Original Article

Serum Interleukin-6 level in children with type 1 diabetes mellitus

Ikhlas K. Hammed* **MBChB, FICMS (Chem.Path).**
Nada F.Rashid* **BSc,MSc**
Baydaa A. Abed** **BSc,MSc**

Summary:

Background: Type 1 diabetes mellitus (T1DM) can be considered as an inflammatory disease of the pancreatic islets in which a process of programmed cell death (apoptosis) is elicited in the β-cells by interaction of activated T-cells and proinflammatory cytokines in the immune infiltrate. Interleukin-6 (IL-6) is a pleiotropic cytokine with a key impact on both immunoregulation and nonimmune events in many cell types .

Objective: to assess the level of serum IL-6 as an inflammatory marker in type 1 diabetic children, with correlation to FBG and HbA1c.

Subjects and methods: 45 type 1 diabetic child (20 males and 25 females), mean age 10.9± 3.4 years who attended the National Diabetic Center, Al-Mustansiria university were included in this study. 45 apparently healthy controls matched for age and sex were participated in this study .Fasting venous blood samples were collected from all the subjects. The serum was used for analyzing HbA1c, Fasting Blood Glucose (FBG) and IL-6, HbA1c was estimated by high performance liquid chromatography ,Serum glucose level was determined enzymatically and serum IL-6 was measured by enzyme linked immune sorbent assay.Analysis of data was performed using the statistically package for social science (SPSS) version 17.0.

Results: Mean serum IL-6 level in type 1 diabetic children were significantly higher compared to the healthy controls (30.9 Pg/ml ±10.85versus 10.57 Pg/ml±1.98 (P ≤0.0001), and positive strong correlation was found between serum IL-6 and FBS, HbA1C ,BMI.

Conclusion: There is a low-level of chronic inflammatory state in type 1 diabetic children reflected by the level of serum IL-6, that may play a key role in the early stages of atherogenesis and the development of microvascular complications.

Keywords: type 1 diabetic children, interleukin-6, inflammation.

Fac Med Baghdad 2012; Vol. 54, No.3
Received June. 2012
Accepted May. 2012

Introduction:

The incidence of type 1 diabetes mellitus(T1DM) is increasing at 3–5% per year worldwide and this increase cannot be accounted for by known genetic factors (1) Type 1 diabetes can be considered as an inflammatory disease of the pancreatic islets in which a process of programmed cell death (apoptosis) is elicited in the β-cells by interaction of activated T-cells and proinflammatory cytokines in the immune infiltrate (2). The immune-mediated β-cell destruction is thought to be initiated by interaction between environmental factors and type 1 diabetes susceptibility gene variants (3). Interleukin IL-6 is a pleiotropic cytokine with a key impact on both immunoregulation and nonimmune events in many cell types and tissues outside the immune system its effects are mediated through interaction with its receptor complex, IL-6Rβ. (4). IL-6 is secreted by macrophages and T lymphocytes following tissue damage (5). Its acute secretion in response to harmful stimuli is therefore physiological and it stimulates the onset of fever, mobilization of acute-phase reactants (such as C-reactive protein), optimization of leukocyte activity against pathogens and in the healing of physical injuries (5, 6). However, chronically elevated systemic IL-6 has been associated with higher risk of cardiovascular events and mortality (7, 8) this association is possibly due to the deposition of excessive glucose and oxidized low-density lipoprotein (LDL-C) and cholesterol in the endothelium (9). These events trigger leukocyte infiltration into the vascular basement membrane, emission of highly toxic reactive oxygen species as hydrogen peroxide, hypochloric acid and other oxygen radicals hydrolyzing proteases, and cytokines (TNF, IL-1, IL-6, and IL-8), causing destruction of the vascular wall and reduction of luminal patency (10). Exaggerated inflammatory and low anti-inflammatory status has been shown to interfere with the preservation of pancreatic - cell functions and maintenance of appropriate metabolic control (12, 13). IL-6 levels positively correlate with higher mortality, unstable angina, left ventricular dysfunction, propensity to diabetes and its complications, hypertension, obesity and several types of cancer(11)

*Department of biochemistry, Al-Kindy college of Medicine, Baghdad University.
**National Diabetic Center,Al-Mustansiria University.

Patients and methods:

Forty five(45) type 1 diabetic children (20 boy and 25 girls), mean age was (10.9± 3.4) years who attended the National

Diabetic Center, Al-Mustansiria university were included in this study, 45 apparently healthy controls matched for age and sex were participated in this study. Fasting venous blood samples were collected from all the subjects. Inclusion criteria includes type 1diabetic children with the duration of DM less than 1.5 years, and without evidence of clinical micro or macrovascular complications and with no other acute or chronic illness.The serum was used for analyzing Fasting Blood Glucose (FBG), IL-6 and HbA1c. HbA1c was estimated by high performance liquid chromatography (supplied by Variant company, USA). Glucose level was determined enzymatically using kits supplied by Randox, UK, serum IL-6 was measured by enzyme linked immune sorbent assay (supplied by Bio source,Europe SA,Belgium).BMI was calculated as weight in kilograms divided by height in meters squared.

