Association of IL-6 with Insulin Resistance in Patients with Type 2 Diabetes Mellitus

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Abstract

Background: Chronic inflammation has been considered as a culprit for setting pathophysiological changes which result in insulin resistance and type 2 diabetes mellitus. IL-6 is one of the several proinflammatory cytokines proposed to have an association with type 2 diabetes. Very few studies on the role of circulatory IL-6 in diabetes have been published in Bangladesh. To see the association of IL-6 with insulin resistance in type 2 diabetes mellitus.

Materials and methods: A hospital-based cross-sectional observational study was carried out in the Department of Biochemistry, Chittagong Medical College, Department of Endocrinology of Chittagong Medical College Hospital and Chattogram Diabetic Hospital. One hundred (100) type 2 diabetes mellitus patients were included in the study by non-probability consecutive sampling. Important variables in this study were serum IL-6, BMI, waist circumference and duration of Diabetes Mellitus.

Results: The mean serum IL-6 level > 5 pg/ml and total IL-6 was 10.90±0.37 pg/ml and 10.08±0.39 respectively in patients with type 2 Diabetes Mellitus. The mean HOMA-IR was 5.56±0.29 in patients with type 2 diabetes mellitus. IL6 more than 5 pg/ml was more likely to have increased waist circumference than IL6 ≤5 pg/ml group. Increased IL6 was more likely to have severe insulin resistance in patients with type 2 diabetes. 74% of the positive outcome would be correctly predicted by the IL-6 value if the value was above or equal to 7.51.

Conclusion: The study results showed higher concentrations of serum IL-6 in the diabetic group when compared with a previous reference value. It suggests that IL-6 being an inflammatory mediator mightbe responsible for some underlying changes which may contribute to the development of insulin resistance in type 2 diabetes.

Key words: Cytokines; IL-6; Insulin resistance; Proinflammatory marker; Type 2 Diabetes.

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Introduction

Type 2 Diabetes Mellitus (T2DM) is a metabolic disorder that affects the metabolism of protein, carbohydrates and fat.[1,2] Insulin resistance is characterized by defective receptors, and glucose uptake in skeletal muscles becomes impaired due to defective regulation of Glucose Transporter Isoform 4 (GLUT4).[2] Insulin resistance syndrome is characterised by high levels of insulin, which significantly raises the chance of developing Type 2 Diabetes Mellitus (T2DM). This syndrome is well-recognised as a key indicator for the onset of T2DM.[3] Peripheral tissues, which account for over 90% of insulin-stimulated glucose utilisation, serve as the primary site for insulin-stimulated glucose disposal. Peripheral tissues, similar to adipocytes, also exhibit the expression of IL-6. However, the precise mechanism by which IL-6 induces insulin resistance in peripheral tissues remains to be fully understood.[4,5,6,7] Several studies have indicated that the duration of exposure and amount of IL-6 can influence insulin sensitivity in peripheral tissues by disrupting the activity of IRS-1-associated PI3K and the tyrosine phosphorylation of STAT3.[4] Multiple experimental research have demonstrated that IL-6 plays an active role in causing insulin resistance in hepatocytes by enhancing numerous inflammatory processes.[4,5,6,7,8] IL-6 carries out this function by engaging multiple molecular pathways, including the JAK/STAT system, STAT3 phosphorylation, transcription of SOCS-3 and suppression of insulin receptor autophosphorylation, as well as tyrosine phosphorylation of IRS-1 and IRS-2.[4,9] Pro-inflammatory substances,

specifically IL-6, TNF-α and other cytokines and chemokines have specific functions in causing oxidative stress and inflammation in the β -cells of pancreatic islets. This finally results in the malfunctioning of the pancreatic islets.[10,11,12] The existence of IL-6 in tissues is a typical occurrence, but its abnormal production is strongly associated with chronic inflammation, which is closely connected to several inflammatory illnesses.[13] IL-6 is also crucial in the transition from acute to chronic inflammation. After an immediate inflammatory reaction, IL-6 attaches to sIL-6R and facilitates trans-signalling via gp130, resulting in the mobilisation of monocytes. Extended exposure to IL-6 results in the occurrence of neutrophilic apoptosis, phagocytosis, and mononuclear accumulation at the site of injury.[14,15] Recent investigations have found that the mRNA expression of IL-6 is increased in the adipocytes of insulin-resistant individuals. Furthermore, individuals with decreased rates of insulin-stimulated glucose elimination have abnormally increased production of IL-6.[16,17] Therefore, this study has been conducted to observe the association of increased IL-6 with insulin resistance in type 2 diabetes mellitus patients.

