



Development of a semi-quantitative tear film based method for public screening of diabetes mellitus

Brijesh Takkar^{a,*}, Sukhes Mukherjee^b, Ramesh Chand Chauhan^c, Pradeep Venkatesh^d

^a Department of Ophthalmology, All India Institute of Medical Sciences, Saket Nagar, Bhopal 462020, India

^b Department of Biochemistry, All India Institute of Medical Sciences, Saket Nagar, Bhopal 462020, India

^c Department of Community and Family Medicine, All India Institute of Medical Sciences, Saket Nagar, Bhopal 462020, India

^d Dr RP Centre for Ophthalmic Sciences, AIIMS, New Delhi 110029, India



ABSTRACT

Diabetes mellitus (DM) is a major health care burden associated with significant morbidity and serious impact on the quality of life. Estimating blood glucose levels is the currently employed method for screening for DM. Due to the invasive nature of access to blood glucose; new methods are being suggested that depend upon different targets than blood or another biochemical pathway altogether. But these are not cost effective and have inherent limitations related to public screening. We hypothesize a simple, non invasive and cheap paper strip method to estimate tear film glucose levels for screening purposes at community level. We also discuss the ideal properties of such a paper strip and the process of validation the technique should undergo before being employed for mass usage.

Introduction

Diabetes mellitus (DM) and its complications are a huge health care burden for all economies. The prevalence of the disease is predicted to double by thirty years in the current millennium, and affect nearly 366 million individuals by 2030 [1]. Given the enormous impact due to lack of effective primary prevention, insidious nature of the disease in majority of the cases, and severe irreversible complications that require lifelong therapies, screening for DM is the cornerstone for a community level intervention [1–3]. Though cost effective and non invasive features of screening methods have been stressed upon [3–6], majority of the developing and underdeveloped nations still don't have adequate and effective active screening protocols [1].

Diagnosis and management of DM and its complications are heavily reliant on determining the body fluid glucose levels and advanced glycation end (AGE) products. The traditionally available methods for detection of glucose in body fluids include glucose oxidase-peroxidase (GOD-POD) (enzymatic method) in clinical laboratories for serum detection, and the Clinitest method using the Benedict's reagent on urine. However, because of difficulty in accessing glucose and urine, and the need for biomedical waste disposal, these methods are far from ideal when one considers a community intervention. For such reasons newer methods are being researched. One of these methods is based on fluorescence spectroscopy, and determines the skin AGEs [4,6]. However the test is complicated by a table top apparatus and is optically intricate and expensive, making it inappropriate for rural or public

screening. Further there are limitations related to ambient temperature, need for cooling devices, consistency of subject posture and dark skin tone [4]. Other methods under evaluation include the infrared absorption spectroscopy [7], reverse iontophoresis through skin [8], metabolic heat measurement method [9], and bio-impedance technology [10]. Apart from the actual biochemical method of glucose detection, another strategy shift is change in target tissue being evaluated. These targets now vary from interstitial fluid and sweat, to saliva and breath [11]. Aqueous humor of the eye has also been evaluated with contact lenses [12], bio fuel cells and fluorescence based optical sensors [13]. All these methods are being promoted for home based continuous DM monitoring, and are highly sophisticated and expensive, requiring optimum study environment [11]. Therefore, they also may not be suitable for public screening purposes.

Recently, Mitsubayashi et al reported a review on the development of cavitas sensors for measuring glucose using tears as samples [14]. Tears appear as a promising biological sample for non-invasive diagnostics due to their easy accessibility and evidential correlation with blood glucose concentration levels [15]. In general, tear fluid is composed of more than 20 components that include minerals, proteins, glucose and metal ions, and most of these components are present in low concentrations [16]. The glucose concentration levels, for example, range from 0.1 to 0.6 mM [16]. In this way, many research groups have analyzed tears using fluorescence [17], mass spectrometry [18], electrochemical [19], and colorimetric detectors [20]. These detection methods have exhibited suitable sensitivity and high selectivity for tear

* Corresponding author.

