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SYMPOSIUM

Utility of Dried Blood Spots for Measurement of Cholesterol and Triglycerides in a Surveillance Study

Ramakrishnan Lakshmy, Ph.D.,[1] Ruby Gupta, Ph.D.,[1] Dorairaj Prabhakaran, D.M.,[2] Uma Snehi, MBBS,[2] and K. Srinath Reddy, D.M.[3]

Abstract

Background:

Developing countries are facing a rise in noncommunicable diseases (NCD), which is a cause for concern. The World Health Organization has recommended a stepwise approach for NCD risk factor surveillance. Screening for risk factors in remote populations is difficult due to lack of resources and technical expertise, including standardized laboratory facilities. The collection of samples on filter paper for the assessment of risk factors circumvents the need for blood processing, storage, and shipment at ultralow temperatures.

Method:

Samples were collected on 3-mm Whatman filter paper from one industry (National Thermal Power Corporation) located in the periphery of Delhi as part of a surveillance carried out in industries from different parts of India. Total cholesterol was measured in serum and dried blood by the cholesterol oxidase/*p*-aminophenazone method and triglycerides by the glycerophosphate oxidase–peroxidase/aminophenazone method. Values obtained by the two methods were compared using Pearson correlation, and Bland–Altman plots were prepared to assess bias.

Results:

The correlation coefficient "*r*" was 0.78 for cholesterol and 0.94 for triglycerides between dried blood spots and serum. Bland–Altman plots suggest that differences in values obtained by the two methods were within two standard deviations for most of the samples.

Conclusions:

Blood samples dried on filter paper can be a successful option for population screening in remote areas, provided preanalytical variations arising due to the method of blood spot preparation and storage are well controlled.

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Author Affiliations: [1]Department of Cardiac Biochemistry, All India Institute of Medical Sciences, New Delhi, India; [2]Center for Chronic Disease Control, New Delhi, India; and [3]Public Health Foundation of India, New Delhi, India

Abbreviations: (NCD) noncommunicable diseases, (SD) standard deviation

Keywords: cholesterol, dried blood spots, risk assessment, triglycerides

Corresponding Author: Ramakrishnan Lakshmy, Ph.D., Department of Cardiac Biochemistry, All India Institute of Medical Sciences, New Delhi, India; email address *lakshmy_ram@yahoo.com*

Introduction

Rising premature morbidity and mortality due to non-communicable diseases (NCD), including cardiovascular diseases, diabetes, obesity, cancers, and chronic lung diseases, are growing concerns in developing countries. Screening for risk factors for NCD in a country is advocated to ascertain the burden of these diseases in the population. The World Health Organization has recommended a three-step approach for NCD risk factor surveillance: (1) gather information through questionnaires, (2) ascertain simple physical measurements, and (3) collect blood sample(s).[1] The measurement of total cholesterol and triglycerides has been recognized as important risk factors for cardiovascular diseases.[2,3] Due to complexities of the measurement involved, biochemical analysis is not undertaken in all developing countries. In a large country such as India, biochemical analysis at remote corners of the country is difficult because of limited resources and technical capacity. Measurement in a good quality central laboratory would be ideal, but the cost and safety of chilled sample transportation are concerns. Transportation of samples in the form of dried blood would circumvent the need for blood processing, storage, and shipment at ultralow temperatures.

A surveillance study was undertaken to assess the risk factors for cardiovascular disease in an Indian industrial population.[4–6] Local laboratories located in close proximity to the industry carried out the analysis. Fasting plasma glucose, total cholesterol, high-density lipoprotein–cholesterol, and triglyceride levels were assessed in the study. The feasibility of using dried blood was evaluated simultaneously in one of the industries located in the periphery of Delhi. The measurement of total cholesterol and triglycerides from dried blood was performed in the Department of Cardio Biochemistry located in the All India Institute of Medical Sciences, New Delhi. Methods for the measurement of cholesterol and triglycerides from dried blood have been reported previously by our group.[7–9] This article presents results of total cholesterol and triglyceride values obtained from dried blood as compared to values obtained in serum.

Methods

A cross-sectional survey was carried out in 11 industries from different parts of India. Males and females between 10 and 64 years old were included in the study from each industry. Biochemical analysis was carried out only in individuals between 20 and 64 years of age from each industry. For quality assurance, three levels of quality checks were exercised in the surveillance study—internal quality control, external quality control, and 10% repeat analysis.[10] At the time of sample collection by venipuncture, for every 10th person, samples were also collected on filter paper from one industry located in the periphery of Delhi. A protocol for the collection of blood on filter paper was prepared and circulated to the participating industry. As per protocol, 10 blood spots per subject were required to be collected on 3-mm Whatman paper, taking the following aspects into consideration: pricking with disposable lancets, wiping of the first drop of blood using a sterile swab, waiting for a large drop of blood to form, allowing the drop to fall on one side of the filter paper without any contact between finger and paper, ensuring uniform penetration, keeping for 2–3 hours at room temperature to allow the spot to dry completely, transferring to resealable bags, and storing at 4°C. Filter papers were transported to a central laboratory in ice packs. Filter papers were stored at 4°C prior to analysis for a minimum of 1 month and a maximum of 6 months.

