Assessment of Interleukin 1B Blood level in type 1 Diabetes Mellitus

BY

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Abstract

Background: Diabetic nephropathy is the leading cause of renal failure in all over the world. Earlier, more sensitive and specific markers of kidney damage might help diagnose and treat diabetic nephropathy at an earlier stage to prevent the progression to renal failure, **Aim:** to measure the level of IL1β in children and adolescents with type1DM and its relation to other factors (HbA1c, eosinophils, fasting free glucose, postprandial blood glucose, albumin creatinine ratio) as early predictor of renal injury, **Subjects and methods:** This was a case-control comparative study carried out at pediatric endocrinology and outpatient clinic of pediatric department of Alzhraa and Tanta hospital on 90 children and adolescents diagnosed asT1DM, during the period from May 2021 to September 2022. divided into two groups: Group 1: included 45 child and adolescents diagnosed as type1 diabetes mellitus, Group 2: included 45 apparent healthy children with age and sex matched, were subjected to full clinical history, complete clinical examination and laboratory tests. **Result**; There was statistically significant positive correlation found between IL-1b and (BMI, HbA1c, eosinophils, fasting free glucose, postprandial blood glucose, albumin creatinine ratio and alkaline phosphatase) while there is negative correlation found between IL-1b and (red blood cell count, hemoglobin, hematocrit, white blood cell count and platelets count). **Conclusion;** IL-1β can be considered as a potential biomarker for T1DM, it was found that circulating levels of IL-1β was highly significant in type 1 diabetic patients compared to healthy controls, and related to BMI but not related to disease duration.

Keywords: Biomarkers; Diabetes; Nephropathy; Microalbuminuria.

Introduction

Type 1 diabetes is a chronic lifelong metabolic disease usually diagnosed in children and young adults, and was previously known as juvenile diabetes in which the body does not produce insulin due to destruction of Beta cells of Islets of Langerhans of pancreas which secrete insulin which is responsible for regulation of blood glucose [(1)]. Diabetic retinopathy, diabetic neuropathy, diabetic nephropathy and cardiovascular disease are related complications to type 1 diabetes [(2)]. Diabetic nephropathy (DN) is one of the most important complications in patients with diabetes, having an adverse effect on morbidity and mortality. At present, albuminuria is widely used as an early clinical marker for the detection of DN [(2)]. Inflammasome-driven release of interleukin (IL)-1β is a central element of many forms of sterile inflammation and has been evident to promote the onset and progression of diabetic kidney disease. When microdissected glomerular and tubulointerstitial samples from kidney biopsies of patients with diabetic kidney disease it was found expression of IL-1β mRNA. Immunostaining of such kidney biopsies across a broad spectrum of diabetic kidney disease stages revealed IL-1β positivity in a small subset of infiltrating immune cell [(3)].

Aim of the study

To measure the level of IL1 β in children and adolescents with type1 DM

Ethical consideration:

1. An informed oral and written was obtained from all parents of both patients and control groups before getting them involved in the study.
2. The researcher explained the stages, the aims, the potential benefits and hazards of the study to all parents of the patients and control groups.
3. The patients had the right to leave the study at any time.
4. Confidentially and privacy were respected.
5. Ethical approval was obtained from the ethics committee of the Pediatrics department at the faculty of medicine for girls at Al-Azhar University: Reg. No. IRB00012239, Date 13September 2019.
6. No conflicts of interest are to be declared, as reported by the authors.
7. FINANCIAL DISCLOSURE / FUNDING: The authors received no financial support for the research, authorship, and/or publication of this article.

Sample size calculation:

The sample size calculation was performed using EpI-Info 2002 software statistical package designed by World Health Organization (WHO) and by Centers for Disease Control and Prevention (CDC).

The sample size was calculated based on the following considerations: 95% confidence level and the sensitivity of IL-1β for T1DM was 70% according to a previous study [(27)] ± 10% confidence limit. Nine cases were added to overcome dropout. Therefore, we will recruit 90 cases.

Inclusion criteria:

A. Age ranges from 6 to 18 years.
B. Children and adolescent diagnosed as T1DM according to (ADA, 2022).
C. Gender: male and female.

Exclusion criteria:

A. Subjects with urinary tract infection.
B. Patients with congenital heart diseases.
C. Other renal diseases or neurological problems.

