Metrics of Glycemic Control and Oxidative Stress in Black and White Pediatric Type 1 Diabetes Patients

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Abstract

Glycated hemoglobin (HbA1c) and blood glucose levels are widely used in the diagnosis and management of diabetes. Thiobarbituric acid reactive substances (TBARS) are used as an index of endogenous lipid peroxidation, a well-established mechanism of cellular injury, and is used as an indicator of oxidative stress. The most abundant lipid peroxidation product is malondialdehyde (MDA), which is a highly reactive three carbon dialdehyde produced as a byproduct of polyunsaturated fatty acid peroxidation. The human body has a complex antioxidant defense system that prevents the initiation of free radical chain reactions caused by lipid peroxidation. We measured multiple metrics of glycemic control and plasma TBARS levels and compared these in black and white pediatric type 1 diabetes patients. We found that there was a significant positive correlation between plasma TBARS levels and plasma glucose levels but no correlation with hemoglobin A1c, mean blood glucose or other metrics. Although metrics of glycemic control differed between black and white patients there was no difference in TBARS levels.

Introduction

Previous studies by our group showed that black type 1 diabetes patients have higher HbA1c levels than white patients independent of the effects of blood glucose concentration (Kamps et al. 2010). It has been suggested that oxidative stress contributes to both inter-individual and inter-racial variation in the relationship between blood glucose and HbA1c. To test this hypothesis we compared plasma levels of thiobarbituric acid reactive substances (TBARS) and metrics of glycemic control (mean blood glucose (MBG), HbA1c, the hemoglobin glycation index (HGI), plasma glucose, βVal1 α glucosylamine, and βVal1 β glucosylamine) in 68 black and white pediatric type 1 diabetes patients. This interim study is part of an ongoing project supported by the Mid-South Transdisciplinary Collaborative Center for Health Disparities Research. The project is also collecting data on clinical, psychosocial, biochemical and social determinants of health.

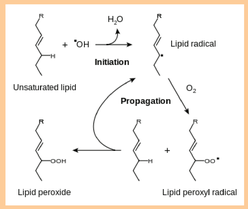

Fig. 1. Why measure TBARS? In this figure an unsaturated lipid reacts with a hydroxide radical to produce a fatty acid radical. The fatty acid is not a stable molecule, so it reacts readily with molecular oxygen forming a peroxy-fatty acid. This radical is also unstable producing a different fatty acid and a lipid peroxide or a cyclic peroxide if it is reacted with itself. The cycle continues as the new fatty acid radical reacts in the same manner.

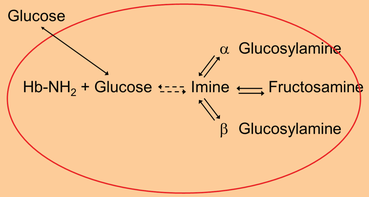

Fig. 2. Why measure βVal1 glucosylamines? Glucosylamines are Maillard reaction intermediates in the non-enzymatic glycation of hemoglobin and fructosamine (HbA1c) synthesis.

Methods

The measurement of Thiobarbituric Acid Reactive Substances (TBARS) is a well-established method for screening and monitoring lipid peroxidation. MDA is formed as a result of lipid peroxidation and reacts with thiobarbituric acid under high temperature and acidic condition.

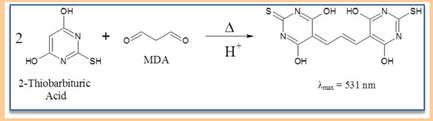

Fig. 3. How the TBARS assay works. MDA reacts with 2 moles of TBA to form an MDA-TBA adduct. This reaction yields a pink MDA-TBA adduct, the product of 2 moles of TBA plus 1 mole of MDA, which is then measured based on absorbance at 531 nm.

TBARS were measured using a commercial kit obtained from Cayman Chemical company. HbA1c was measured by the Children's Hospital laboratory using a National Glycohemoglobin Standardization Program certified immunoassay. βVal1 α and β glucosylamines were measured by dynamic capillary isoelectric focusing (Hempe et al. 2012). MBG levels over a 30 d period were downloaded from patient glucose meters. HGI was calculated as the difference between a patient's observed HbA1c and a predicted HbA1c based on the patient's MBG. Plasma glucose was measured using a commercial enzyme (glucose oxidase) assay. Statistical analyses were performed using SAS 9.3. Results were compared between groups using a non-parametric Kruskal-Wallis test. Results were considered statistically significant at $p<0.05$.

