Noninvasive Type 2 Diabetes Screening: Superior Sensitivity to Fasting Plasma Glucose and Glycosylated Hemoglobin

Received for publication 20 November 2006 and accepted in revised form 10 February 2007.

Noninvasive Type 2 Diabetes Screening

*(running title)*

J.D. Maynard, MS¹, M. Rohrscheib, MD², J.F. Way, BS³, C.M. Nguyen, BSc³ and M.N. Ediger, PhD¹

¹VeraLight, Inc
²University of New Mexico School of Medicine
³InLight Solutions

Corresponding author:

M.N. Ediger

VeraLight

800 Bradbury SE, Suite 217

Albuquerque NM 87106

woody.ediger@veralight.com

ClinicalTrials.gov ID: NCT00358254

Copyright American Diabetes Association, Inc., 2007
Objective

This study compared the performance of a novel noninvasive technology to the fasting plasma glucose (FPG) and glycosylated hemoglobin (A1c) tests for detecting undiagnosed diabetes and impaired glucose tolerance (IGT).

Research Design and Methods

The design was a head-to-head evaluation in a naïve population. Consented subjects received FPG, A1c and oral glucose tolerance (OGTT) tests. Subjects were also measured by a noninvasive device that detects the fluorescence of skin advanced glycation endproducts. A total of 351 subjects participated.

Results

Subjects with 2-hour OGTT values equal to or exceeding 140 mg/dL defined the positive screening class. A total of 84 subjects (23.9% prevalence) screened as positive. The performances of the noninvasive device, FPG and A1c were evaluated for sensitivity and specificity against this classification. At the IFG threshold (FPG = 100 mg/dL), the FPG sensitivity was 58% and the specificity was 77.4%. At that same specificity, the sensitivity for A1c was 63.8%, while the noninvasive sensitivity was 74.7%. The sensitivity advantage of the noninvasive device over both blood tests for detecting diabetes and precursors was statistically significant (p < 0.05).

Conclusions

The noninvasive technology showed clinical performance advantages over both FPG and A1C. The sensitivity differential indicated that the noninvasive device is capable of identifying 28.8% more individuals in the OGTT-defined positive screening class than FPG and 17.1% more than A1c. The combination of higher sensitivity and greater convenience – rapid results with no fasting or blood draws - make the device well-suited for opportunistic screening.
Introduction

The U.S. is facing a dangerous epidemic in type 2 diabetes. Of the estimated 20.6 million individuals with diabetes, approximately thirty percent of them are undiagnosed [1]. Another 54 million people have some form of pre-diabetes and many will progress to frank diabetes within three years [1-3]. Numerous studies have shown that with early detection and effective intervention, diabetes can be prevented or delayed [2-7]. In patients with diagnosed diabetes, other studies have shown that glucose control can lower the incidence of complications [8,9].

Diagnosis is typically initiated during a physical exam with a primary care physician. However, current screening methods for type 2 diabetes and pre-diabetes are inadequate due to their inconvenience and inaccuracy. Specifically, the most widely applied screening test in the U.S., the fasting plasma glucose (FPG), has convenience barriers in the form of an overnight fast and a blood draw. FPG also suffers from poor sensitivity (40-60%) contributing to late diagnoses [10]. In fact, about one-half of diabetes patients present with one or more irreversible complications at the time of diagnosis [11,12]. A more accurate and convenient screening method could dramatically improve early detection of type 2 diabetes and its precursors, facilitating interventions that can prevent or at least delay the development of type 2 diabetes and its related micro and macrovascular complications.

Several studies including DCCT and EDIC have demonstrated that elevated skin advanced glycation endproducts (AGEs) are biomarkers of diabetes, highly correlated with the complications of diabetes and are predictive of future diabetic retinopathy and nephropathy [13-15]. A person with diabetes will accumulate skin AGEs faster than individuals with normal glucose regulation [16]. Thus, skin AGEs constitute a sensitive, summary metric for the integrated glycemic exposure that the body has endured.

However, until the recent development of novel noninvasive technology to measure advanced glycation endproducts, a punch biopsy was required to quantify skin AGE levels. This method for “Spectroscopic measurement of dermal Advance Glycation Endproducts” - hereafter referred to as SAGE - measures skin fluorescence due to AGEs in vivo and provides a quantitative diabetes risk score based on multivariate algorithms applied to the spectra [17]. SAGE does not require fasting and creates no biohazards. It automatically compensates for subject-specific skin differences caused by melanin, hemoglobin, and light scattering. The measurement time is approximately one minute and thus provides an immediate result.

