ORIGINAL ARTICLE

A new ratio derived from inflammasome markers can serve as a marker of assessment of glycemic index in children with Type 1 diabetes

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Background: Mature inflammasome markers play a role in the development of Type 1 diabetes (T1D). This cross-sectional study aimed to derive ratios from the serum levels of interleukins (ILs): IL-1β and IL-18 and to relate their values with glycemic index and anti-inflammatory markers (IL-4 and IL-10) in children with T1D. **Materials and Methods:** This study was conducted at Hawler Medical University in Erbil-Iraq from April to July 2018. Healthy subjects (Group I, $n = 40$) and patients (Group II, $n = 76$) were recruited from primary schools and the Center of Diabetes in Erbil, respectively. Glycemic indices (including fasting serum glucose, insulin, glycosylated hemoglobin, and peptide C) and pro- and anti-inflammatory markers (including high-sensitivity C-reactive protein, IL-1β, IL-18, IL-4, and IL-10 and the ratio of neutrophil or platelet to lymphocyte) were determined. **Results:** Cutoff values of 105 pg/mL, 85 pg/mL, and 1.235 for serum IL-1β, IL-18, and IL-1β to IL-18 ratio, respectively, were found to be significant discriminators of glycemic index and anti-inflammatory markers with respect to the calculated area under the curve. **Conclusion:** A ratio of IL-1β to IL-18 adjusted to 1.235 can serve as a useful marker of assessment of glycemic index. This ratio does not discriminate the status of anti-inflammatory markers (IL-4 and IL-10) in children with T1D.

Keywords: Glycemic index, inflammasome, Type 1 diabetes

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INTRODUCTION

Type 1 diabetes (T1D) is an autoimmune disease characterized by the presence of autoantibodies in circulation which act against pancreatic β-islet cells. Evidence of existed inflammation in T1D had been documented by significantly high levels of cytokines and other pro- and inflammatory markers.[1-3] Interleukin-1β (IL-1β) is a member of IL-1 family that is found in inflammasome as immature cytokines, and it is released into circulation after two steps of inflammasome activation.[4] IL-1β can cause death of islet β-cell of pancreas in patients with insulin-dependent diabetes (T1D), and a high serum level of IL-1β had been observed significantly.[5] On the other hand, serum IL-1β is significantly high in Type 2 diabetes and is inversely correlated with serum insulin level, indicating that IL-1β suppresses the secretion of insulin from β-cells.[6] IL-18 is also a member of inflammasome, and it is secreted into the circulation after stimulation of inflammasome by certain triggers, for example, silicon particle and uric acid.[7] One of the important functions of IL-18 is inhibiting the production of IL-1β.[8] Active inflammasome plays a crucial role in complications due to diabetes in the form of both IL-1β- and IL-18-induced cell death by pyroptosis.[9] Activated inflammasome by hyperglycemia plays an important role in inducing cell death by pyroptosis and enhancing fibrosis by activating fibroblasts.[10] An imbalance between IL-1β and IL-18 secretions from the active inflammasome may interfere the production of anti-inflammatory cytokines,

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for example, IL-4 and IL-10, which may affect the glycemic status.[11] Therefore, this cross-sectional study aimed to determine the cutoff levels of serum IL-1β, IL-18, and IL-1β to IL-18 ratio and to relate these values with glycemic index and anti-inflammatory markers in children with T1D.

MATERIALS AND METHODS

This cross-sectional study was approved by the Ethical Scientific Committee of Hawler Medical University according to the guidelines established by the university. The authors ensured the parents or the proxy of the patients that any treatment or device used will not harm the patient, and the patient can withdraw from the study at any time.

The parents or the proxy of the patients signed a consent form before their child was included in the study. Eligible patients were children of both genders aged less than 13 years old. T1D patients without clinical evidence of complications, those with short duration of illnesses, and those with positive autoantibody test (islet cell and glutamic acid decarboxylase-67 antibodies) were included in the study. Patients with renal or liver or autoimmune disease, those with inflammatory disease, those with recent infectious or connective tissue diseases, or those using medicines related with steroids or nonsteroidal anti-inflammatory drugs were excluded from the study. This cross-sectional study was conducted in the Department of Microbiology/Immunology-College of Health Sciences at Hawler Medical University in cooperation with the Center of Diabetes in Erbil-Iraq from April to July 2018. Healthy subjects and patients were recruited from the primary schools (Group I, n = 40) and from the Center of Diabetes (Group II, n = 76), respectively. The sample size of Group II patients was calculated by using margin of errors (α =0.05, β =0.2), two tails, and 95% confidence interval.

