Inflammatory Profiles of Black and White Pediatric Type 1 Diabetes Patients

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Introduction

Type 1 Diabetes Mellitus results from the autoimmune destruction of pancreatic beta cells that secrete insulin. Metrics of glycemic control used to diagnose and treat diabetes are fasting blood glucose, mean blood glucose (MBG), and HbA1c. Previous research has found biological variation in HbA1c levels independent of MBG. The hemoglobin glycation index (HGI) is a newly researched quantitative measure of this variation. HGI is calculated from the deviation of observed HbA1c from predicted HbA1c based on MBG. HGI was found to be highly correlated with increased risk of diabetic complications such as retinopathy and nephropathy.[1]

Hyperglycemia, iatrogenic hypoglycemia, and even insulin itself can lead to the increased release of *inflammatory cytokines*[2,3,6]. Research has discovered that chronic inflammatory cytokines can cause insulin resistance[4] and alter the regulation of the MAPK/PKC pathway, a pathway highly active in times of excess glucose and linked to the development of diabetes complications such as cardiovascular disease (CVD), retinopathy, and nephropathy[2]. Chronic inflammation can: (1) cause **insulin resistance** through the lowering of blood flow to skeletal muscle effectively decreasing glucose disposal in the tissue[4]; (2) cause or worsen CVD because inflammation is a major and initial cause of atherosclerosis, which leads to cardiovascular disease[5]; (3) cause or worsen **proliferative diabetic retinopathy** through the induction of angiogenesis or **diabetic macular edema** by decreasing renal blood flow, causing vascular leakage[2]; (4) advance **nephropathy** by accelerating the progression of hyperglycemic damage - resulting in renal fibrosis and scarring – and affecting hypertension, which has a recognized role in exacerbating diabetic kidney disease.[8]

This study aimed to: (1) create inflammatory profiles of black and white pediatric type 1 diabetes patients and test for an association to parameters of glycemic and metabolic control (MBG, HbA1c, HGI, BMI, and SBP); (2) identify high-risk type 1 diabetes individuals in the study population and evaluate whether inflammation should be targeted clinically to reduce risks.

Methods

This study included 38 children with Type 1 Diabetes who attended the Pediatric Diabetes Clinic at Children's Hospital in New Orleans. Patients were recruited for a larger project under the MSTCC research organization, were only included in the study if they met certain criteria, and were enrolled with informed consent as approved by the LSUHSC Institutional Review Board. Features of the study population are presented in Table 1.

Inflammatory biomarkers were measured in the plasma of the 38 patient study population using a fluorescent bead-based immunoassay from EMD Millipore. The samples were stored at -80°C and underwent two freeze-thaw cycles before being analyzed. All specimens were analyzed for **IFNγ**, IL-**1β,** IL-1ra, IL-4, IL-6, IL-8, IL-10, IL-12 (p70), **TNFα**, VEGF, and CRP. The first 10 analytes were measured using the human cytokine/chemokine magnetic bead panel, a 96 well plate assay. CRP was measured using the human cardiovascular disease panel 3, a 96-well plate assay. [Note: Bolded cytokines are pro-inflammatory; Non-bolded cytokines are anti-inflammatory.]

Both assays were performed according to the instructions provided by the manufacturer. The first layer in the plate was highly specific immobilized antibodies. The second layer contained the antigens (test sample). The cytokine measurements were performed in 25µL of undiluted plasma in duplicates. CRP measurements were performed in 25µL diluted plasma (1:40) in duplicates. The third layer was made of the labeled antibodies that emitted fluorescence.

The MAGPIX instrument was used to gather the data. Standard curves were generated for each analyte using the premixed lyophilized standards that came in the kit. Median fluorescence intensities (MFIs) were then collected on the instrument using the MILLIPLEX Analyst 5.1 software. The measured MFI is considered directly proportional to the amount of antigen that reacted with the antibody, and thus could be transformed into cytokine concentrations (recorded in pg/mL for cytokines and ng/mL for CRP).

Simple statistical analyses such as Pearson correlations, Wilcoxon Rank Sum scores, and the Kruskal-Wallis test were performed. Other trends were presented as XY scatterplots, bar graphs, or vertical scatterplots.

Results

Table 1: Features of the Study Population

n	38
Male/Female	20/18
Black/White	19/19
Age (y)	14 ± 3.4
Duration of Diabetes (y)	14.3 ± 3.5
HbA1c (%)	9.8 ± 2.3
MBG (mg/dl)	224.3 ± 77.5
HGI (%)	0.07 ± 1.7
BMI (kg/m^2)	22.67 ± 6.1
SBP (mmHg)	120.2 ± 11.5
DBP (mmHg)	70.1 ± 7.2
HR (beats/min)	82.8 ± 12.0