The concept of quantifying dermal AGEs noninvasively was successfully tested in a previous in vitro study. In that work, concentrations of a well-studied fluorescent AGE, pentosidine, were accurately quantified in a porcine dermis model by noninvasive fluorescence spectroscopy [18]. Subsequently, an early noninvasive prototype was evaluated in a diabetic vs. normal (case-control) human subject study, demonstrating that SAGE could accurately classify disease in a case-control population...
This led to the premise of the current work: we hypothesize that SAGE can detect undiagnosed diabetes and pre-diabetes with sufficient performance to serve as a screening tool.

**Research Design and Methods**

The present study is a direct comparison of SAGE with the fasting plasma glucose (FPG) and glycosylated hemoglobin (A1c) using the 2-hour oral glucose tolerance test (OGTT) to determine truth (i.e., the “gold standard”). The threshold for impaired glucose tolerance (IGT) - a 2-hour OGTT value of 140 mg/dL or greater - delineated the screening threshold for “abnormal glucose tolerance.” A subject is classified as having abnormal glucose tolerance if they screen positive for either IGT (OGTT: 140-199 mg/dL) or type 2 diabetes (OGTT: ≥ 200 mg/dL). The abnormal glucose tolerance group encompasses all subjects needing follow-up and diagnostic confirmation. The study was conducted in a naïve population - subjects who have not been previously diagnosed with either type 1 or 2 diabetes.

In order to demonstrate superior sensitivity at 80% power with 95% confidence, an abnormality in 80 subjects was required [20]. At that prevalence and for a projected SAGE sensitivity of 68%, the power calculations yield a 95% confidence interval for test sensitivity of 57.8% - 78.2%.

Study subjects were selected from persons who responded to flyers and newspaper advertising. Subjects were recruited until the target prevalence of abnormal glucose tolerance was comfortably achieved. Selection criteria were one or more risk factors for diabetes per the American Diabetes Association (ADA) standard of care guidelines [21]. Individuals with a previous diagnosis of diabetes were excluded. Ages in the cohort ranged between 21 and 86 years while the ethnic and racial composition mirrored the demographics of Albuquerque, New Mexico. The cohort demographics are summarized in Table 1 under the N column heading. The study protocol was approved by the University of New Mexico School of Medicine Human Research Review Committee. When recruiting concluded, 84 subjects with abnormal glucose tolerance had been identified within a cohort of 351 participants.

Subjects were asked to fast overnight for a minimum of 8 hours prior to participation. All provided their informed consent. Blood was drawn from subjects for clinical chemistry tests. The glucose assays were run on a Vitros 950™ clinical chemistry analyzer while the A1c assay was performed on a Tosoh G7 HPLC™ [22].

The prototypical SAGE instrument is a table-top apparatus. The subject sits in a chair beside the instrument and rests his/her left forearm in an ergonomically-designed cradle. A custom fiber-optic probe couples output from near-ultraviolet and blue light-emitting diodes (LEDs) to the subject’s volar forearm and collects the resulting skin fluorescence and diffuse reflectance. The sequentially-illuminated LEDs have peak wavelengths at 375, 405, 420, 435 and 460 nm. The optical radiation emitted from the skin is dispersed in a modified research-grade spectrometer.
and detected by a charge-coupled device (CCD) array. The optical exposure from SAGE was compared to the International Electrotechnical Commission (IEC) ultraviolet skin exposure limits [23]. Skin exposure from the screening device was a factor of 250 times smaller than the exposure limit. Hence, the risk of skin erythema or other damage due to optical radiation from the SAGE is negligible.

Melanin and hemoglobin are optical absorbers at the wavelengths of interest and reduce light amplitude and distort the skin’s spectral characteristics. In addition, subject-specific tissue characteristics such as wrinkles, dermal collagen concentration and organization, and hair follicles scatter light in the skin. Previous studies developed techniques that were applied in the prototype instrument to mitigate the impact of skin pigmentation, hemoglobin content and light scattering on the noninvasive measurement [18]. Also, skin AGEs accumulate naturally over time in all people. An algorithm compensated for patient age to remove this trend. Principal-components analysis (PCA) was applied to the spectra from 267 subjects with normal glucose regulation with ages ranging 22-85 years. PCA reduces the dimensionality of the data set, transforming the fluorescence spectra into eigenvalues and eigenvectors [24]. Linear regression determined the age-related slope of the eigenvalues. The age-dependence is then removed from all spectra to compensate for subject age. The pigmentation and age corrected spectra comprise the ‘intrinsic’ dermal fluorescence spectra.