Clinical data and measurements

The consultant endocrinologists and the authors examined each patient and measured the anthropometric variables, including the weight (kg) and height (m). Calculated body mass index (kg/m^2) was determined by using the Quetlet's formula. A 12-h night fasting venous blood was drawn from each patient, which was divided into two portions: one portion was added into ethylenediaminetetraacetic acid (EDTA)-tubes for the determination of hematological indices by using automated coulter hematological analyzer, and glycosylated hemoglobin (HbA1c) was determined by using colorimetric methods after separating plasma by centrifugation, washing the cell pellet, and hemolyzing the red cells. The non-EDTA blood portion was centrifuged at 3000 rpm for 15 min, and the sera were separated for determining the following:

1. Determination of serum autoantibodies (islet cell and glutamic acid decarboxylase 67) using enzyme-linked immunosorbent antibodies (ELISA). Patients (Group II) with a negative test as well as healthy adolescents (Group I) with positive tests were excluded. Fasting serum glucose was determined by enzymatic method using a visible spectrophotometer
2. Determination of glycemic indices, included fasting serum insulin and C-peptide levels, was made by using ELISA
3. Measurement of inflammatory markers, including high-sensitivity C-reactive protein (hS-CRP), IL-1β, IL-4, IL-10, and IL-18 in the sera of the patients, was done by using ELISA technique.

The neutrophil-to-lymphocyte (NLR) ratio and platelet-to-lymphocyte ratio (PLR) were calculated, which served as inflammatory markers.

Statistical analysis

The results were expressed as number, percentage, and mean ± standard deviation. A two-tailed, independent two-sample t-test was performed to compare the laboratory measurements of the groups. Correlations between cytokine markers and glycemic parameters were assessed by bivariate linear correlation (Spearman's test). The sensitivity and the specificity of the IL-1β (cutoff = 105 pg/mL), IL-18 (cutoff = 85 pg/mL), the ratio of IL-1β to IL-18 (cutoff = 1.235), and the area under the curve (AUC) were calculated using the inflammasome markers as discriminators. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 20 (IBM corporation product, Armonk, New York, USA).

RESULTS

Table 1 shows that Group I and Group II were of age and gender matched. The mean value of the body mass index of Group II patients was significantly higher than the corresponding value of Group I by 10.7% [Table 1]. Glycemic indices including fasting serum glucose and glycosylated hemoglobin were significantly high, whereas the fasting serum C-peptide and insulin were significantly low in Group II [Table 1]. The mean serum levels of inflammatory markers, including hS-CRP, IL-1β, and IL-18, significantly increased in Group II when compared with the corresponding values of Group I. The serum levels of anti-inflammatory markers, including IL-4 and IL-10, were significantly low in Group II when compared with the corresponding values of Group I [Table 1]. NLR was significantly higher in Group II patients compared to Group I patients, which increased by 18%, whereas the PLR declined by 24.5% [Table 1]. The ratio of IL-1β to IL-18 increased by 12.27% in T1D patients in reference

to the healthy subjects. Bivariate Spearman's correlation between the ratio of Il-1β to IL-18 with body mass index, glycemic indices, and inflammatory or anti-inflammatory markers did not reach significant levels in both groups except with glycosylated hemoglobin which significantly and inversely correlated (r = −0.539, P < 0.001) [Table 2]. 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 [Table 3 and Figure 1]. The AUC (using IL-18 as a discriminator with a cutoff value ≥85 pg/mL) was significantly high for glycosylated hemoglobin [Table 3 and Figure 1]. The AUC (using the ratio of IL-1β to IL-18 as a discriminator with a cutoff value ≥1.235) was significantly low for glycosylated hemoglobin [Table 3 and Figure 1].

Table 1: Characteristics of the participants enrolled in the study

Variables	Group I (*n*=40)	Group II (*n*=76)	*P*
Gender (male: female)	20:20	38:38	1.000
Age (years)	13.3±1.0	13.1±1.1	0.480
Body mass index (kg/m^2)	21.4±2.1	23.7±1.5	<0.001
Fasting serum glucose (mg/dL)	91.9±3.5	303.7±12.1	<0.001
HbA1c (%)	4.9±0.2	9.5±1.0	<0.001
Serum C-peptide (ng/mL)	2.19±0.2	1.12±0.4	<0.001
Fasting serum insulin (mU/L)	4.64±0.65	1.85±0.45	<0.001
hs-CRP	0.45±0.15	1.76±0.48	<0.001
IL-4 (pg/mL)	23.8±1.7	20.2±2.8	<0.001
IL-10 (pg/mL)	52.3±9.9	44.6±9.9	<0.001
IL-1β (pg/mL)	0.6±0.4	115.5±8.8	<0.001
Il-18 (pg/mL)	6.3±0.8	95.8±13.4	<0.001
IL-1β to IL-18 ratio	0.1±0.07	1.227±0.189	<0.001
NLR	2.00±0.40	2.36±0.27	<0.001
PLR	186.9±44.3	141.2±24.2	<0.001