STUDY PROCEDURE:

This case-control comparative study that was done on 90 Children and adolescents, recruited from pediatric endocrinology and outpatient clinic of pediatric department of Al-zharaa and Tanta university hospital during the period from May 2021 to September 2022. It included 45 child with age 6-18 diagnosed as type 1 diabetes (serve as patients group) according to (ADA, 2021) compared with 45 healthy children with age and sex matched (serve as control group)

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All studied Patients and controls were subjected to:

1- full clinical history and

11- complete clinical examination. (general and systemic)

111-Laboratory evaluation including:

1) A complete blood count was done using fully automatical cell counter (Systmex KX 21N, Kobe, Japan).

2) Fasting blood glucose,

3) post prandial blood glucose,

4) liver function tests (ALT, AST, ALP, Albumin, bilirubin)

5) kidney functions (Creat. and Urea) were done using fully automatic chemistry analyzer (Cobas C311, Germany).

6) Glycosylated haemoglobin (HbA1c); HbA1c was measured by (Cobas C311, Germany) by Immune turbidity method.

7) Urinary albumin was measured using Calorimetric method (kits micrototal protein. Spectrum Lot. MTPS0103019). The urine albumin concentration first calculated as abs. of specimen/ abs. of standard x 150. The concentration/24 h urine = concentration/dl x urine volume /ml / 100.

Both albumin and create in urine measurement were done using (Cobas C311, Germany). The albumin (mg/L) in each urine sample is compared against it's concentration of creat. Finally the ratio was expressed in mg/g creatine.

Diagnosis was classified into: Normal if A/C ratio<30mg/g, microalbuminuria if A/C ratio30-300mg/g and macroalbuminuria if A/C ratio >300mg/g[(4)].

MDRD GFR Equation was used to estimate glomerular filtration rate, based on creatinine and patient characteristics. This calculator used 4-variables age, six, creatinine, race.

According to MDRD GFR equation: GFR in mL/min per 1.73 m^2 = 175 x SerumCr 1.154 x age-0.203 x 1.212 (if patient is black) x 0.742 (if female).

Name of the kits of IL-1B manufactured by Sunred.

Statistical Analysis:

Data was collected, revised, coded and entered to the statistical package for social science (SPSS) version 17 and the following were done: Qualitative data were presented as number and percentages while quantitative data were presented as mean, standard deviations, ranges, median interquartile range (IQR) and Z score (=individual variable-mean value of reference population/standard deviation (SD) of reference population. The comparison between two groups with qualitative data were done by using Chi-square test and/or Fisher exact test was used instead of Chi-square test when the expected count in any cell was less than 5. The comparison between two independent groups with quantitative data were done by using In-dependent t-test when the data were parametric. Comparison between more than two groups with parametric data were done by using One –Way ANOVA. The confidence interval was set to 95% and the margin of error accepted was set to 5%.

RESULTS

Table (1): Comparison between DM group and control group regarding clinico- demographic data and characteristics of the studied patients

		DM group	**Control group**	**Test value**	**P-value**
		No. = 45	**No. = 45**		
Age (year)	Mean±SD Range	11.78 ± 2.15 6 – 17	12.04 ± 2.31 8 – 18	-0.567•	0.572
Sex	Females Males	25 (55.6%) 20 (44.4%)	24 (53.3%) 21 (46.7%)	0.045*	0.832
Height (cm)	Mean±SD Range	147.87 ± 14.00 108 – 172	149.87 ± 14.40 123 – 190	-0.668	0.506
Height z-score	Median (IQR) Range	-0.13 (-0.77 – 0.72) -2.89 – 1.63	-0.13 (-0.56 – 0.64) -1.83 – 2.90	-0.428≠	0.669
Weight (kg)	Mean±SD Range	42.49 ± 6.80 23.91 – 54.33	38.14 ± 6.39 26.63 – 57.4	3.127	0.002
Weight z-score	Median (IQR) Range	0.27 (-0.29 – 0.97) -2.37 – 2.03	-0.50 (-0.90 – 0.23) -1.98 – 2.47	-3.240≠	0.001
BMI (kg/m2)	Mean±SD Range	19.36 ± 0.60 18.1 – 20.5	16.91 ± 0.50 15.8 – 18	20.932•	0.000
BMI z-score	Median (IQR) Range	1.01 (0.57 – 1.16) -0.03 – 1.76	-0.92 (-1.14 – -0.69) -1.73 – -0.10	-8.176≠	0.000

Family history	No	36 (80.0%)	43 (95.6%)	5.075*	0.024
	Yes	9 (20.0%)	2 (4.4%)		

P>0.05: Non significant (NS); P <0.05: Significant (S); P <0.01: Highly significant (HS)

*: Chi-square test; •: Independent t-test

Table (1) shows that there was statistically significant increase in the BMI in diabetes group than control group. And statistically significant increase in the weight in diabetes group than control group. And statistically significant increase in weight z-score and BMI z-score in diabetes group than control group. And also, statistically significant increase in the percentage of patients with family history of DM in diabetes group than control group.

