Cardiometabolic Risk Screening using Simple and Convenient Dried Blood Spot
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Abstract

Background: Cardiovascular and metabolic risk factors are gaining more attention as potential targets of intervention in the prevention and treatment of cardiovascular disease and type 2 diabetes mellitus.  Additionally, patients with high cardiovascular and metabolic conditions have increased risk of adverse events. In this study, we present an improved high through screening method for estimation of insulin, high-sensitivity CRP, triglycerides and hemoglobin A1c in dried blood spot. As insulin resistance and CRP are known to be associated with morbidity and mortality, measurement of CRP levels along with insulin may help in the assessment of cardiovascular risk.  The role of triglycerides as risk factor in cardiovascular and coronary artery disease has been stressed by several investigators.  ADA has suggested that HbA1c is a better screening tool for diabetes and prediabetes than oral glucose.

Methods

Bloodspot Insulin
Fasting and non-fasting healthy subjects contributed a blood spot sample from a finger-stick and a blood sample through venipuncture for serum isolation. The blood spots obtained from finger sticks were air-dried at room temperature after collection and stored at -20°C for 24 hr of collection. Serum was stored frozen at -20°C. Standards and controls for blood spots were prepared by mixing washed red blood cells 50:50 with insulin kit standards and controls and drying on blood spot filter paper.  Serum and rehydrated blood spots were tested in parallel for insulin using the commercial ELISA kits from DRG.

Bloodspot hs-CRP
Serum and dried blood spots from volunteers were collected and stored as explained above. One 6.0 mm disk of dried blood spot was punched and 200 µL of extraction buffer was added and incubated at room temperature for two hours. A 20 µL of extracted sample and 100 µL of CRP enzyme conjugate from the assay kit was incubated at room temperature for 45 minutes in a 96 well plate. After washing with distilled water, 100 µL of auto-TMB solution was added and incubated for 20 minutes. The reaction was stopped by adding 100 µL of stop solution and the plate was read at 450 nm within 15 minutes.

Bloodspot Triglycerides
Bloodspot extract was prepared by punching out two 6.0 mm disks from bloodspots and 200 µL of methanol was added to each well.  The plate was then incubated for 2 hours at 37°C.  Triglycerides were measured by modifying the method of Qurashi et al. 50 µL of standards, controls or blanks were added to 250 µL of triglyceride reagent (Randox) and incubated at 37°C for 10 minutes.  The absorbance was read using the Wallac Victor2 Multiplate Counter set to 490 nm.  Serum triglyceride levels were determined using the same assay kit.

Hemoglobin A1c
The method is based on the interaction between antigen and antibody to directly determine the HbA1c in extracted blood from paper disks. HbA1c in blood is known to bind to latex particles. Therefore, a latex-HbA1c/mouse anti-human HbA1c antibody complex is formed when mouse anti-human HbA1c monoclonal antibody is added. Further, addition of goat anti-mouse IgG polyclonal antibody forms an agglutination by interacting with the monoclonal antibody. The concentration of HbA1c is proportional to the total amount of agglutination. The amount of agglutination is measured as absorbance. The HbA1c value is obtained from a calibration curve. One large spot was punched using the multipuncher and 500 µL of lys buffer was added. The extraction plate was incubated for 2 hours at RT.  Twenty microliters of this hemolysate was incubated with anti-human HbA1c monoclonal antibody for five minutes at 37°C followed by addition of 60 µL goat anti-mouse IgG polyclonal antibody. Microtiter plate was incubated at 37°C for 5 minutes and read at 620 nm.

Results

Discussion

Cardiovascular and metabolic factors are gaining attention as potential targets of intervention in the prevention and treatment of cardiovascular disease and type 2 diabetes mellitus.  New tools will enable clinicians to formulate treatment strategies for prevention and management of cardiovascular and metabolic conditions.  In this study, we present an improved high through screening method for estimation of insulin, high-sensitivity CRP, triglycerides and hemoglobin A1c in dried blood spot. As insulin resistance and CRP are known to be associated with morbidity and mortality, measurement of CRP levels along with insulin may help in the assessment of cardiovascular risk.  The role of triglycerides as risk factor in cardiovascular and coronary artery disease has been stressed by several investigators.  ADA has suggested that HbA1c is a better screening tool for diabetes and prediabetes than oral glucose.

Conclusion

The stability, efficient recovery, and excellent correlation between levels of these markers in dried bloodspot and serum makes high-sensitivity CRP and convenient tool for screening of cardiometabolic risk factors.  A bloodspot panel consisting of fasting insulin, hs-CRP, HbA1c and triglycerides along with blood pressure and cholesterol level assessments is recommended for a population at risk and also for those with diagnosed cardiovascular disease or type 2 diabetes mellitus.

References