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Original Contribution

LEVELS OF SECRETORY IgA IN DIABETIC CHILDREN

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ABSTRACT

The aim of this study was to determine whether the levels of secretory IgA (slgA) correlated with the vascular complications in patients with Type I (insulin-dependent) diabetes mellitus. The baseline study population consisted of 61 children (28 boys and 33 girls) with Type 1 diabetes mellitus (mean age 12.3±4 years) and control group consisted of 20 healthy children of similar age (13.6±4.2). The patients were divided in two groups based on the presence (group 1; n=31) or absence (group 2; n=30) of vascular complications. Sera of tested groups were studied by ELISA for slgA. Group 1 and 2 showed non-significant slgA level than healthy controls.

Key words: Secretory IgA , diabetes mellitus, microvascular complication

INTRODUCTION

Secretory IgA (sIgA)[3] is the main antibodies secreted by the mucosa of the airways, gastrointestinal tract, and other mucosal tissues [15]. A high proportion of Ig-producing cells in the mucosal lymphoid tissue is committed to the IgA isotype, and the majority of IgA in mucosa is derived from local synthesis [7]. Receptors for the Fc portion of IgA (FcR) have been established on a variety of cell types, including lymphocytes, [6,9] monocytes/macrophages [14], neutrophils[2,l 1], and eosinophils[12]. The importance of IgA in mucosal immunity is further supported by the presence of secretory component (SC) in S-IgA[4], Epithelial cells express the polymeric Ig receptor, which binds and mediates the transepithelial transport of IgA from the basolateral side of the cells into the lumen[5,15]. The SC is a soluble proteolytic cleavage fragment of the extracellular domain of membrane-bound polymeric Ig receptor[13]. Addition of SC to IgA confers increased stability on the resultant S-IgA by helping to hold the IgA monomers together [10] and by masking proteolytic sites from proteases present in mucosal secretions[8]; however, the effect of the addition of SC on the biologic activity of IgA is unknown.

Diabetes mellitus type 1 is autoimmune disease and immune complexes formed by different antigens and corresponding antibodies are pro-atherogenic and pro-inflammatory [1]. Considering that microalbuminuria is a risk factor for coronary heart disease and that common pathogenic factors for atherosclerosis and glomerulo-sclerosis exist.

Our aim was to determine whether the levels of slgA correlated with the vascular complications in patients with Type I (insulin-dependent) diabetes mellitus.

MATERIALS AND METHODS

Subjects

The baseline study population consisted of 61 patients (28 boys and 33 girls) with Type 1 diabetes mellitus (mean age 12.3±4 years) diagnosed according to the WHO definition. The control group consisted of 20 healthy children of similar age (13.6±4.2) and sex with no family history of diabetes, atherosclerosis, and/or nephropathy. The patients are all diabetic children residing in the vicinity of the Pleven University Hospital.

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All patients were treated with a standard dose of human insulin obtained from Novo Nordisk Industri, Copenhagen, Denmark. None of the subjects were taking antihypertensive medication prior to the appearance of persistent microalbuminuria. None of the patients had a diagnosis of renal disease unrelated to diabetes.

The mean duration of diabetes was 5.2±3.7 years. All subjects were being administered 2 to 4 subcutaneous doses insulin per day.

The patients were divided into two groups based on the presence (group 1) or absence (group 2) of vascular complications. Group 1 consisted of 31 diabetics - 17 had microalbuminuria, 3 elevated blood presure, 5 both retinopathy and microalbuminuria, and 3 HBP and microalbuminuria. The clinical data of the patients are present on Table 1.

Table 1. *Clinical characteristics of diabetic patients wilh or without vascular complications*

Clinical data	Group I (with vascular complications) n — 31	Group 2 (without vascular complications) n — 28	P
Age (years)	14.03 +3.6	10.5 +3.9	0.0007
Mean duration (years)	6.9 +3.2	3.6 +3.43	0.0004
Mean glycated hemoglobin (%)	10.35 +1.6	10.4+1.3	NS
Systolic blood pressure (rtimHg)	110.3 +14.3	104.8 +11.2	NS
Diastolic blood pressure (miriHg)	75.5 +11.1	68.8 +7.9	0.01
Triglycerides (minol/1)	1.37 +0.58	1.14 +0.54	NS
Total cholesterol (mmol/1)	4.71 +0.84	4.82 +0.74	NS
Dose(U/kg/24h)	1.11 +0.29	0.85 +0.28	0.001
Microalbuminuria (p.g/riiin)	37.76 +16.9	10.14 +3.18	0.0001
High density lipoprotein **(mmol/1)**	1.33 +0.34	1.16 +0.19	NS
slgA E494	0.53 +0.31	0.46 +0.29	NS

Microalbuminuria was defined as a persistent urinary albumin excretion rate (AER) in the range of 20 and 200 pg/min in urine. Levels of hemoglobin Ale (HbAlc), total serum cholesterol, triglycerides and AER were measured as described earlier (Nicoloff et al., 2000b). Levels of HDL were quantified using the quantitative enzyme method for direct detection (test-kits HDL-C plus, Roche). Serum fructose was detected by Farb-test (Roche).

Ethical approval was obtained from the local research ethics committee and the parents of all subjects gave a written informed consent prior to enrolment in the study.

Procedure

ELISA for IgA

In brief, each well of the microtiter plate was sensitized with 100 μĩ anti-secretory component antibodies (dilution) at room temperature for 3h followed by an overnight incubation at 4o C. The plate was washed with phosphate-buffered saline (PBS) containing *0.05%* Tween 20. Then, 100 μĩ serum sample (dilute 1:5) were added to the plates and incubated for 60 min at 37o C, washed several times, and bound 100 μl of mouse anti human IgA peroxidase conjugates diluted 1:800 with PBS containing 1% BSA and 0.05% Tween 20 and o-phenylen-ediamine, 4 mg/ml in 10 ml 0.05 M citrate buffer, pH 5.0+0.01% H202 for 1 h at room temperature in a dark chamber. The reaction was stopped by adding of 4M H2S04 to each polystyrene well.

Statistical Analyses

All values are expressed as mean±SD. Statistical analyses were performed using the computer programs Excel and Statgraphics plus for Windows. The Student's t-test and

ANOVA were used to assess differences between study groups (LSD, Tukey HSD, Scheffe, Bonferroni, Newman-Keuls, and Duncan). For select data sets, correlation analysis was performed and data considered significant with a p value of less than 0.05.

RESULTS

Group 1 and 2 showed non-significant slgA level than healthy controls. Diabetics showed correlation with: age and slgA (r=-0,2589 p= 0,0317); duration and slgA (r= -0,2612 p=0,0457); frukt and slgA (r= 0,2565 p=0,0499);

Group 1 showed correlation with fructose and slgA (r=0.39 p=0.025); In subgroup with microalbuminuria (n=18); cholesterol and slgA (r=-0.55 p=0.03); slgA and HDL(r=-0.9820(6) p=0.0005); cholesterol and slgA (r=0.8576 (7) p= 0.0136); fruktose and slgA (r=0.7643 (7) p=0.0454); doza and slgA (r=-0.9013 (6) p= 0.0141)

In subgroup with elevated blood pressure (n=9) slgA and triglycerides (r=0.69 p=0.038) cholesterol and sIgA(r=0.67 p=0.046); fructose and slgA (r=0.79 p=0.01); HDL and slgA (r=-0.93 p=0.002)

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