The results are expressed as mean±SD. *P* value is calculated by using two-tailed independent two-sample *t*-test. Group I=Healthy subjects; Group II=Type 1 diabetic patients; HbA1c=Glycosylated hemoglobin; hs-CRP=High-sensitivity C-reactive protein; IL=Interleukin; NLR=Neutrophil-to-lymphocyte ratio; PLR=Platelet-to-lymphocyte ratio; SD=Standard deviation

Table 2: Correlations between the ratio of interlekin-1β to interleukin-18 and the glycemic, inflammatory, and anti-inflammatory markers in healthy subjects (Group I) and type 1diabetic patients (Group II)

Determinants	Group I (*n*=40)		Group II (*n*=76)	
	r	*P*	*r*	*P*
Body mass index (kg/m^2)	0.043	0.793	-0.069	0.552
Fasting serum glucose (mg/dL)	-0.059	0.715	-0.165	0.155
HbA1c (%)	-0.091	0.577	-0.539	<0.001
Serum C-peptide (ng/mL)	0.062	0.704	0.010	0.931
Fasting serum insulin (mU/L)	0.067	0.683	-0.210	0.068
hs-CRP (mg/L)	-0.180	0.266	-0.064	0.583
IL-4 (pg/mL)	-0.249	0.165	0.189	0.103
IL-10 (pg/mL)	-0.125	0.441	-0.052	0.675
NLR	-0.175	0.281	-0.090	0.493
PLR	0.011	0.945	-0.085	0.464

r=Spearman's correlation coefficient; *P*=Probability; HbA1c=Glycosylated hemoglobin; hs-CRP=High-sensitivity C-reactive protein; IL=Interleukin; NLR=Neutrophil-to-lymphocyte ratio; PLR=Platelet-to-lymphocyte ratio

DISCUSSION

The results of this study show that the levels of IL-1β and IL-18 were reversed in T1D compared with those of healthy subjects. A significant inverse correlation between the ratio of IL-1β to IL-18 and glycosylated hemoglobin indicates that this ratio is a useful marker in assessing glycemic index. A cutoff value of ≥1.235 can discriminate patients with low glycosylated hemoglobin. The results of this study show that the serum levels of IL-1β and IL-18 did not correlate with the serum levels of hS-CRP, IL-4, and IL-10. Previous studies have showed a link between polymorphism of IL-4, IL-1β gene, and risk of T1D.[12] Other studies have demonstrated a relationship between the ratio of IL-4 and IL-13 with the development of T1D, which claimed that the effects of Th-1 and Th-17 cytokines in animals deficient in IL-13 gene can induce T1D.[13] In this study, a new ratio derived from the markers of inflammasomes can potentially serve as a marker of control of T1D. A cutoff value of 1.235 that represents the ratio of IL-1β to IL-18 indicates that a higher level of IL-1β is necessary to regulate the glycemic index. There is evidence that low serum IL-1β is associated with high titer of autoantibodies.[14] Moreover, it has been shown that in children with early-onset T1D with positive CAD65 autoantibodies, the levels of plasma IL-1β are significantly higher (9.3 ± 7.3 pg/mL) than the corresponding values of healthy children (4.9 ± 3.8 pg/mL), which accounted 1.9 folds. However, IL-1β is necessary to overcome the effect of autoantibodies against the islet β-cells, but at the same time, IL-1β involves in inducing apoptosis of β-cells. Recent experimental animal models that mimic T1D demonstrate that as a result of inhibition of caspase-3, the levels of IL-1β get reduced accompanied by an improvement in the glycemic profile.[15,16] Therefore, it is necessary to obtain a balance between the release of IL-1β and IL-18. Pancreatic cells produce IL-18 which plays a role in inducing inflammation and thereby pancreatic cell injury.[17,18] Therefore, upregulation of IL-1β to counteract autoantibodies and downregulation of IL-18 to counteract the insulinitis can offer an improvement in glycemic index as the present study demonstrates a ratio of 1.235.

