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Evaluation of the Role of CXCL8 and NOx in Pediatric Type 1Diabetes Mellitus

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Abstract

Background and aim of the work: Genetic and environmental factors may play a role in the etiology of type 1diabetes (T1D) but a well-accepted view is that autoimmunity is the predominant effector.The aim of this study is to investigate the profile and the relationships between interleukin (IL, CXCL) -8 and nitric oxide metabolite (NOx) in T1D and to reveal their possible role in the development and progression of the disease and its complications.

Material and method: Twenty children with Type 1 diabetes (T1D) were enrolled for the study and compared to twenty healthy age and gender matched non-diabetic controls.

Results: The data revealed that children with T1D established high glycated hemoglobin (HbA1c %) values versus the control group (P<0.0001). Significantly higher serum CXCL-8 concentration (23.54±11.92pg/ml) was detected in T1D children versus the control group(5.69±1.67pg/ml). 0n the other hand, serum nitric acid metabolite (NOx) showed a significant reduction in the T1D children (2.38±1.14 mmol/l) compared to the control group (4.63±1.2 mmol/l). Correlation analysis showed positive correlation between CXCL-8 with duration of the diabetes and with HbA1c.

Conclusion: It could be concluded that CXCL-8 and NO may play important roles in the pathophysiology and progression of T1D with increased possibility to develop premature atherosclerosis which should be considered in the development of new strategies for monitoring the disease as well as for developing effective preventive and therapeutic interventions.

Keywords: Pediatric T1D, CXCL-8, NOx, endothelial dysfunction, premature atherosclerosis.

Introduction

Diabetes mellitus is very common in KSA (25-30% of the population), and T1D accounts for about 5-10% of all cases of diabetes (1). The etiology of T1D is not clearly known but it is well-accepted to be genetically predisposed as it is closely linked to HLA-DR3/4 and DQ2/8 antigens and commonly precipitated by virus or food factor leading to a progressive chronic autoimmune process (2). This process is mediated by autoreactive CD4+ and CD8+ T cells, the formation of antibodies to islet autoantigens (AAgs), and the release of high amounts of cytokines and chemokines especially interleukin-8 (IL-8) (2,3). Interleukin-8 belongs to a chemokine family known as CXC chemokines, because the two first cysteines in their molecules are spaced by another amino acid, so it is currently known as CXCL8 (CXC ligand 8) (4, 5). CXCL8 governs the traffic of inflammatory and immune cells, playing the major role in the infiltration of the pancreas with mononuclear cells which leads to progressive destruction of the islet beta-cells and the final outcome is insulin deficiency and hyperglycemia (2, 3, 5). CXCL-8 with other chemokines stimulates the adhesion of the monocytes to the endothelial cells leading to its dysfunction and subsequent development of atherosclerosis (6, 7). Endothelial dysfunction will lead to decrease in the activity of the endothelial nitric oxide synthase (eNOS) with consequent reduction in the levels of plasma nitric oxide (NO), the main molecule derived from the endothelium and is involved in vascular homeostasis (8-10). A low level of NO is strongly linked to endothelial dysfunction and is considered to be the earliest sign of atherosclerosis (11, 12). Nitric oxide has a fast half life, so we can measure the nitric oxide metabolite (NOx) which is the sum of nitrite and nitrate levels and it is used to reflect the bioactivity of NO and eNOS (11). The aim of this study is to investigate the profile and the relationships between interleukin (IL, CXCL) -8 and nitric oxide metabolite (NOx) in T1D and to reveal their possible role in

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the development and progression of the disease and its complications.

Material and Methods: This was a prospective controlled clinical study conducted at King Abdul Aziz Specialist Hospital in Taif, Saudi Arabia from June 2012 to April 2013. Twenty children who were diagnosed as type 1diabetes (T1D) were enrolled for the study and another 20 healthy age and gender matched non-diabetic children served as controls.The study was conducted after approval of the ethical committee of the hospital and informed consents were obtained from the parents of all the enrolled children.