Materials and methods

This hospital-based cross-sectional observational study was carried out in the Outpatient Department of Endocrinology, Chittagong Medical College Hospital in collaboration with the Department of Biochemistry, Chittagong Medical College, Chattogram Diabetic Hospital. The study was conducted from July 2019 to January 2023. The study was undertaken after approval by the Ethical Review Committee of Chittagong Medical College and the concerned Departments. Informed consent was obtained from each subject.

After conducting consecutive non-probability sampling methods one hundred (100) patients (60 male and 40 female) a mean age of 53.04±8.83 of type 2 diabetes mellitus patients were enrolled in this study (Table I).

Inclusion criteria

- Patients with Type 2 Diabetes Mellitus.
- Age > 35 yrs.

Exclusion criteria

- Chronic liver disease.
- Acute infection.
- Autoimmune disease and malignancy.
- Sepsis, burn.
- Pregnancy.

Subjects were selected from the Outpatient Department (OPD) of the Department of Endocrinology, Chittagong Medical College Hospital and Chattogram Diabetic Hospital who had come for their regular checkup for Diabetes. After taking a brief history and preliminary selection each subject and their informed verbal consent was taken. Then they were requested to report to the Department of Biochemistry, Chittagong Medical College in the morning between 8.00 and 9.00 am following an overnight (8-12 hours) fasting. When the subjects were reported, informed written consent was taken. A predesigned case record form was used to record relevant clinical, medical, demographic and socio-economic data from subjects.

The cut-off point of normal IL-6 was considered by ≤5pg/ml.[18]

Serum Interleukin-6 was measured by a commercially available IL-6 kit which is an in-vitro chemiluminescence immunoassay for quantitative determination (MAGLUMI 2000). BMI and WC (Waist circumference) were measured by standard procedure. The fasting serum blood glucose was measured by glucose oxidase enzymatic kinetic method using an auto analyzer. 2HPPBS was measured by an auto-analyzer Simens dimension EXL 200.

HOMA-IR.[19,20] HOMA-IR is computed as follows: fasting insulin (μIU/ml) × fasting glucose (mmol/l)/22.5

HOMA-β: Normal Insulin secretory capacity 50-70%.[19,21] HOMA-β was calculated using the following formula: (20 × fasting insulin (μIU/ml)/fasting glucose (mmol/ml) -3.5)%.

All the data has been processed and analyzed using IBM-SPSS (Statistical Package for Social Science) v 25.0 for Windows. p-value < 0.05 will be considered to be statistically significant. Variables expressed as mean ± Standard Error of Means (SEM)/SD. The normality of the distribution of the data has been tested by the

Kolmogorov-Smirnov Test and a p-value greater than 0.05 indicated that the observed distribution of a variable is not statistically different from the normal distribution. The Pearson correlation test has been applied whenever necessary to see the statistical significance. A chi-square test was done to see the association between categorical variables. ROC curve analysis was observed to determine the sensitivity and specificity of IL-6 with the severity of insulin resistance. Necessary permission was obtained before start the study from the proper authorities.

Results

Table I Baseline Anthropometric and sociodemographic parameters of the Study population (Type 2 Diabetes Mellitus) patient (n=100)

Variables of Type II Diabetes Mellitus Patients	Mean ± Standard Deviation			Range (Min-Max)	Median
	Male	Female	Aggregate		
Age (Years)	55.40±8.9	49.50±7.3	53.04±8.83	40-82	52.50
Height (cm)	163.05±6.7	157.28±6.9	160.74±7.3	137-186	160.50
BMI (kg/m^2)	27.96±4.0	28.0±4.3	27.93±4.08	21-48	28
Waist circumference (cm)	100.38±7.2	95.15±11.7	100.21±8.3	75-150	100
Duration of Diabetes Mellitus (Years)	6.5±4.03	5.5±4.10	6.15±4.10	1 month to 22 years	6
Gender distribution					
	Male		Female		
	60		40		