Linear-discriminant-analysis (LDA) was applied to the intrinsic spectra to assess noninvasive disease classification performance [25]. In this method, the intrinsic dermal fluorescence spectra were first decomposed by PCA. From the resulting spectral scores, multidimensional spectral distances were determined. These distances (Mahalanobis distances) represent the effective distance of each spectra with respect to the normal (D0) and abnormal groups (D1). From the difference between the distances (D1 - D0), posterior probabilities ranging from 0 to 100 are computed. A posterior probability - the SAGE output value - represents a likelihood metric for that subject belonging to the abnormal class.

During each SAGE testing session, subjects were measured three times, lifting and replacing their arm into the cradle between measurements. In addition, subjects were tested by SAGE in two sessions in order to assess any effect due to subject fasting status. The first SAGE session always occurred in a fasting state. Approximately 60% of the study cohort received both FPG and OGTT during a single visit. For the remaining group, the OGTT was administered on a subsequent day. For all subjects, their second SAGE session occurred at least one hour after ingestion of the glucose load - near the anticipated peak of the acute blood glucose level due to the OGTT glucose bolus. Subject convenience dictated whether they participated via one or two visits. In all cases, subjects were in a non-fasting state during their second SAGE session. In principle, SAGE should be independent of fasting...
status since AGE concentration is not influenced by acute blood glucose levels. SAGE dependence on fasting status was evaluated by comparing classification performance stratified by first versus second session. Artifacts in the fluorescence spectra arising from subject movement or poor contact with the optical probe were identified by objective spectral outlier metrics. This quality control step rejected < 10% of the data set as ‘spectral outliers.' SAGE values (LDA posterior probabilities) were determined for all ‘clean’ spectra. Thus, the SAGE classification output contains multiple values per subject and the subsequent analysis includes this inherent measurement uncertainty. The redundant measurements also enable computation of the intra-subject variance.

To quantitatively assess the impact of skin coloration on the noninvasive classification performance, subject skin pigmentation was objectively quantified from diffuse reflectance measurements and classified into light and dark subgroups. Noninvasive disease classification performance was then evaluated for each subgroup. The screening performance of FPG, A1c and SAGE were assessed by comparing their respective sensitivities at a relevant clinical threshold. An appropriate comparative threshold for screening is the FPG threshold for impaired fasting glucose (IFG). All three tests were evaluated at the specificity corresponding to this FPG value (100 mg/dL).

**Results**

The OGTT identified abnormal glucose tolerance in 84 of the 351 subjects (23.9% prevalence). Of the 84 subjects with abnormal glucose tolerance, IGT was found in 55 subjects and frank type 2 diabetes in 29 subjects. Prevalence of abnormal glucose tolerance by age, gender and ethnicity is provided in Table 1. The table also details the specific numbers of subjects with normal and abnormal glucose tolerance (NGT and AGT) in these demographic categories. A comprehensive comparison of OGTT and FPG screening categorization is presented in Figure 1.

Using the normal vs. abnormal classification determined by OGTT, the receiver-operator characteristics (ROC) for FPG, A1c and SAGE were computed. The IFG threshold of 100 mg/dL corresponds to a FPG specificity of 77.4% - the critical specificity for comparing the tests. At 77.4% specificity, the FPG sensitivity was 58.0%, the A1c sensitivity was 63.8% and SAGE sensitivity was 74.7%. The test values corresponding to the critical specificity were 100 mg/dL for FPG, 5.8% for A1c and 50 for SAGE. The ROC plots are shown in Figure 2 and test performance is summarized in Table 2. The 95% confidence interval for SAGE sensitivity was 65.4% - 84%. Thus, the sensitivity differences between SAGE and both FPG and A1c are statistically significant (p < 0.05). The actual confidence interval differs from that estimated by the power calculations in the methods section, since the study found higher prevalence and increased SAGE sensitivity at the IFG-defined critical specificity. The absolute sensitivity advantage of the noninvasive device compared to FPG and A1c were 16.7 and 10.9 percentage points,
respectively. The relative sensitivity advantage for SAGE versus FPG was 28.8%, and for A1c the relative advantage was 17.1%. These values estimate the additional fraction of abnormal glucose tolerance subjects that are detected by SAGE but are missed by the conventional blood tests.

