ± 14.468	22-72 50	*0.000 For 1 vs 3*
Controls 50	Male 38	26.370 ± 7.295	16-43 27	*0.808 For 3 vs 4*
	Female 12	25.420 ± 5.900	20-38 18	*0.000 For 2 vs 4*

1: Male Patients with Renal Failure, 2: Female Patients with Renal Failure, 3: Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

Method: Sandwich-ELISA technique was applied to estimate levels of lectin in the serological samples of the two current study groups using Human CLEC4E Kit that prepared by Elabscience Company, China.

Analysis of Data: The statistical analysis was done using SPSS software for windows, version 22. The results were expressed as Mean ± S.D., maximum, minimum and range. Student's independent *t*-test was used to analyze the data of studied parameters. One-way Analysis of Variance (ANOVA) was used to compare parameters in different studied subgroups. Pearson's correlation coefficient was applied to determine the relations between the age and lectin in the present study. *p-values* less than 5% ($p < 0.05$) were considered statistically significant.

RESULTS AND DISCUSSION

According to the data in the information form used, the study did not find the effect of age on the incidence of renal failure, where the injury included persons in the second decade until the eighth decade, moreover; the current study indicates that more than 87% of diabetic patients suffered renal failure were over 40 years of age, full distribution of the diabetic patients undergoing to hemodialysis was summarized in the Figure 1. The study showed that the largest percentage of people with diabetes who suffered from kidney failure as a result of a large and uncontrolled increase in blood sugar levels, which required the use of hemodialysis mechanism to help the kidney to perform its vital functions, although at the lowest levels were in the middle age groups (between 50 -70 years), as shown in Figure 1. This result confirms that the renal impairment caused by diabetes is a long-term disorders and does not show the farthest years of developing diabetes and this is consistent with the results confirmed in previous research and documented in the recommendations of the World Health Organization (WHO).

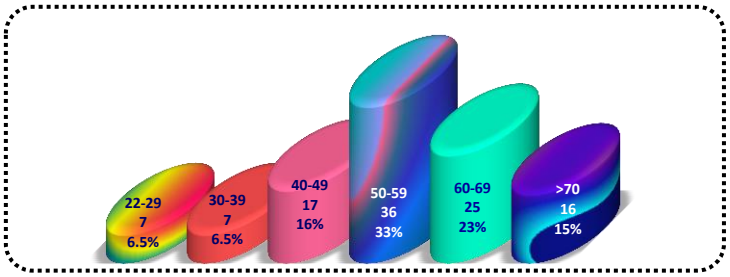

Figure 1: Distribution of T2DPatients According to Their Ages

In more detailed terms, the study showed that the largest hemodialysis patient with type 2 diabetes was male in 80 years old, while the youngest patients was a woman at the age of 22 years. Moreover; only 8 out of 71 (11%) male patients were under 40 years of age, and 11 patients (16%) were under 50 years of age, while the largest number (30 male patients) of those were aged between 50 and 60 years (42% of total male diabetic patients). In the group of women patients applying for hemodialysis included 6 female patients are among the total 50 participants (12%) in the current work under the age of 40 years, while the largest number of female patients (15 patients, 30% of total female diabetic patients) with renal failure were in the age group between 60 and 69 years. These findings indicated that the progression of complications from diabetes is higher in males than females, on the other hand, males may be more likely to survive compared to females. When the current study included 12 male patients over the age of 70 of them had no less than 20 years of diabetes, while the study included only 3 women aged 70 and older at age 72 and no more than 15 years of diabetes. The statistical analysis using the *Student's t-test* showed a significant difference ($p=0.001$) when comparing the two study groups (renal failure patients undergoing hemodialysis and healthy individuals), where the recorded results showed an increase in the levels of measured lectin (CLEC4L) in the sera of the patients group comparison to the controls group as shown in Table 2.

Table 2: Levels (Mean± S.D.) of CLEC4LConcentrations (pg/ml) in the Sera Samples of Renal Failure Patients and Controls Groups

Study Groups (n)	*CLEC4L Concentrations (pg/ml)Mean ± S.D.*	*Min.-Max. Range*	*p-value*
Patients 108	**3.532 ± 0.312**	**0.026- 4.482 4.456**	***0.001***
Controls 50	**2.135 ± 0.646**	**0.136-4.469 4.333**	

The Mean Difference is Significant at 0.05 Level

In order to investigate the effect of gender on the levels of serum lectin, the statistical ANOVA was applied to compare the recorded results in the study samples after classification the participants in the two groups according to their gender. The present study found a statistically significant raise (p=0.000) of the measured lectin in sera samples of males with renal failure compared with their peers in the control group, this finding was consistent with what was observed when comparing the levels of lectin in infected female samples with those in the control group (p=0.000). Moreover, the present study found significant gender differences within the same group, it was observed that the levels of the measured lectin in males (in both studied groups) were higher than in females, (p=0.001 for comparison in the patients group, and p=0.043 for comparison in the controls group, respectively).