Table I contain the mean and standard deviation of anthropometric and sociodemographic parameters reflecting 60% of the study population was male and 40% was female. the mean age of men was 55.40±8.9 years and of the women 49.50±7.3 years. The mean BMI of the men was 27.96±4.0 kg/m^2 and of the women 28.0±4.3 kg/m^2. The mean waist circumference of the men was 100.38±7.2 cm and of the women 95.15±11.7 cm. In the study population, the mean age was 53.04±8.83 years. The mean BMI was 27.93±4.08 kg/m^2. The mean waist circumference was 100.21±8.3. The mean duration of diabetes mellitus was 6.15±4.10 years. The Median age of the patients was 52.50 years, the median BMI was 28 kg/m^2, the median waist circumference was 100cm and the median duration of diabetes was 6 years.

Table II Distribution of Risk factors of insulin resistance in Type 2 Diabetes Mellitus (n=100)

Risk factors of insulin resistance		Percentage (%)
BMI	Normal	4%
	Overweight	14%
	Obese	82%
Waist Circumference	Male ≥ 90cm	60%
	Female ≥ 80 cm	40%
HOMA-IR	1.5-2.5	5%
	>2.5-3	12%
	>3	83%
HOMA-Beta	< 50%	86%
	> 50%	14%
Duration of Diabetes Mellitus (Years)	≥ 5yrs	65%
	< 5yrs	35%
History of Diabetes Mellitus in 1 relative	Yes	76%
	No	24%

Table II contains 14% & 82% of type 2 Diabetes Mellitus patients were overweight and obese. The male had an increased frequency of waist circumference than the female. About 83% of the sample had severe (HOMA-IR >3) insulin resistance. 86% of Type 2 Diabetes Mellitus Patients had < 50% insulin secretory defect. 65% of Type 2 Diabetes Mellitus patients had a history of more than 5 years of Diabetes. Diabetes Mellitus in 1° relative was 76%.

Table III Descriptive statistics of Biochemical data in Type 2 Diabetes Mellitus patients (n=100)

Parameter (n=100)		Mean ± SEM	Median	Range
Fasting Blood Sugar (mmol/L)		11.39±0.36	10.65	7.0-20.8
2 hours Postprandial Blood glucose (mmol/L)		17.25±0.52	15.75	8.5-31.9
Serum IL6 (pg/ml) >	5pg/ml	10.90±0.37	11.03	5.31-19.86
	≤5pg/ml	4.56±0.07	4.58	4.14-4.87
	Total	10.08±0.39	9.82	4.14-19.86
Serum Fasting Insulin (µIU/ml)		10.84±0.39	9.93	4.31-24.61
HOMA-IR		5.56±0.29	4.74	1.70-17.07
HOMA-β		33.0±1.76	30.83	7.83-93.54

Table III contains Mean Fasting Blood sugar is 11.39 mmol/L, the mean 2 hours postprandial blood glucose is 17.25 mmol/L, the mean serum IL6 concentration is 10.01 pg/ml, the mean serum fasting Insulin 10.95 µIU/ml mean HOMA-IR value is 5.56, mean HOMA-β is 33%.

Table IV Chi-square test (χ^2) significance (n=100) to test the association of serum IL6 and waist circumference among type 2 diabetes mellitus patients

IL6 status	Waist circumference			
	Male (n=60)		Female (n=40)	
	Normal	Increased	Normal	Increased
IL6 > 5 pg/ml	7	49	25	25
IL6 < 5 pg/ml	2	2	0	10
p-value	Significant at < 0.05		Significant at < 0.05	

A chi-square test for independence with $\alpha = 0.05$ has been used to assess whether waist circumference is associated with serum IL6 concentration. The chi-square test is statistically significant, χ^2 (Male, df,1, N=60, 4.11 and Female, df,1; N=40,7.06), $p < 0.05$, with a Phi coefficient of 0.26 for male and for female 0.46, indicating a moderate relationship. As seen in Table IV, IL6 more than 5 pg/ml is more likely to have increased waist circumference than IL6 $\leq$ 5 pg/ml group.