Results

Table 1: Characteristics of the Study Population

	Black	White
Male/Female	12/11	24/21
Age (y)	13.1 ± 0.8	14.6 ± 0.5
Duration of Diabetes (y)	13.5 ± 0.9	15.0 ± 0.5
HbA1c (%)	10.7 ± 0.5	8.8 ± 0.2
MBG (mg/dl)	250.2 ± 18.7	197.0 ± 5.8
HGI (%)	0.5 ± 0.3	-0.2 ± 0.1
Plasma Glucose	207.2 ± 19.0	176.4 ± 13.5
βVal1 α glucosylamine	2.6 ± 0.1	2.0 ± 0.1
βVal1 β glucosylamine	2.2 ± 0.2	1.9 ± 0.1
SA1C1	14.7 ± 0.8	11.8 ± 0.3
TBARS	18.0 ± 0.8	17.0 ± 1.8

**Values are means ± SEM*

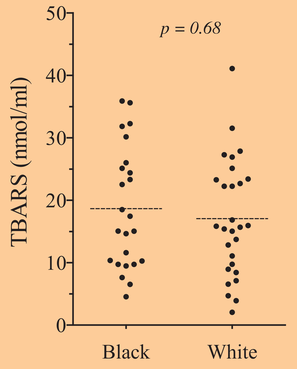

Fig. 4. No difference in TBARS levels. TBARS were not significantly different (p>0.05) in black and white pediatric type 1 diabetes patients.

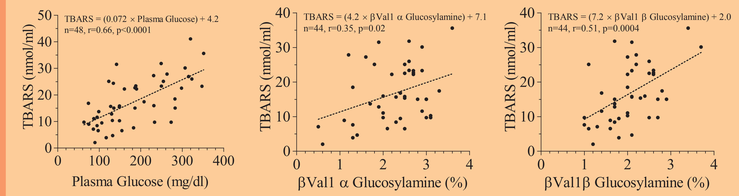

Fig. 5. Simple linear regression comparing TBARS and metrics of glycemic control. TBARS levels were positively correlated with ambient plasma glucose levels and with both βVal1 α glucosylamine and βVal1 β glucosylamine. Both glucosylamines are intermediates in the formation of HbA1c (Fig. 2) and are strongly correlated with plasma glucose.

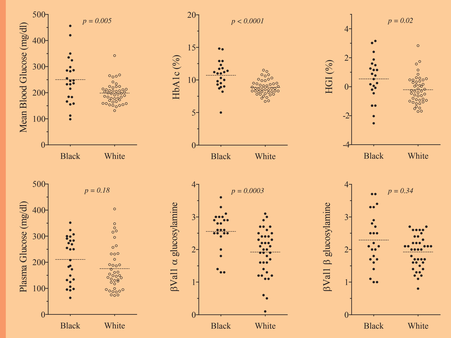

Fig. 6. Racial differences in metrics of glycemic control. MBG, HbA1c, HGI, and βVal1 α glucosylamine were significantly different in black and white pediatric diabetes patients. Plasma glucose and βVal1 β glucosylamine levels were not significantly different.

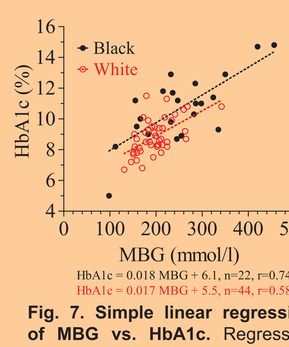

Fig. 7. Simple linear regression of MBG vs. HbA1c. Regression equations for black and white diabetes patients had similar slopes but different intercepts.

Conclusion

1. MBG, HbA1c and HGI were significantly higher in black type 1 diabetes patients compared to white patients as previously reported (Kamps et al, 2010).
2. Plasma TBARS levels were not significantly different in black and white diabetes patients.
3. Plasma TBARS levels were positively correlated with plasma glucose levels and endogenous levels of both glucosylamines.

References:

Kamps, J.L., Hempe, J.M., and Chalew, S.A. (2010). Racial disparity in A1C independent of mean blood glucose in children with type 1 diabetes. Diabetes care *33*, 1025-1027.

Hempe, J.M., McGehee, A.M., Hsia, D., and Chalew, S.A. (2012). Characterization of unstable hemoglobin A1c complexes by dynamic capillary isoelectric focusing. Analytical biochemistry *424*, 149-155.

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