The children included in this study were then classified into two groups:

T1D group; they were 11 males and 9 females and their ages ranged between 4 – 14 years with mean age of 9.05 ± 3.14 years. The study included patients under insulin treatment and those under oral hypoglycemic medications were excluded. Children with acute or chronic diabetic complications were also excluded.

Control group; this group included twenty apparently healthy children (10 males and 10 females). Their ages ranged between 2 –15 years with a mean age of 9.12 ±3.70 years. They had no family history of diabetes, or any other autoimmune disease.

Both groups were subjected to the following:

Clinically:

Full medical history was taken and thorough physical examination was performed for all the enrolled children.

Laboratory investigations:

Serum concentration of CXCL-8 was assayed by enzyme-linked immunosorbent assay (ELISA) technique.

Nitric oxide metabolite (NOx) was measured using the Griess reaction.

Fasting plasma glucose and fasting glycated hemoglobin (HbA1c) were assayed.

Statistical analysis: Results were expressed as mean ± standard deviation and the analyses were performed using SPSS version 15. Means of biochemical concentrations were compared by Student`s "*t*" test. Relationships between different quantitative parameters were assessed by simple linear regression analysis ,and Pearson *(r)* correlation coefficients were presented .The results were considered significant whenever *p* values <0.05 and highly significant when *p* values <0.001were observed .

Results

The results are demonstrated in tables 1-3 and figures 1-2. The data in table (1) reveals that, the diabetic group displayed significant higher levels of fasting blood glucose and glycated Hb% versus controls with P<0.0001. Table (2) shows that serum CXCL-8 level exhibited a significant increase while the nitric oxide level showed a significant decrease in the T1D children versus the healthy controls.

Table (3) shows established pilot correlation in a trial to find out the effect of age, duration of diabetes, fasting blood glucose and HbA1c on log CXCL-8, and log NO in T1D children. A positive correlation was found between duration of diabetes and HbA1c with log CXCL-8. No correlation was detected between other parameters. Figure (1) shows comparison between serum CXCL-8 in controls and type 1D. Figure (2) shows comparison between serum NOx in controls and T1D

Discussion: Type 1diabetes is the most common metabolic disease of childhood and it is accepted that autoimmunity is the predominant effector in the pathophysiology of the disease in genetically susceptible patients(2, 13). Though, the disease can occur at any age, but its onset most often occurs in childhood (2). In this study the mean age of the patients was 9.05 ±3.14 years. Many authors agreed that the early onset of the disease with insulin deprivation increase the risk of development of complications as diabetic ketoacidosis (DKA) which may be the 1st presentation in addition to micro and macrovascular complications, especially premature atherosclerosis (14-16). The results of our study revealed that 4/20 children (20%) presented initially by DKA.

Several studies revealed that viral infection, particularly of the upper respiratory tract, may precipitate T1D through enhancement of the production of antibodies that trigger an autoimmune response against the antigenically similar beta cells (17, 18). In the current study, all children had past history of recurrent upper respiratory tract infections in their infancy and early childhood and they had one attack at least within the last few months before the onset

of the disease. These findings are in accordance with the study of Beyerlein et al. (18). However, other viral infections are also important especially enterovirus infection in infants previously exposed to cow's milk before 3 months of age (19).This past history was not clarified in our study.

Many investigators declared that the pro-inflammatory cytokines released from CD4+T cells play a major role in the pathogenesis and progression of T1D and their mediators facilitate the pancreatic mononuclear-cell infiltration and acceleration of beta cell destruction (5, 20, 21). The current study verified a significant increase in serum CXCL-8 levels in diabetic children versus healthy controls. The same result was established by Erbagci et al. (21)and Lo et al. (22). Haskins et al. (23), found that human CD4+ T cells produce high amounts of CXCL8 (IL-8), and other CXC family chemokines in addition to activation of CD8+ T cells and stimulation of macrophages to produce more pro-inflammatory cytokines and nitrogen free radicals. Similar results were obtained by Nomura et al. (24). Hoffman et al. (25), added that IL-8 sustains the inflammatory process and associates with tissue damage and it is secreted in high levels in diabetic ketoacidosis with other inflammatory chemokines.