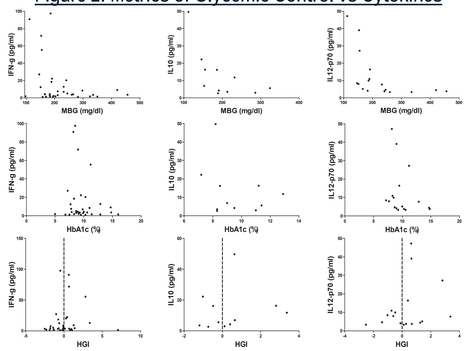
Figure 1: Cytokine Concentration by Race

Pro-inflammatory Biomarkers

Anti-inflammatory Biomarkers

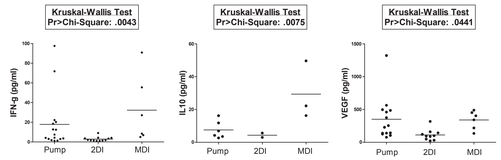
Cytokine concentrations were not significantly different between races. Results showed whites had higher concentrations of IFN-γ, IL12(p70), IL6, VEGF, and IL-1ra compared with blacks, and blacks had higher concentrations of IL1β, IL8, TNF-α, CRP, IL10, and IL4 compared with whites.

Figure 2: Metrics of Glycemic Control vs Cytokines



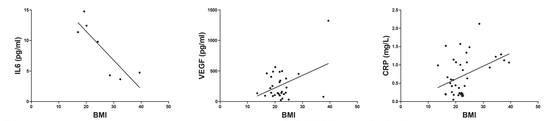
Mean blood glucose (MBG) was inversely correlated with IFN-γ (r= -0.33875, p= .0500*), IL10 (r= -0.62603, p= .0394*), and IL12p70 (r= -0.52596, p= .0301*). HbA1c and HGI were not significantly correlated. HGI did show a general trend towards higher inflammatory markers in patients with higher HGIs. [For HGI graphs, the line at x=0 represents the cut-off between high (>0) and low (<0) HGI class.]

Figure 3: Cytokine Concentration by Treatment Type



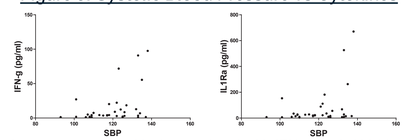
A significant difference in medians was found when comparing IFN-γ, VEGF, and IL10 across treatment types. This relationship was trending across all cytokines that were measured. Patients on intensive treatment regimens (pump and MDI) had higher inflammatory profiles than those using less intensive methods (2DI). [2DI = twice daily injections; MDI = multiple daily injections.]

Figure 4: Body Mass Index vs Cytokines



BMI was inversely correlated with IL-6 (r= -0.85767, p= .0136*) and positively correlated with VEGF (r= 0.42194, p= .0202*) and CRP (r= 0.41439, p= .0097*). Data also showed BMI trending towards inverse correlations with IL-1β and TNF-α.

Figure 5: Systolic Blood Pressure vs Cytokines



Systolic blood pressure was positively correlated with IFN-γ (r= 0.40341, p= .0180*) and IL-1ra (r=0.36587, p=.0282*). Data also showed SBP trending towards a positive correlation with VEGF.

Discussion

1. RBCs have pro- or anti-glycation intracellular environments. Kinetic modifiers of this environment include pH, temperature, glutathione, and possibly other unknown genetic or environmental factors. Research has found that HbA1c and HGI are typically higher in blacks than whites independent of variation in blood glucose concentration.[1]

2. Type 1 diabetes patients with high HbA1c levels are usually prescribed high doses of insulin. High insulin increases risk for hypoglycemia. Hypoglycemia, as well as insulin, have been shown to increase pro-inflammatory mediators.[3, 6]

3. Previous research found an inverse relationship between pro-inflammatory biomarkers and metabolic markers (BMI, subcutaneous and visceral fat stores). This relationship is not well understood; research postulates that acute inflammation may be normal for development and metabolic homeostasis in children.[7] BMI's inverse relationship with IL-6 but positive relationship with VEGF and CRP might be evidence that some cytokines are more beneficial than others. Chronic inflammation in children is thought to have deleterious effects later in life. VEGF and CRP have been directly linked to retinopathy and CVD, respectively.

4. Blood pressure changes are known to exacerbate diabetes complications. The positive correlation between SBP and inflammatory biomarkers thus shows a relationship between inflammation and the progression of diabetes complications. Further research is warranted to determine the mechanistic relationship between these variables.

Conclusions

1. Cytokine concentration was not statistically different between blacks and whites in this small sample. Further research with a larger sample size is necessary to elucidate the racial disparity seen in diabetes and its complications.

2. Inflammation is significantly associated with various glycemic and metabolic parameters (i.e., MBG, BMI, and blood pressure). Therefore, it is important to consider its role in chronic disease complications.

Future Directions

1. Multivariate analysis will be used to determine whether multiple levels of independent variables on their own or in combination have an effect on the dependent variables.

2. The results of this study will be used to conduct power analyses to determine sample sizes needed to detect differences in cytokine levels between ethnic, treatment, or metabolic subgroups of pediatric diabetes patients.

3. A Geographical Information System will be used to identify social determinants of health and test the hypothesis that stressful environments are associated with adverse inflammatory profiles in pediatric diabetes patients.

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