Table V Chi-square test showing the association between IL6 and HOAM-IR (n=100)

		HOMA-IR			Chi-square Value	p-Value
		> 3	≤ 3	Total	25.796	< 0.01
IL6	> 5pg/ml	78	8	86		
	≤ 5pg/ml	5	9	14		
	Total	85	15	100		

A chi-square test for independence with $\alpha = 0.05$ has been used to assess whether insulin resistance is associated with serum IL-6 concentration. The chi-square test is statistically significant, χ^2 (df,1, N=100) = 25.79, $p < 0.01$, with a Phi coefficient of 0.508, indicating a strong relationship. As seen in Table V, IL6 more than 5 pg/ml is more likely to have severe insulin resistance than IL6 $\leq$ 5 pg/ml group.

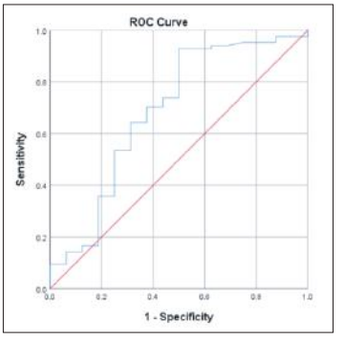

Figure 1 Receiver Operating Characteristic Curve for Severe Insulin resistance

ROC curve Test the Test variable IL-6 value with the outcome variable severity of Insulin resistance. The value of 7.51 as a cut-off value was seen that the sensitivity is 74% and that the False Positive rate is equivalent to 44 % (i.e. a Specificity of 56%). It means that if 7.51 was chosen as a cut-off point, 74% of the positive outcome would be correctly predicted by the IL-6 value if the value is above or equal to 7.51. On the other hand, 44% of the positive outcomes would be incorrectly predicted. The coordinates (0.44,0.74), lead to a cutoff value 7.51 from the table below :-

Area Under the Curve				
Test Result Variable(s): IL-6				
		Asymptotic Sig.	Asymptotic 95% Confidence Interval	
Area	Std. Error	p-value	Lower Bound	Upper Bound
0.686	0.083	0.019	0.524	0.849

Here 0.5<AUC<1, (0.686) so there was a high chance that the classifier could distinguish the positive class values from the negative ones. This was so because the classifier can detect more numbers of True positives and True negatives than False negatives and False positives.

Discussion

This study was designed and conducted to observe the association of serum IL-6 with insulin resistance in type 2 diabetes mellitus. It was observed by Van, Greevenbroek. et al. that excess visceral obesity, particularly excess visceral adiposity, could lead to chronic low-grade inflammation.[22] In this study type 2 diabetes mellitus patients have a mean IL-6 of 10.08±0.39 pg/ml (Table III) and the value was more than normal. Laishram, V. et al. and Marques-Vidal, p. et al. also observed such kind of increased mean IL-6 in their study.[23,24]

A greater number of adipose tissue macrophages were held accountable for the increased plasma concentration of pro-inflammatory cytokines including IL-6 as cited by Stephens, J. W. et al.[25] According to the findings of this study, the serum IL-6 concentration in type 2 Diabetes Mellitus patients was associated with waist circumference (Table IV). The mean waist circumference in this study was 100.21±8.3 cm (Table I). Such Adiposity and insulin resistance are commonly found in type 2 diabetes mellitus as cited by Park, H. S., et al.[26]

The mean HOMA-IR in the study was 5.56±0.29 and the median was 4.74. The study conducted by Amina Nadeem, et al. found mean HOMA-IR was 4.78. El-Byoumy, I. also found a mean increase in HOMA-IR about 6.3.[27,28] There was a significant association between IL-6 and HOMA-IR in type 2 diabetes mellitus patients (Table V).

The value of 7.51 as a cut-off value showed that the sensitivity is 74% and that the False Positive rate is equivalent to 44 % (i.e. A Specificity of 56 %) (Figure 1). Mieldazis et al. examined the correlation between BMI, HOMA-IR, and insulin levels in children who were obese.[29] They determined a robust correlation between hyperinsulinemia and obesity, and that when the Body Mass Index (BMI) increases, so does the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). In this study, it was observed that there was hyperinsulinemia of mean 10.84 µIU/ml (Table III) with increased frequency of HOMA-IR > 3 (83%) (Table II) in patients with type 2 diabetes mellitus.