Recent studies documented the autoimmune origin of atherosclerosis revealing the involvement of CD4+ T cells with its cytokines and chemokines in addition to the formation of Anti-endothelial cell antibodies (6, 7, 16, 26, 27). CXCL8 acts as a fast secretagogue of proteases which help to generate immunodominant peptides that attract autoimmune cells; including monocytes chemotactic protein1 (CCL2; CC ligand2) which is the main chemokine recruiting monocytes from blood to early atherosclerotic lesion (6, 7). CXCL-8 and CCL2 stimulate the adhesion of the monocytes to the endothelial cells leading to its dysfunction and subsequent development of atherosclerosis (7). However, these effects are preventable, Esparza et al.(28), in their study showed that immunodepressive and anti-inflammatory agents, can inhibit the development of atherosclerosis by inducing apoptosis of activated CD4 T lymphocytes and affecting the cascade of immunomodulatory cytokines and chemokines, such as interleukin-6, CXCL8 and CCl2.

Hemoglobin A1c is the product of irreversible glycation of the beta chain of hemoglobin by plasma glucose and its level is increased with hyperglycemia providing an estimate of plasma glucose levels during the preceding 1-3 months (29). Hemoglobin A1c level of 6.5% or higher is considered a criterion for diabetes diagnosis and predicts the progression of diabetic microvascular complications (30). The present study revealed a significant increase in HbA1c level in Type 1dibetes children versus the control group and positive correlation was found between duration of diabetes and HbA1c level with CXCL-8, a finding which reflects the progress of the inflammatory process and defective control of hyperglycemia. These results were in agreement with the recorded by Lo et al. (23),and the study of Shalitin and Phillip (31),who found a positive correlation between HbA1C, pro-inflammatory chemokines, and angiopathy.

In addition to the effect of the chemokines and cytokines on the monocytes, these mediators may affect the nitric oxide (NO) pathway which is the main molecule derived from the endothelium and is involved in vascular homeostasis (9). Several studies have shown that the reduction in NO and in its bioactivity is strongly linked to endothelial dysfunction which reduces the activity of eNOS and many authors correlated this to the autoimmune process and the associated elevation in inflammatory mediators in addition to formation of anti-endothelial antibodies (8-10). Balarini et al. (32), revealed in their study on the apolipoprotein E knockout mice that the drugs which restore the bioactivity of NO as sildenafil improved endothelial dysfunction and prevented the development of atherosclerotic plaques.

The findings of our study illustrated significantly lower NOx levels which reflect the reduced bioactivity of eNOS and NO in diabetic children compared to the control group .This agreed with the study performed by Kuboki et al., (33) and Correa and Alfieri (34) and Lo et al., (22), who found that

diabetic patients have significantly lower circulating NO level; however, they claimed low nitric oxide levels to the absence of the stimulatory effect of insulin on eNOS. Type 1diabetes is more prevalent in children and the development of endothelial dysfunction would eventually progress to premature atherosclerosis (16). Low nitric oxide plasma levels is considered by many investigators to be the earliest sign of atherosclerosis, before ultrasound or angiogram show evidence of atherosclerotic plaque formation (16, 34).

This study and other studies have been verified that the progress of T1D and its complications are multifactorial but most of the factors are closely related to the autoimmune process including AAs, activated T cells with the release of cytokines, chemokines and other chemical mediators (2-7, 20-29). However, the loss of the beta cell mass is not complete and there is a significant reserve of functioning beta cells, and it was found that the disease and most of its complications are preventable and can be treated or at least, this functional beta-cell reserve can be preserved by immunomodulation (2). Researches to produce Type 1 diabetes vaccine are promising and recent studies have shown that, cytokines and chemokines that induce enzymes, could be antagonized by biosynthetic chemokine antagonists and might become useful in the treatment of autoimmune diseases including Type 1 diabetes in addition to the researches about the effect of immunotherapy and gene therapy to treat the disease and its complications (2, 35, 36).

Conclusion: It could be concluded that CXCL-8 and NO may play important roles in the pathophysiology and progression of T1D with increased possibility to develop premature atherosclerosis which should be considered in the development of new strategies for monitoring the disease as well as for developing effective preventive and therapeutic interventions.