Alternatively, the tests can be compared via their equal error rate (EER) - the point toward the top-left corner of the respective ROCs where sensitivity and specificity are equal. The SAGE equal error rate was 24.1% (sensitivity = specificity = 75.9%) , while the EER for A1c and FPG were 27.7% and 32.2%, respectively.

The general performance metric of area-under-the-curve (AUC) shows a statistically significant advantage (p < 0.05) for SAGE (AUC = 79.7%) vs. the FPG (72.1%). The AUC values for SAGE (79.7%) vs. A1c (79.2%) were not statistically separable. The Hoorn coefficient of variation of the SAGE measurement, quantifying the inter-session reproducibility of the noninvasive instrument, was 9.4%.

SAGE performance was assessed for high and low melanin concentration sub-groups that were divided by their measured skin diffuse reflectance. At IFG threshold noted above (critical specificity = 77.4%), sensitivity for detecting abnormal glucose tolerance in subjects with lighter skin was 70.1%, while in those with darker skin it was 82.1%. Compared to the results for the entire cohort, the performance for sub-cohorts stratified by skin melanin content are not statistically different. In other words, SAGE sensitivity is not impaired by inter-subject skin melanin variations.

Classification performance was also stratified by subject fasting status. SAGE sensitivity for first session (fasting) was 78.4%, while the sensitivity for second session values (non-fasting) was 72.7%. The session-stratified sensitivities are not significantly different from that of the full cohort. Alternatively, the correlation coefficient between fasting and non-fasting SAGE measurements was r = 0.87 (p < 0.001). Consequently, the SAGE performance is independent of the ambient blood glucose level.

**Conclusions**

SAGE significantly out-performs FPG and A1c for detection of abnormal glucose tolerance. SAGE identified ~29% more individuals with undiagnosed abnormal glucose tolerance than FPG and ~17% more than A1c. In addition, SAGE provides rapid results and does not require fasting or blood draws – factors that are convenience barriers to opportunistic screening.

The low sensitivity for FPG detection of abnormal glucose tolerance reported here is not unexpected. A review of studies of FPG screening for undiagnosed diabetes is found that sensitivities ranged from 40 to 65% [10]. Since negative screening results are not subject to confirmatory testing, the large false-negative rate for FPG is a latent problem and contributes to the growing number of undiagnosed, ‘silent’ cases of type 2 diabetes.

The results presented here are consistent with the pathogenesis of abnormal glucose regulation, in which excessive post-prandial glucose levels accelerate accumulation of skin AGEs although fasting levels may remain normal. Since dermal AGEs represent
the integrated damage due to hyperglycemia, noninvasive measurement of these biomarkers is a promising means for early detection of abnormal glucose regulation. Given the increasing worldwide prevalence of type 2 diabetes and pre-diabetes, a move to earlier detection and treatment is necessary to help mitigate the diabetes epidemic. In the United States, if current trends continue the prevalence of diabetes is expected to more than double by 2025 and affect 15% of the population [26]. The recent estimate of $135 billion for annual diabetes-related healthcare costs in the United States means that the costs of the diabetes epidemics threatens to overwhelm the nation's healthcare system [27]. Fortunately, once detected, diabetes is now more treatable than ever before. Large clinical studies such as the DCCT and UKPDS have shown that tight control of glucose levels has significant health benefits to those with established diabetes [8,9]. Moreover, if pre-diabetes is detected and treated, progression to frank type 2 diabetes can be delayed or prevented. The DPP, FDPS and DREAM trials have shown that it is possible to prevent or at least delay the development of type 2 diabetes in patients with pre-diabetes [3-5]. This may be accomplished with aggressive diet and exercise modification and/or therapeutics such as metformin (DPP) and rosiglitazone (DREAM). The combination of accuracy and convenience of SAGE make it well-suited for opportunistic screening and earlier detection of diabetes and pre-diabetes. This noninvasive technology is a promising tool to facilitate early intervention for preventing or delaying the development of diabetes and its devastating complications.
References


21 Standards of Medical Care in Diabetes - 2006. Diabetes Care, 29(Supplement 1):S4-S42, 2006

22 The assays adhered to internal standard operating procedures, “CHEM-081: Glucose, Serum or CSF by Vitros Slide Technology” or “HEM-003: Hemoglobin A1C, Tosho G7.”