Table (2): Comparison between both studied group regarding laboratory finding.

		DM group	**Control group**	**Test value**	**P-value**
		No. = 45	**No. = 45**		
Red blood cell count (x10^12/L)	Mean±SD	4.27 ± 0.11	4.75 ± 0.18	-15.59•8	0.000
	Range	4 - 4.5	4.3 - 5.1		
Hemoglobin (g/dL)	Mean±SD	13.05 ± 0.38	14.05 ± 0.41	-12.05•6	0.000
	Range	12.2 - 13.7	13.2 - 14.8		
Hematocrit (%)	Mean±SD	39.58 ± 1.46	41.70 ± 1.50	-6.799•	0.000
	Range	36.2 - 42.4	39.3 - 44.4		
Platelet count (x10^9/L)	Mean±SD	217.89 ± 33.23	235.82 ± 36.40	-2.441•	0.017
	Range	132 - 285	164 – 340		
White blood cell count (x10^9/L)	Mean±SD	5.89 ± 1.21	6.53 ± 1.42	-2.310•	0.023
	Range	3.1 - 8.8	3.5 - 10.1		
HbA1c (%)	Mean±SD	10.68 ± 0.51	4.90 ± 0.18	71.923•	0.000
	Range	9.7 - 11.9	4.5 - 5.2		
Fasting blood glucose (mg/dl)	Mean±SD	148.72 ± 14.11	84.69 ± 6.82	27.416•	0.000
	Range	118.2 - 176.1	71.3 - 100.2		
Postprandial blood glucose (mg/dl)	Mean±SD	189.72 ± 13.69	123.95 ± 7.21	28.521•	0.000
	Range	159.2 - 215.1	109.3 - 139.2		

Creatinine (mg/dL)	Mean±SD Range	0.77 ± 0.12 0.54 - 0.98	0.75 ± 0.12 0.46 - 0.93	0.837•	0.405
Urea (mg/dL)	Mean±SD Range	32.53 ± 5.82 22 - 42.9	31.62 ± 6.12 19.4 - 47.6	0.725•	0.470
Urea/Creatinine ratio	Mean±SD Range	42.20 ± 1.39 39.1 - 45.2	42.11 ± 2.50 39.4 - 51.2	0.208•	0.835
Urinary albumin excretion (mg/L)	Mean±SD Range	10.98 ± 4.20 2 – 18	10.98 ± 4.09 4 – 18	0.000•	1.000
Pus cells in urine	Mean±SD Range	3.09 ± 0.85 2 - 4	2.80 ± 1.04 1 - 4	1.448•	0.151
Albumin creatinine ratio	Mean±SD Range	54.28 ± 14.47 8.2 - 90.3	6.14 ± 2.04 2.2 - 12.1	22.103•	0.000
GFR (ml/min/1.73 m2)	Mean±SD Range	127.37 ± 29.72 79.5 - 205.6	133.53 ± 39.65 78.5 - 249.3	-0.834•	0.407
ALP (U/L)	Mean±SD Range	84.71 ± 20.25 43 - 128	76.36 ± 18.26 21 - 120	2.056	0.043
Albumin (g/dL)	Mean±SD Range	4.27 ± 0.34 3.6 - 5	4.13 ± 0.35 3.5 - 5	1.916	0.059
Bilirubin (mg/dL)	Mean±SD Range	0.65 ± 0.25 0.2 - 1.1	0.58 ± 0.23 0.1 - 1	1.316	0.191
IL-1b (pg/mL)	Mean±SD Range	853.47 ± 833.78 306.9 - 4092.1	284.06 ± 83.00 114.8 - 536.2	4.559•	0.000

P>0.05: Non significant (NS); P <0.05: Significant (S); P <0.01: Highly significant (HS)

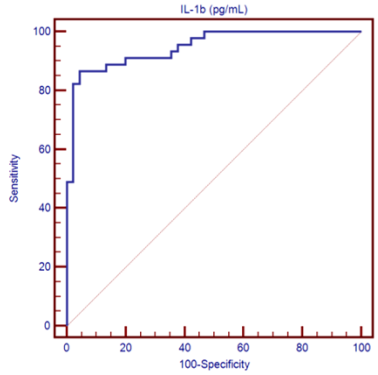
•: Independent t-test

Table (2) shows that there was statistically significant decrease in the platelet count in patients group than control group and also highly statistically significant decrease in red blood cells, hemoglobin and hematocrit in patients group than control group.

there was statistically significant increase in the HbA1c, fasting blood glucose and postprandial blood glucose in patients group than control group. Also, there was statistically significant increase in the Albumin creatinine ratio in patients group than control group, while there was no statistically

significant difference found between diabetes group and control group regarding creatinine, urea, urea/creatinine ratio, urinary albumin excretion, Pus cells in urine and GFR.