Table 3: Levels (Mean±S.D.) of CLEC4L Concentrations (pg/ml) in the Study Subgroups

Study Groups (n)	*Gender (n)*	*CLEC4L Concentrations (pg/ml) Mean ± S.D.*	*Min.-Max. Range*	*p-value*
Patients 108	**Male 71**	**3.901 ± 0.322**	**0.060 – 4.482 4.422**	***0.001 For 1 vs 2***
	Female 37	**2.899 ± 0.824**	**0.026 – 4.587 4.561**	***0.000 For 1 vs 3***
Controls 50	**Male 38**	**2.348± 0.851**	**0.406 – 4.469 4.063**	***0.043 For 3 vs 4***
	Female 12	**1.467 ± 0.634**	**0.136 – 3.016 2.880**	***0.000 For 2 vs 4***

1: Male Patients with Renal Failure, 2: Female Patients with Renal Failure,3:Healthy Male Control, and 4: Healthy Female Control. The Mean Difference is Significant at 0.05 Level

In more detail, the highest level of lectin recorded in the present study (4.587 pg/ml)was found in a sample of a woman with renal failure and undergoing repeated hemodialysis for more than a year. This woman was diagnosed with type 2 diabetes 33 years ago. It was also found the lowest level of CLEC4L (0.026pg/ml) in a sample of a 57-year-old woman who was diabetic only 12 years and undergoing hemodialysis only two months.

The results of the investigation in the previous literature and literature did not indicate the existence of a study to evaluate the levels of lectin CLEC4E in samples of healthy individuals or samples of people with diabetes to one of its complications, which makes the present study an attempt to provide a full study on the levels of this class of lectin in healthy samples and others with renal failure on the other hand, the present study was designed as an attempt to present this type of lectin as a function to investigate the most vulnerable to the development of renal injury caused by chronic elevated sugar levels leading to renal failure. The results of the study showed that this type of lectin is produced naturally in humans, but its levels are very high in people with type 2 diabetes, where the study recorded an increase in levels of CLEC4E in 85 out of 108 samples evaluated (**~79%**), in addition, it was found that the levels of this protein increase relatively and steadily with age. The study indicated that there were differences in the levels of this type of proteins between the sexes within the same group, whether for individuals infected or healthy, while the study showed that the lower levels of this protein in females than in males, while the sample of infected females recorded individual levels higher than recorded in males; in general, however, male lectin levels (both healthy and patients) were found to be higher than females. Actually, the observed increase in the level of CLEC4E in diabetics can be explained by the hypothesis of the potential role of lectin in inducing diabetes as an inflammatory condition and may be implicated in the pathogenesis of diabetic vascular complications by inducing the development of complications related to the body's external efficiency. Previous studies indicated isolation of different types of lectin from different human samples[Anja 2001, Rasha 2010, and Kelany 2012],whereas levels of a large number of lectin were evaluated in satisfactory and unsatisfactory samples[Daniel 2001, Damon 2003, Søren 2009, Humam 2019].

Results of the present study indicate a positive correlation in more than three-quarters (78.23% at $p<0.005$) of renal failure samples, when it had been recorded a simultaneous rise in the new lectin (CLEC4L) levels assessed in the current work with the age of patients, this observation highlighted by Figure 2 A. While the study recorded the absence of statistical significance for the negative correlation ($r=-0.2$ at $p<0.05$) of age with the concentration of CLEC4L in the group of healthy individuals, as shown in Figure 2 B.

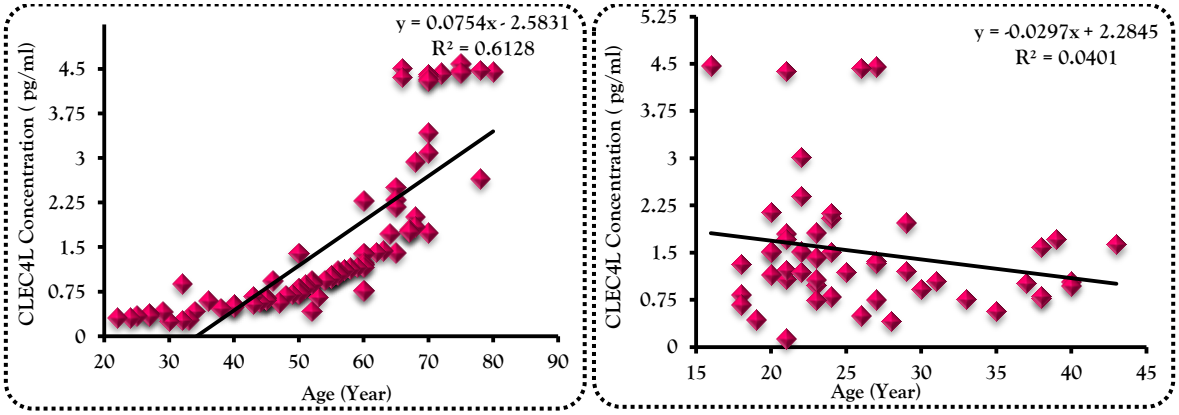

Figure 2: Relationship Between Age and CLEC4L Concentration in Sera Samples of (A): Patients and (B):Healthy Individual

Actually, yet no references have been found to indicate the relationship of this type of lectins with age of individuals with diabetes mellitus or renal failure.

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