CONCLUSION

We conclude that the ratio of IL-1β to IL-18 adjusted to 1.235 can serve as a useful marker of assessment of glycemic index by the significant evidence of an inverse correlation between this ratio and glycosylated hemoglobin and a significant low AUC of glycosylated hemoglobin at a cutoff of 1.235 of this

Table 3: The area under the curve of the glycemic, inflammatory, and anti-inflammatory markers using the ratio of interleukin-1β to interleukin-18, interleukin-1β and interleukin-18 as discriminators

Determinants	IL-1β (cutoff=105)			IL-18 (cutoff=85)			Ratio IL-1β/IL-18 (cutoff=1.235)		
	AUC	95% CI	*P*	AUC	95% CI	*P*	AUC	95% CI	*P*
Body mass index (kg/m^2)	0.473	0.314-0.632	0.762	0.656	0.430-0.700	0.437	0.541	0.409-0.522	0.454
Fasting serum glucose (mg/dL)	0.638	0.463-0.812	0.119	0.659	0.484-0.834	0.058	0.391	0.260-0.522	0.104
HbA1c (%)	0.700	0.544-0.857	0.024	0.882	0.774-0.990	<0.001	0.198	0.099-0.296	<0.001
Serum C-peptide	0.769	0.658-0.880	0.002	0.551	0.389-0.713	0.544	0.499	0.367-0.631	0.987
Fasting serum insulin (mU/L)	0.305	0.163-0.426	0.027	0.462	0.300-0.625	0.653	0.379	0.250-0.508	0.072
hs-CRP	0.600	0.439-0.760	0.261	0.479	0.318-0.641	0.804	0.436	0.306-0.566	0.342
IL-4	0.682	0.495-0.869	0.040	0.539	0.363-0.714	0.643	0.536	0.405-0.667	0.595
IL-10	0.395	0.209-0.581	0.235	0.490	0.330-0.651	0.906	0.505	0.373-0.638	0.937
NLR	0.306	0.146-0.467	0.029	0.529	0.362-0.696	0.729	0.486	0.354-0.618	0.832
PLR	0.256	0.360-0.692	0.767	0.662	0.520-0.805	0.053	0.446	0.315-0.576	0.420

The cutoff values are 105 pg/mL, 85 pg/mL, and 1.235 for serum IL-1β, IL-18, and IL-1β to IL-18 ratio, respectively. AUC=The area under the curve; CI=Confidence interval; *P*=Probability; HbA1c=Glycosylated hemoglobin; hs-CRP=High-sensitivity C-reactive protein; IL=Interleukin; NLR=Neutrophil-to-lymphocyte ratio; PLR=Platelet-to-lymphocyte ratio

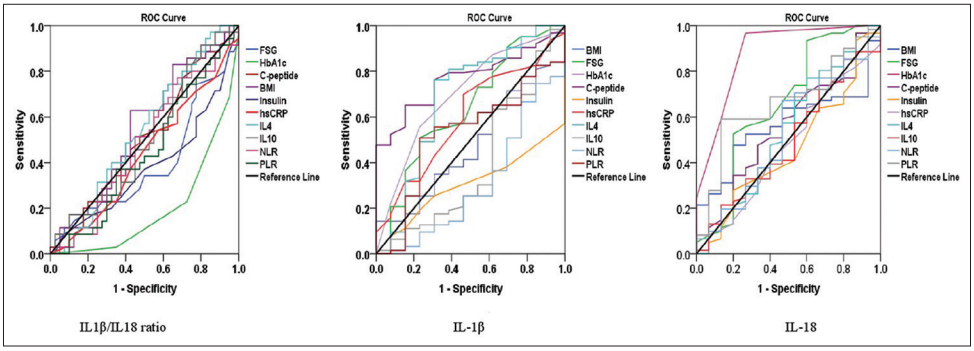

Figure 1: The area under the curve of the glycemic, inflammatory, and anti-inflammatory markers using the ratio of interleukin-1β/interleukin-18, interleukin-1β, and IL-18 as discriminators. The cutoff values are 105 pg/mL, 85 pg/mL, and 1.235 for serum IL-1β, IL-18, and IL-1β to IL-18 ratio, respectively

ratio as a discriminator. This ratio does not discriminate the status of anti-inflammatory markers (IL-4 and IL-10) in children with T1D.

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Conflicts of interest

There are no conflicts of interest.

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