E-mail addresses: britak.aiims@gmail.com (B. Takkar), sukhes.biochemistry@aiimsbhopal.edu.in (S. Mukherjee).

glucose analysis. In addition, the required sample volume in each mentioned system is considerably low (ca. 10 μL) [16]. All these features are advantageous for exploiting tear samples for non-invasive diagnostics while offering great potential in a clinical scenario. Yet, majority of these methods utilized in research needed invasive methods for actual tear acquisition. A single technique where both tear film acquisition and glucose measurement may be done in a non-invasive and inexpensive way would be very useful for large-scale community interventions.

Hypothesis

Herein, we hypothesize a new method for DM screening based on detection of tear film glucose levels using a paper strip impregnated with a suitable reagent. This method is semi-quantitative in nature and has the potential to identify, low, normal and high blood glucose levels.

Theory

Our hypothesis revolves around a sterile paper strip impregnated with a test reagent capable of reacting at the glucose levels present in tear film of normal and diabetic patients. This reagent may be coupled to a coloring reagent that shows change in color according to glucose concentration. Following appropriate analysis, the color change shall be proven to be associated to a specific range of tear glucose levels. As tear glucose levels are well known to fluctuate proportionately to serum glucose levels [15], a simple observation of color change on the paper can guide the observer to low, normal or high blood glucose levels. Thus, a semi quantitative test can be developed.

The reagent and the development of the strip

The strip may be designed in a simple format (25 mm \times 10 mm). It shall have 5 zones. Zone 1, or inlet zone, shall be the part that comes into contact with the tear lake or tear collection in fornix. Zone 2 shall be the transition zone that is intended to be junction of folding (Fig. 1). Zone 3 shall be a control zone intended to identify false negative

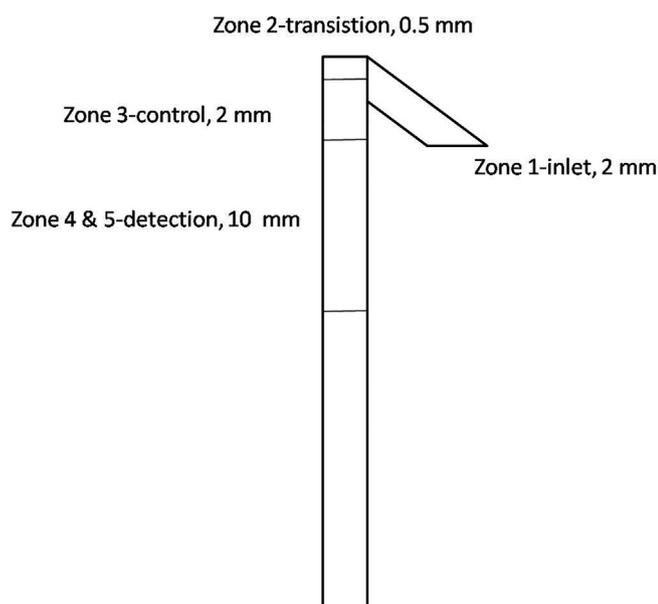


Fig. 1. A schematic representation of the proposed paper strip showing the 5 zones. Zone 1 is the reagent free inlet zone that shall be in contact with the tears in the conjunctival fornix. Zone 2 is the transition zone of folding over the eye lid. Zone 3 shall be the control zone not having the coloring reagent. Zone 4 and 5 shall be spotted with reagents responsible for biochemical reaction and colorimetric detection of glucose.

results. Zone 4 shall be the reagent rich detection zone of the strip that actually demonstrates the color change reaction, and zone 5 shall show deepening of color intensity. Color change in zone 5 would confirm adequate wetting of zone 4.