In summary, it was seen in all of the cited studies in this context that, there was a significant association of pro-inflammatory markers like IL-6 with insulin resistance (HOMA-IR) in type 2 diabetes mellitus.

Limitations

The study had a small sample size that might not reflect the generalization of the findings to the reference population. Univariate association of IL-6 with the grading of HOMA-IR can not be conducted due to the lack of an age-gender-matched control group.

Conclusion

From this study, it can be concluded that there was a significant association of increased IL-6 with insulin resistance in type 2 diabetes mellitus patients. Abdominal obesity which is reflected by increased waist circumference is considered to be an important risk factor for increased levels of IL-6 concentration in type 2 diabetes. The ongoing subclinical chronic inflammation in peripheral tissue could have an explanation for insulin resistance and poor glycemic control in type 2 diabetes patients.

Recommendations

A case-control study is indicated to see the univariate association with the increased serum IL-6 concentration in type 2 diabetes mellitus. The correlation between IL-6 and Hs-CRP needs to be evaluated. A feasibility study may be aimed to convey awareness regarding the measurement of inflammatory marker serum IL-6 and Hs-CRP in type 2 Diabetes Mellitus and its complications.

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Contribution of authors

SD-Conception, interpretation of data, data analysis, drafting, acquisition of data & final approval.

PC-Data analysis, critical revision & final approval.

MRMI-Acquisition of data, interpretation of data, drafting & final approval.

NC-Conception, critical revision & final approval.

MH-Conception, critical revision & final approval.

AMMEH-Interpretation of data, critical revision & final approval.

MHI-Interpretation of data, critical revision & final approval.

Disclosure

All the authors declared no conflict of interest.

References

1. Akash MSH, Rehman K, Chen S. Spice plant allium cepa: dietary supplement for treatment of type 2 diabetes mellitus.Nutrition (Burbank). 2014;30(10):1128–1137.

2. Akash MSH, Rehman K, Chen S. Effects of coffee on type 2 diabetes mellitus. Nutrition (Burbank). 2014;30(7–8):755–763.

3. Casazza K, Willig AL, Gower BA, Nagy TR, Hunter GR, Wallace S, Amaya M, Franklin F, Beasley M, Fernandez JR. The role of European genetic admixture in the aetiology of the insulin resistance syndrome in children: Are the effects mediated by the fat accumulation? J Pediatrics. 2010;157(1):50–6. e51.

4. Kim HJ, Higashimori T, Park SY, Choi H, Dong J, Kim YJ, Noh HL, Cho YR, Cline G, Kim YB, Kim JK. Differential effects of interleukin-6 and -10 on skeletal muscle and liver insulin action in vivo. Diabetes. 2004;53(4):1060–1067.

5. Rieusset J, Bouzakri K, Chevillotte E, Ricard N, Jacquet D, Bastard JP, Laville M, Vidal H. Suppressor of cytokine signalling 3 expressions and insulin resistance in skeletal muscle of obese and type 2 diabetic patients. Diabetes. 2004;53(9):2232–2241.

6. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, Wolsk-Petersen E, Febbraio M. The metabolic role of IL-6 produced during exercise: Is il-6 an exercise factor? Proc Nutr Soc. 2004;63(2): 263–267.

7. Klover PJ, Zimmers TA, Koniaris LG, Mooney RA. Chronic exposure to interleukin-6 causes hepatic insulin resistance in mice. Diabetes. 2003;52(11):2784–2789.

8. Senn JJ, Klover PJ, Nowak IA, Zimmers TA, Koniaris LG, Furlanetto RW, Mooney RA. Suppressor of cytokine signaling- 3 (socs-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. J Biol Chem. 2003;278(16):13740–13746.

9. Rehman K, Akash MSH, Liaqat A, Kamal S, Qadir MI, Rasul A. Role of interleukin-6 in development of insulin resistance and type 2 diabetes mellitus. Crit Rev Eukaryot Gene Expr. 2017;27(3):229–236.