Statistical analysis:Analysis of data was performed using statistically package for social science (SPSS) version 17.0. Results are expressed as mean ± SD or SEM as appropriate. Student t- test was used to compare the significance of the difference in the mean values of any two groups, P≤0.05 was considered statistically significant, linear regression analysis was used to study the correlation between the parameters.

Results:

Table 1: Demographic and biochemical characteristic of the studied population

	Control No:45	Type 1 Diabetic No:45	P-Value
Girls:boys	25:20	23:22	
Age	10.56±3.42	10.98±3.45	NS
BMI(kg/m^{r})	14.98±3.34	15.24±3.98	NS
FBS(mmol/L)	4.98±1.19	12.43±4.2	0.00001**
HbA_{1C}	5.12±0.32	8.35±2.45	0.00001**
IL-6(pg/ml)	10.57±1.98	30.9±10.85	0.00001**

Results are expressed as mean± SD

P≤0.05 is considered statistically significant*,if ≤0.001 it is highly significant **

Table 2: Correlation between Interleukin-6 and different parameters in type 1 DM children

Parameters	R
IL6-age	0.288
IL6-FBS	0.967**
IL6-HbA_{1c}	0.882**
IL6-BMI	0.911**

P≤ 0.05 is statistically significant*, if ≤ 0.001 it is highly significant **

Discussion:

The study showed serum IL-6 concentrations were significantly higher in diabetic patients with a strong positive correlation with both the FBG and HbA1C , Hyperglycemia stimulates the, monocytes to secrete increased amounts of IL-6 via upregulation of Protein kinase C (PKC), mitogen-activated protein kinase (p38 MAPK) and Nuclear factor(NF-κB) activity, leading to increased IL-6 transcription and release (14) the etiology of diabetes-related inflammation is complex and includes at least two major components: an "intrinsic" component, i.e., the permanent exaggeration of inflammatory mechanisms related to the presence of diabetes per se including hyperactivation of specific leukocyte subtypes(15) ,where T lymphocytes (as CD4 ,CD8,regulatory T) are the main players in T1DM though other leukocytes such as B lymphocytes, dendritic cells, macrophages, and Natural Killer cells are also implicated . Leukocyte infiltration into the pancreatic islets, called insulitis, contributes to a gradual loss of pancreatic β cells, leading to insulin insufficiency, a hallmark of T1DM . This infiltration is thought to be regulated by chemokine receptors and chemokines (15,16) while the "reversible" diabetes-related inflammation component, includes the inflammatory exacerbations associated with chronic and acute hyperglycemia (15) Except for the few months or years immediately following diabetes onset, the latter component appears quantitatively more important, being related to the frequency and duration of hyperglycemia (17).However, this inflammatory component is also susceptible to correction through strict glycemic control (9). This may be particularly important for T1DM children, in whom increased long-term cardiovascular risk may build gradually over many years starting very early in life, through chronic, subclinical inflammation modulated by hyperglycemic fluctuations(18). While the molecular mechanisms that link chronic hyperglycemia to diabetic vascular complications are still incompletely defined, dysregulation of systemic inflammatory status is now believed to play an important role (15, 19) Several studies have found an increase in serum IL-6 concentrations (20); however other studies reported no difference (21) or even decreased (22) IL-6 levels in type 1 diabetic patients. The strong positive correlations between BMI and circulating IL-6 concentrations are in concordance with other studies, that found plasma IL-6 concentrations were positively correlated with weight, BMI, waist circumference, hip circumference, and waist-to-hip ratio in obese and nonobese subjects from both sexes (23). adipose tissue is an important source of proinflammatory cytokines (24) and adipocytes metabolism is influenced by cytokines, especially by IL-6 that stimulates lipolysis in human adipocyte or preadipocyte cultures (25).

Conclusion:

The evidence from this study supports the possibility that in young adults with type 1 diabetes, there is a low-level chronic inflammatory state, as reflected by levels of serum IL-6, that may play a key role in the early stages of atherogenesis and in the development of microvascular disorders. This hypothesis lends itself to testing the use of interventions to influence IL-6 secretion and actions.

References:

1. Janet K. Snell-Bergeon, Nancy A. West, Elizabeth J. Mayer-Davis, Angela D. Liese,Santica M. Marcovina et al,

Inflammatory Markers Are Increased in Youth withType 1 Diabetes: The Search Case-Control Study.J Clin Endocrinol Metab, June 2010, 95(6):2868–2876

2. Maigan A. Hulme, Clive H. Wasserfall, Mark A. Atkinson ,Todd M. Brusko. Central Role for Interleukin-2 in Type 1 Diabetes. Diabetes January 2012 vol. 61 no. 1 14-22

3. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. Lancet 2001; 358:221–229.