The paper strip or technically the micro fluidic paper-based analytical device (μPAD) can initially be modified with chitosan and linked with siloxane3-aminopropyltriethoxysilane (APTMS) as probe for colorimetric μPAD . First, a chitosan solution can be prepared in acid acetic. Then, a volume of 2.5 μL of this solution shall be added into the control and detection zones, and allowed to dry at room temperature for 15 min. Afterwards, the detection zone shall be spotted with a chromogenic solution composed of tetra-methyl-benzidine (TMB) and APTMS as a probe for colorimetric μPAD , followed by addition of an enzymatic mixture containing GOx and HRP prepared in buffer solution. The control zone shall be spotted only with the enzymatic solution. Colorimetric detection following the procedure of the test may be performed with scanners during validation and manually during community exposure.

Procedure of the test

The actual test can easily be performed by either trained nursing personnel, or a relative of the subject, or even by the subject himself. The folded part of the sterile strip shall be inserted into the lower conjunctival fornix (much like the paper strip used commonly for evaluating dry eye) [21]. This part of the paper shall not have any reagent on it to prevent introduction of irritants into the fornix. The strip shall acquire tears by capillary action, which shall then wet the remaining zones of the paper strip leading on to the biochemical color coded reaction which shall be read by the observer later.

Test interpretation

The time required for exposure of the strip to tears can be judged after adequate experiments, but is expected to be less than half a minute in normal tear abundant individuals if one considers the pattern of wetting of the Schirmer's strip in normal individuals [21]. Following the chemical reaction, the color change can be read by the observer. A color coded chart shall be provided with the strips for comparison and understanding as to what color of the strip corresponds to what range of blood glucose.

Discussion

The tear film has lower glucose concentrations than blood [14,15], and therefore the ability of the test to function at low glucose concentrations is a concern with regards to our hypothesis. Gabriel et al showed that the modification of the paper surface with chitosan promotes noticeable improvements in the sensitivity and detection levels. The improvement in terms of color intensity is associated with the formation of chitosan films on cellulose fibers with APTMS acting as probe for the colorimetric μPAD . This creates a suitable environment for the fast electron transfer between the enzyme and sensing surface. The use of a chromogenic solution composed of 4-AAP and DHBS (4 mM:8 mM) had provided a limit of detection (LOD) value of 23 μM in that study, one of the lowest LODs reported for glucose using μPADs [20]. Such an enhanced analytical performance will allow the detection of glucose in human tears.

Steps to validation of utility

In this current scenario, the main goal is to develop a simple cost effective strip for measuring glucose levels in tear samples using colorimetric detection. This novel method proposed by us is likely to be advantageous in being cheap, easy to use, non-invasive and repeatable. The observer training required for its usage is also likely to be minimal.

The only burden of bio-medical waste disposition shall be that of the paper itself and no body fluid as such, and therefore the proposed method shall perform much better than methods dependent on needles, blood or urine. However before putting its use into practice, the test needs to undergo multiple steps of scientific validation.

Following production of such a paper strip, the acceptability, repeatability and validity of the test would be evaluated through a community based validation study. The test will be assessed for observer variation (intra-observer and inter-observer) and biological variations, and errors related to technical methods will be recorded. The validity of the test (sensitivity, specificity, positive predictive value and negative predictive value of the test) will be calculated. Next, the test should be compared with the standard diabetes screening methods in practice and various benefits including cost effectiveness should be assessed.

To conclude, we have suggested a potentially simple, non invasive and cost effective semi-quantitative way of glucose detection for community screening of DM. If proven safe and efficacious, it may find immense use in basic health care systems in resource limited settings.

Disclosures

None of the authors reports any conflicts of interest, funding source or acknowledgments.

Source(s) of support

The authors have not received grant support or research funding and do not have any proprietary interests in the materials described in the article.

Presentation at a meeting

Nil.

Acknowledgement

Nil.

References

- [1] Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. *Australas Med J* 2014;7:45–8.