10. Akash MS, Rehman K, Chen S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. J Cell Biochem. 2013;114(3):525–531.

11. Akash MS, Shen Q, Rehman K, Chen S. Interleukin-1 receptor antagonist: A new therapy for type 2 diabetes mellitus. J Pharmaceut Sci. 2012;101(5):1647–1658.

12. Rehman K, Akash MS. Mechanisms of inflammatory responses and development of insulin resistance: How are they interlinked? J Biomed Sci. 2016;23(1):87.

13. Hodge DR, Hurt EM, Farrar WL. The role of il-6 and stat3 in inflammation and cancer. Eur J Cancer. 2005;41(16): 2502–2512.

14. Gabay C. Interleukin-6 and chronic inflammation. Arthrit Res Ther. 2006;8(2):1.

15. Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin- 6. Biochim Biophys Acta. 2011;1813(5):878–888.

16. Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olsen DB, Saltin B, Hawley JA, Febbraio MA. Interleukin-6 and tumour necrosis factor-alpha are not increased in patients with type 2 diabetes: Evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. Diabetologia. 2004;47(6):1029–1037.

17. Carey AL, Febbraio MA. Interleukin-6 and insulin sensitivity: Friend or foe? Diabetologia. 2004;47(7):1135–1142.

18. Naila Abdul Sattar, Sumera Shaheen, Siraj-Uddin Sajid. Association of Bio-Inflammatory Markers (CRP, IL-6) with Glucose Level in Obese T2DM Pakistani Patients. 2018;19(6);282-286.

19. Svensson E, Mor A, Rungby J, Berencsi K, Nielsen JS, Stidsen JV, et al. Thomsen RW. Lifestyle and clinical factors associated with elevated C-reactive protein among newly diagnosed Type 2 diabetes mellitus patients: A cross-sectional study from the nationwide DD2 cohort. BMC Endocr Disord. 2014;14:74.

20. Maurer R. Insulin Resistance/T2 Diabetes: Map Your Test Results. The Blood code. 2014.

21. Ferrannini E, Gastaldelli A, Miyazaki Y, Matsuda M, Mari A, DeFronzo RA. beta-Cell function in subjects spanning the range from normal glucose tolerance to overt diabetes: A new analysis. J Clin Endocrinol Metab. 2005;90(1):493-500.

22. Van Greevenbroek MMJ, Schalkwijk CG, Stehouwer CDA. Obesity-associated low-grade inflammation in type 2 diabetes mellitus: causes and consequences. Neth J Med. 2013;71(4):174–187.

23. Laishram V, Lamabam C, Laikangbam S, Dubey A, Longkumer C, Sharma S, et al. Interleukin-6 in obese type 2 diabetes with hypertension. Int J Res Med Sci. 2016;896–901.

24. Marques-Vidal P, Bastardot F, von Känel R, Paccaud F, Preisig M, Waeber G, et al. Association between circulating cytokine levels, diabetes and insulin resistance in a population-based sample (CoLaus study). Clin Endocrinol (Oxf). 2013;78(2):232–241.

25. Rodrigues KF, Pietrani NT, Bosco AA, Campos FMF, Sandrim VC, Gomes KB. IL-6, TNF-α and IL-10 levels/polymorphisms and their association with type 2 diabetes mellitus and obesity in Brazilian individuals. Arch Endocrinol Metab. 2017;61(5):438–446.

26. Park HS, Park JY, Yu R. Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Res Clin Pract. 2005;69(1):29-35.

27. Amina Nadeem, Abdul Khaliq Naveed, Muhammad Mazhar Hussain, Syed Irfan Raza. Correlation of inflammatory markers with Type 2 Diabetes Mellitus in Pakistani patients. JPMI. 2012;27(03):267–273.

28. El-Byoumy I. Interleukin 6 as an inflammatory marker and insulin resistance in obese Kuwaiti adolescents. Integr Obes Diabetes. 2017;3(3).

29. Mieldazis SFA, Azzalis LA, Junqueira VBC, Souza FIS, Sarni ROS, Fonseca FLA. Hyperinsulinism assessment in a sample of prepubescent children. J Pediatr (Rio J). 2010;86(3):245–249.