4.Kamimura D, Ishihara K, Hirano T: IL-6 signal transduction and its physiological roles: the signal orchestration model. Rev Physiol Biochem Pharmacol 2003; 149:1–38

5. Kristiansen OP, Mandrup-Poulsen T. Interleukin-6 and diabetes: the good, the bad, or the indifferent? Diabetes 2005; 54: S114–S124.

6. Opal SM, DePalo VA. Anti-inflammatory cytokines. Chest 2000; 117: 1162–1172.

7. Cesari M, Penninx BWJH, Newman AB, Kritchevsky SB, Nicklas BJ,Sutton-Tyrrell K, Rubin SM, Ding J, Simonsick EM, Harris TB, Pahor M. Inflammatory markers and onset of cardiovascular events:results from the health ABC study. Circulation 2003;108: 2317–2322.

8. Lindmark E, Diderholm E, Wallentin L, Siegbahn A. Relationship between interleukin 6 and mortality in patients with unstable coronary artery disease: effects of an early invasive or noninvasive strategy. JAMA 2001; 286: 2107–2113.

9. Jaime S. Rosa, Rebecca L. Flores, Stacy R. Oliver, Andria M. Pontello, Frank P. Resting and exercise induced IL-6 levels in children with Type 1 diabetes reflect hyperglycemic profiles during the previous 3 days. J Appl Physiol 2010; 108:334-342

10. Baynes JW, Thorpe SR. Role of oxidative stress in diabetic complications:

a new perspective on an old paradigm. Diabetes1999; 48: 1–9

11. Larsen CM, Faulenbach M, Vaag A, Volund A, Ehses JA, Seifert B, Mandrup-Poulsen T, Donath MY. Interleukin-1-receptor antagonist in type 2 diabetes mellitus. N Engl J Med 2007; 356: 1517–1526

12. Pfleger C, Mortensen HB, Hansen L, Herder C, Roep BO, Hoey H, Aanstoot HJ, Kocova M, Schloot NC. Association of IL-1ra and adiponectin with C-peptide and remission in patients with type 1 diabetes.Diabetes 2008; 57: 929–937

13.Alexander Tenenbaum.. The ubiquitous interleukin-6: a time for reappraisal.Cardiovascular Diabetology 2010, vol 9

14.G. Kavitha, G.Ramani, Priya K Dhass, Rita Mary Aruna. Oxidative stress,interleukin -6 and atherogenic index of plasma in diabetic nephropathy.International journal of applied biology and Pharmecutical technology 2011; Volume: 2: Issue-2:211-217

15. Yamagishi S, Imaizumi T. Diabetic vascular complications: pathophysiology,

Biochemical basis and potential therapeutic strategy. Curr Pharm Des 2005;

11: 2279–2299.

16. Ming-Yi Shen, Yu-Ping Lin, Bei-Chang Yang, Yu-Song Jang, Chih-Kang Chiang,et al. Catenarin Prevents Type 1 Diabetes in Nonobese Diabetic Mice via Inhibition of Leukocyte Migration Involving the MEK6/p38 and MEK7/JNK Pathways.Evidence-Based Complementary and Alternative Medicine J, 2012 ;13.

17. Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation, 2002; 106: 2067–2072

18. The Diabetes Control and Complications Trial (DCCT)/The Epidemiology of Diabetes Interventions and Complications (EDIC) Research Groups. eneficial effects of intensive therapy of diabetes during adolescence: outcomes after the conclusion of the Diabetes Control and Complications Trial (DCCT). Pediatrics 2001; 139: 804–812.

19. El-Osta A, Brasacchio D, Yao D, Pocai A, Jones PL, Roeder RG,Cooper ME, Brownlee M. Transient high glucose causes persistent epigenetic changes and altered gene expression during subsequent normoglycemia. J Exp Med, 2008; 205: 2409–2417.

20. Targher G, Zenari L, Bertolini L, Muggeo M, Zoppini G. Elevated levels of interleukin-6 in young adults with type 1 diabetes without clinical evidence of microvascular and macrovascular complications. Diabetes Care. 2001; 24(5):956–957

21. Kulseng B, Skjak-Braek G, Folling I, Espevik T. TNF production from peripheral blood mononuclear cells in diabetic patients after stimulation with alginate and lipopolysaccharide. Scandinavian Journal of Immunology. 1996;43(3):335–340

22. Geerlings SE, Brouwer EC, Van Kessel KC, Gaastra W, Stolk RP, Hoepelman AI. Cytokine secretion is impaired in women with diabetes mellitus. European Journal of Clinical Investigation. 2000 ;30 (11):995–1001

23. 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; 69:29–35

24. Coppack SW. Pro-inflammatory cytokines and adipose tissue. Proc Nutr Soc 2001; 60:349–356

25. Trujillo ME, Sullivan S, Harten I. Interleukin-6 regulates human adipose tissue lipid metabolism and leptin production in vitro. J Clin Endocrinol Metab 2004; 89:5577–5582.