Also, there was statistically significant increase in the ALP in patients group than control group, while there was no statistically significant difference found between diabetes group and control group regarding albumin and Bilirubin. While, there was statistically significant increase in the IL-Ib in patients group than control group.



Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>377.5 (pg/mL)	0.947	86.67	95.56	95.1	87.8

Figure (1): Receiver operating characteristic curve (ROC) for IL-1b to differentiate between diabetes and the control groups

Table (3): Correlation of IL-1b with the studied parameters

	IL-1b (pg/mL)	
	r	p-value
Weight	0.209*	0.048
BMI (kg/m2)	0.701**	0.000
Eosinophils (x10^9/L)	0.409**	0.000
Pus cells in urine	-0.013	0.900
HbA1c (%)	0.675**	0.000
Fasting free glucose (mg/dl)	0.645**	0.000
Postprandial blood glucose (mg/dl)	0.640**	0.000
Albumin creatinine ratio	0.632**	0.000

Spearman correlation coefficients

**: Significant at the level of <0.01

Table (3) shows that there was statistically significant positive correlation found between IL-1b and BMI, weight, HbA1c, eosinophils, fasting free glucose, postprandial blood glucose, albumin creatinine ratio and alkaline phosphatase (ALP).

DISCUSSION

The aim of this study is to measure the level of IL-1β in children and adolescents with type 1 DM.

This case-control comparative study was conducted on 45 Children and adolescents diagnosed as T1 DM, recruited from pediatric endocrinology and outpatient clinic of pediatric department of Alzhraa and Tanta university hospital. This study was also included 45 healthy controls.

Regarding demographic data, there was statistically significant increase in the BMI in diabetes than control group and statistically significant increase in the weight in diabetes than control group, and statistically significant increase in weight z-score and BMI z-score in diabetes group than control group.

Our results were in agreement with ***Lee et al.*** [5] who revealed no significant difference between patients and controls regarding gender with p-value (p=0.599). Also, our results were in agreement with other studies previous studies ***Suh et al.*** [6], ***Mohammed et al.*** [7], ***Dezayee & Al-Nimer*** [8] ***and DaCosta et al.*** [9].

In the current study (20%) of the patients group had positive family history of having DM and this was in agreement with ***Mohammed et al.*** [7]. Also, our results were in agreement with other studies previous studies ***Šipetić et al.*** [10], ***Turtinen et al.*** [11] and ***Majeed and Hassan*** [12].

It was well established that DM is diagnosed by testing the level of sugar or HbA1C in the blood [13]. Target A1C goal may vary depending on the age and various other factors, but the American Diabetes Association (ADA) generally recommends that A1C levels be below 7 percent, which translates to an estimated average glucose of 154 mg/dL [13].

As regard HbA1c, normally some of the glucose in the blood stream attaches itself to proteins in our body. Hemoglobin, which is part of our red blood cells, is one of those proteins. This process is called glycosylation and is permanent. Once the glucose is attached to the hemoglobin, it stays there for the life of red blood cells, which is about 100 days (3 months). The higher the level of blood sugar, the more sugar attaches to hemoglobin and the higher the percent of hemoglobin which is glycosylated (HbA1c). This is why the results are given as percentage [14].

So, it is used as an indicator of long-term glycemic control. The more consistently low the HbA1c, the better the metabolic control

and the less severe and delayed onset of the microvascular complications [15].

The comparison between DM and control groups regarding blood glucose parameters, showed that there was statistically significant increase in the HbA1c, fasting blood glucose and postprandial blood glucose in patients than the control group.

In agreement with the current study ***Mohammed et al.*** [7] revealed that there was a highly significant difference between the cases and controls groups regarding fasting blood glucose and glycosylated hemoglobin.

Also, in agreement with our results ***Dezayee & Al-Nimer*** [8] revealed that HbA1c, fasting blood glucose and postprandial blood glucose were significantly higher in T1D patients compared to controls.

Children and adolescents with Type 1 diabetes mellitus (T1DM) are at risk for hyperfiltration and elevated urinary albumin-to-creatinine ratio (ACR), which are early indicators of diabetic nephropathy [16].

In agreement with the current study ***El Helaly et al.*** [17] showed that the children and adolescents with type 1 diabetes mellitus have significantly higher ACR compared to control group.