26 Barriers to Chronic Disease Care in the United States of America: The Case of Diabetes and its Consequences. Yale University Schools of Public Health and Medicine and the Institute for Alternative Futures, 2005

Table 1 - Summary of study demographics

<table>
<thead>
<tr>
<th>Age (yrs)</th>
<th>N</th>
<th>NGT</th>
<th>AGT</th>
<th>Prev (%)</th>
<th>Gender</th>
<th>N</th>
<th>NGT</th>
<th>AGT</th>
<th>Prev (%)</th>
<th>Race/Ethnicity</th>
<th>N</th>
<th>NGT</th>
<th>AGT</th>
<th>Prev (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>21-30</td>
<td>17</td>
<td>15</td>
<td>2</td>
<td>11.8</td>
<td>Male</td>
<td>128</td>
<td>100</td>
<td>28</td>
<td>21.9</td>
<td>Caucasian</td>
<td>187</td>
<td>149</td>
<td>38</td>
<td>20.3</td>
</tr>
<tr>
<td>31-40</td>
<td>52</td>
<td>44</td>
<td>8</td>
<td>15.4</td>
<td>Female</td>
<td>223</td>
<td>167</td>
<td>56</td>
<td>25.1</td>
<td>Hispanic</td>
<td>128</td>
<td>92</td>
<td>36</td>
<td>28.1</td>
</tr>
<tr>
<td>41-50</td>
<td>99</td>
<td>75</td>
<td>24</td>
<td>24.2</td>
<td>Total</td>
<td>351</td>
<td>267</td>
<td>84</td>
<td>23.9</td>
<td>African Am</td>
<td>11</td>
<td>10</td>
<td>1</td>
<td>9.1</td>
</tr>
<tr>
<td>51-60</td>
<td>88</td>
<td>71</td>
<td>17</td>
<td>19.3</td>
<td>Native Am</td>
<td>17</td>
<td>11</td>
<td>6</td>
<td>35.3</td>
<td>Asian</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>33.3</td>
</tr>
<tr>
<td>61-70</td>
<td>65</td>
<td>41</td>
<td>24</td>
<td>36.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>East Indian</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>71-80</td>
<td>22</td>
<td>14</td>
<td>8</td>
<td>36.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Other</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>50.0</td>
</tr>
<tr>
<td>81+</td>
<td>8</td>
<td>7</td>
<td>1</td>
<td>12.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

N: subject count per category; NGT and AGT: normal and abnormal glucose tolerance; Prev (%): prevalence of abnormal glucose tolerance (AGT/N)
Comparison of sensitivities for SAGE, FPG and A1c for detecting abnormal glucose tolerance. The FPG threshold for IGT (100 mg/dL) set the critical specificity (77.4%) for this comparison. Thresholds for each test at the critical specificity are indicated. The right section notes the performance advantage of SAGE over the two blood-based tests in terms of absolute and relative sensitivity.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity Threshold</th>
<th>SAGE Sensitivity Advantage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Absolute</td>
<td>Relative</td>
</tr>
<tr>
<td>SAGE</td>
<td>74.7%</td>
<td>50</td>
</tr>
<tr>
<td>FPG</td>
<td>58.0%</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>A1c</td>
<td>63.8%</td>
<td>5.8%</td>
</tr>
</tbody>
</table>
Figure 1 - Comparison of ADA-accepted, blood glucose screening tests: OGTT vs. FPG (n = 351). Dashed lines delineate screening state categories (e.g., NFG: normal fasting glucose; IFG: impaired fasting glucose, etc.). Category counts lay inside their respective axis. The solid box denotes the overlap of IFG and IGT categories (n = 24).
Figure 2 - Test performances for detecting abnormal glucose tolerance compared via receiver-operator-characteristic (ROC) plots. A1c results are depicted by the dotted line, the FPG by the dot/dash line and SAGE by the solid line. The open circles denote test performance at the critical specificity corresponding to the IGT threshold (FPG = 100 mg/dL). The error bars on the SAGE measurement indicate the 95% confidence interval in sensitivity.