- [2] Unnikrishnan RI, Anjana RM, Mohan V. Importance of controlling diabetes early—the concept of metabolic memory, legacy effect and the case for early insulinisation. *JAPI (Suppl)* 2011;50:8–12.
- [3] Chatterjee R, Narayan KM, Lipscomb J, et al. Screening for diabetes and prediabetes should be cost-saving in patients at high risk. *Diabetes Care* 2013;36:1981–7.
- [4] Mohan V, Rani CS, Regin BS, et al. Noninvasive type 2 diabetes screening: clinical evaluation of SCOUT DS in an asian indian cohort. *Diabetes Technol Ther* 2013;15:39–45.
- [5] Peterson S, Sheffer S, Roth SL, Bennett PA, Lloyd L. Noninvasive screening for risk factors of type 2 diabetes in young, rural, caucasian children. *J Sch Nurs* 2010;26:301–9.
- [6] Maynard JD, Rohrscheib M, Way JF, et al. Noninvasive type 2 diabetes screening: superior sensitivity to fasting plasma glucose and A1C. *Diabetes Care* 2007;30:1120–4.
- [7] Kasahara R, Kino S, Soyama S, Matsuura Y. Noninvasive glucose monitoring using mid-infrared absorption spectroscopy based on a few wavenumbers. *Biomed Opt Exp* 2017;9:289–302.
- [8] Tamada JA, Garg S, Jovanovic L, et al. Noninvasive glucose monitoring: comprehensive clinical results. *JAMA* 1999;282:1839–44.
- [9] Cho OK, Kim YO, Mitsumaki H, Kuwa K. Noninvasive measurement of glucose by metabolic heat conformation method. *Clin Chem* 2004;50:1894–8.
- [10] Geng Z, Tang F, Ding Y, Li S, Wang X. Noninvasive continuous glucose monitoring using a multisensor-based glucometer and time series analysis. *Sci Rep* 2017;7:12650.
- [11] Bruen D, Delaney C, Florea L, Diamond D. Glucose sensing for diabetes monitoring: recent developments. *Sensors* 2017;17:1866.
- [12] Reid RC, Minter SD, Gale BK. Contact lens biofuel cells tested in a synthetic tear solution. *Biosens Bioelectron* 2015;68:142–8.
- [13] Badugu R, Lakowicz JR, Geddes CR. Ophthalmic glucose monitoring using disposable contact lenses—A review. *J Fluoresc* 2004;14:617–33.
- [14] Mitsubayashi K, Arakawa T. Cavitas sensors: contact lens type sensors & mouth-guard sensors. *Electroanalysis* 2016;28:1170–87.
- [15] Baca JT, Finegold DN, Asher SA. Tear glucose analysis for the noninvasive detection and monitoring of diabetes mellitus. *Ocul Surf* 2007;5:280–93.
- [16] Witkowska NE, Kundys M, Jeleń PS, Jönsson-Niedziółka M. Electrochemical glucose sensing: Is there still room for improvement? *Anal Chem* 2016;88:11271–82.
- [17] Badugu R, Lakowicz JR, Geddes CD. Fluorescence sensors for monosaccharides based on the 6-methylquinolinium nucleus and boronic acid moiety: potential application to ophthalmic diagnostics. *Talanta* 2005;65:762–8.
- [18] Baca JT, Taormina CR, Feingold E, Finegold DN, Grabowski JJ, Asher SA. Mass spectral determination of fasting tear glucose concentrations in nondiabetic volunteers. *Clin Chem* 2007;53:1370–2.
- [19] Andoralov V, Shleev S, Arnebrant T, Ruzgas T. Flexible micro(bio)sensors for quantitative analysis of bioanalytes in a nanovolume of human lachrymal liquid. *Anal Bioanal Chem* 2013;405:3871–9.
- [20] Gabriel EFM, Garcia PT, Cardoso TM, Lopes FM, Martins FT, Coltro WKT. Highly sensitive colorimetric detection of glucose and uric acid in biological fluids using chitosan-modified paper microfluidic devices. *Analyst* 2016;141:4749–56.
- [21] Korb DR. Survey of preferred tests for diagnosis of the tear film and dry eye. *Cornea* 2000;19:483–6.