IL1b is a potent pro-inflammatory cytokine that exhibits elevated serum and urinary levels in patients with DKD, showing significant and direct correlations with urinary albumin excretion levels and with the evolution of albuminuria[18].

The current study showed that there was statistically significant increase in the IL-Iβ in patients' group than control group.

In consistency with the current study ***Gouda et al.***[19] revealed that the pro-inflammatory cytokine IL-1β showed higher serum level in T1D patients compared to controls.

In agreement with our results ***Dezayee & Al-Nimer*** [7] revealed that the pro-inflammatory cytokine IL-1β was significantly higher in T1D patients compared to controls.

The systematic review by ***Cano-Cano et al.*** [20] revealed that, compared with controls, IL-1β determined by immunoassays was significantly elevated in T1DM. The compared IL-1β levels in patients <18 years was significantly elevated.

This result is in the same line with ***Sesterheim et al.***[21] who observed that this cytokine serves in an autoimmune damage of pancreatic β-cells which favor the process of autoimmune and inflammatory response typical of T1D.

Furthermore, our results are in accordance with results obtained by ***Dogan et al.***[22] who detected high concentrations of inflammatory markers in recently diagnosed T1D patients, what suggests that systemic inflammation is contributed to the onset of the disease. However, the level of this marker is known to stay high during DM progression, and it can probably relate to the development of complications.

Receiver operating characteristic curve (ROC) for IL-1β (pg/mL) to differentiate between diabetes group and control group, showed that at cutoff of >377.5 it has Sensitivity and Specificity of 86.6% and 95.5% respectively.

Our results were supported by ***Gouda et al.***[19] who revealed that IL-1β can be used to differentiate between diabetes group and

control group. Furthermore, they reported that IL10 was the best to discriminate T1DM with cut-off value of 5.95 pg/ml and, at this value, the sensitivity was 96% and specificity was 90%.

Also, ***Dezayee & Al-Nimer***[8] showed that The AUC (using IL-1β as a discriminator with a cutoff value ≥105 pg/mL) was significantly high for glycosylated hemoglobin, fasting serum C-peptide, and serum IL-4, while it was significantly low for serum IL-10 and fasting serum insulin. The AUC (using IL-18 as a discriminator with a cutoff value ≥85 pg/mL) was significantly high for glycosylated hemoglobin).

Similar outcomes were achieved by previous study of ***Pérez-Marín et al.*** [23] who detect elevated concentration of IL-1β in early diagnosed children with diabetes. ***Molloul et al.*** [24] proposed that suppression of cytokine induced β-cells destruction may be a possible current approach for β-cell protection.

There was statistically significant relation between IL-Iβ and sex increasing in females.

In agreement with our results ***Aribi et al.*** [25] reported that there was statistically significant relation between higher IL-Iβ level and female gender.

Our results also showed that there was statistically significant positive correlation found between IL-Iβ and BMI, weight, HbA1c, eosinophils, fasting free glucose, postprandial blood glucose, albumin creatinine ratio and ALP while there is negative correlation found between IL-Iβ and red blood cell count, hemoglobin, hematocrit, white blood cell count and platelets count.

Our results were supported by ***Gouda et al.*** [19] how revealed that insulin was positively associated with IL-1β. Furthermore, regarding age, the study reported a positive correlation with both of BMI and IL-1β.

Also, ***Dezayee & Al-Nimer*** [8] showed that there was significant association between IL-1β with only HbA1c but other parameters were non-significantly correlated with IL-1β in T1DM group.

The current study failed to demonstrate significant association between IL-1β and kidney functions results, may be due to the low sample size and the low incidence of diabetic nephropathy in the current study.

However, ***Mahmoud et al.*** [26] reported that there was a marked relationship between Bcl-2, IL-1β, IL-17, and IL-33 levels and the onset and progression of diabetic nephropathy.

CONCLUSION

IL-1β can be considered as a potential biomarker for T1DM, it was found that circulating levels of IL-1β was highly significant in type 1 diabetic patients compared to healthy controls, and related to BMI but not related to disease duration. This is highly expressive of the convenience of this cytokine to support in further investigative type 1 diabetes pathophysiology and monitoring pharmacological interventions to interpose with the progress and evolution of diabetes.

Our results showed that the serum level of IL-1β was significantly higher in females, but we

failed to demonstrate significant association between IL-1β and kidney functions results, these results need to be reassessed in larger studies.

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RECOMMENDATIONS:

Further studies in larger sample size and on large geographical scale to emphasize our conclusion and to determine the relation of level of IL1β in children and adolescents with type1DM.

LIMITATIONS:

It was a single center study, relatively small sample size and the patients did not have enough follow up period

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