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Table (1): Characteristics and the laboratory investigations of the children with T1D and controls

Parameters	Controls (n=20)	T1D(n=20)	P-values
Age (years), Mean ± SD	9.12± 3.70	9.05±3.14	N.S.
Gender (M/F)	10/10	11/9	
Duration of diabetes (years), Mean ± SD		3.5±2.4	
History of recurrent chest infection early in childhood		100%	
Initial presentation by diabetic ketoacidosis		20%	
Fasting blood glucose (mg/dl), Mean ± SD	79.66± 8.87	139.47± 25.75	<0.0001
HbA1c (%),Mean ± SD	5.56± 1.22	8.9± 1.33	<0.0001

Table (2): Mean ±SD serum concentrations of CXCL-8 and Nitric oxide metabolite in T1D children and controls.

Parameters	**Controls (n=20)**	T1D **(n=20)**	**P-values**
CXCL-8 (pg/ml) (Mean ± SD)	5.69±1.67	23.54±11.92	<0.0001
NOx (mmol/l) (Mean ± SD)	4.63±1.2	2.38±1.14	<0.05

Table (3): Correlation between age, duration of the disease, fasting blood glucose and HbA1c with log CXCL-8 and log NOx in T1D children by simple linear correlation

	Age(years)		**Duration of the disease**		**Fasting blood glucose**		**HbA1c %**	
	R	**P- values**	**r**	**P-values**	**r**	**P-values**	**r**	**P-values**
Log CXCL-8	0.1883	N.S	0.6271	<0.05	0.1589	N.S	0.5539	<0.05
Log NOx	0.0891	N.S	0.5354	<0.05	0.2315	N.S	0.1102	<0.05

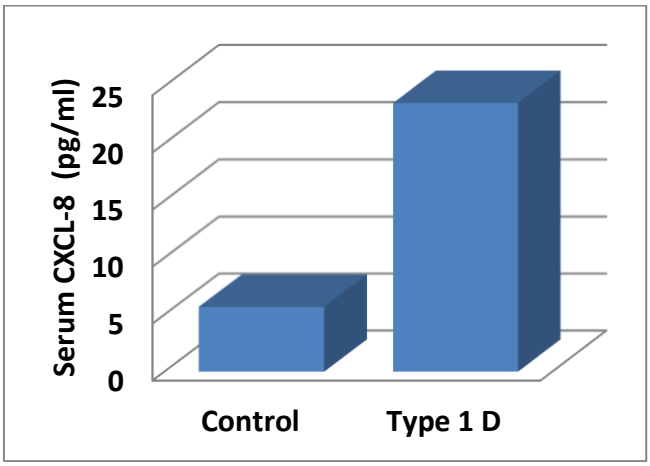

Figure 1: Comparison between serum CXCL-8 in controls and type 1D

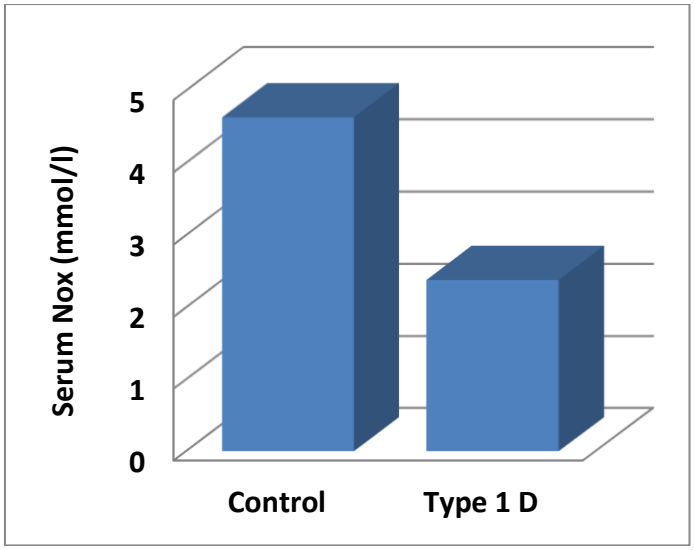

Figure 2: Comparison between serum NOx in controls and type 1D