Total cholesterol was measured by the cholesterol oxidase/*p*-aminophenazone method and triglycerides by the glycerophosphate oxidase–peroxidase/aminophenazone method using enzymatic kits from Randox Laboratories Ltd., United Kingdom. Total cholesterol and triglycerides in serum were estimated in the laboratory affiliated with the industry on a semiautoanalyzer (RA-50, Bayers) using the standard procedure described by the kit manufacturer (Bayers Diagnostics, Germany).

For dried blood lipid measurement, one disk was punched out and put in a tube with a Teflon screw cap. One hundred microliters of methanol (analytical grade, Qualigens, Glaxo Limited, India) was added to the tube. Tubes were incubated at 37°C for 2 hours, with shaking at 100 rotations per minute in an Environ Shaker (Lab Line Inc., Illinois). For the measurement of total cholesterol and triglycerides in the eluate, 20 µl of the extract was placed into a microtiter plate and 200 µl of the commercially available enzymatic reagent was added. The reaction mixture was stirred on a vortex mixer with a microplate attachment, incubated at 37°C for 15 minutes, and then measured at 540 nm on a microplate reader (Rayto Inc., China) using a whole blood zero standard as the blank. To minimize matrix differences and maximize comparability between calibrators and test samples,

dried blood spot standards and controls were prepared by mixing washed red blood cells with the cholesterol and triglyceride standard provided with the enzymatic kit and three levels of quality control. The lipid standard was diluted serially in normal saline, and washed erythrocytes were mixed in 50:50 (v/v) proportions to get whole blood calibrators. Blood-based quality controls were prepared similarly by adding washed erythrocytes in 1:1 dilutions.

Statistical Analysis

Pearson correlations were computed using SPSS version 16.0 (SPSS Inc., Chicago, IL). Bland–Altman plots were prepared to ascertain bias.

Results

Cholesterol values in the 85 samples analyzed in dried blood ranged from 104 to 351 mg/dl. Fifty-six samples had values ≤200 and 29 samples had cholesterol values >200 mg/dl. The mean [standard deviation (SD)] cholesterol value obtained from dried blood was 194.35 (39.12) mg/dl and the mean cholesterol from corresponding serum was 186.67 (30.11) mg/dl. Triglyceride values in the 85 samples ranged from 69 to 342 mg/dl with 57 values less than 150 mg/dl. The mean values obtained with dried blood and serum was 140.62 (50.34) and 129.71 (55.04) mg/dl, respectively. **Figure 1** shows the scatter plot of all values of cholesterol and triglycerides obtained from dried blood and serum. A correlation coefficient of 0.78 for cholesterol and 0.94 for triglycerides was evident between dried blood and serum. Bland–Altman plots suggest that the difference in values obtained by the two methods was within the 2 SD limits for most of the samples (**Figures 2** and **3**). Less than 5% of the values were outside the 2 SD limits.

Discussion

Blood spotted on filter paper has been found to be suitable in large-scale population screening programs for a number of assays.[11–17] In the present study, dried blood collected in the field in a community-based study was evaluated for its suitability for cholesterol and triglyceride estimations. Cholesterol and triglyceride measurements from dried blood have been reported previously under controlled laboratory conditions.[7–9] The collection of blood samples by finger pricks from subjects living in places with limited resources and transportation to a distant laboratory will be useful for screening with a wider reach. However, the suitability

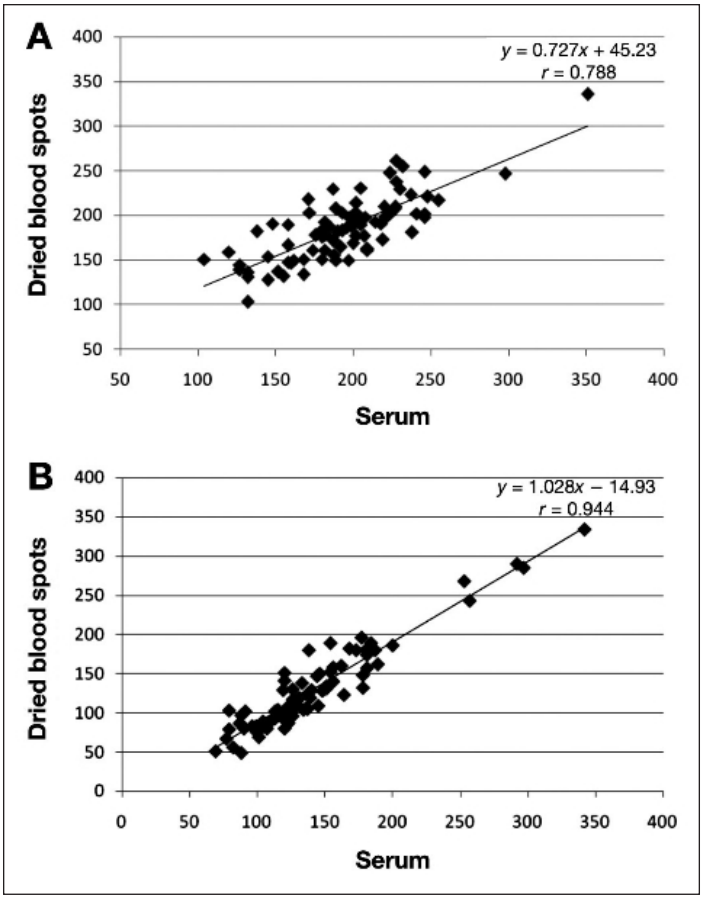

Figure 1. Scatter plot of cholesterol **(A)** and triglycerides **(B)** from serum and dried blood.

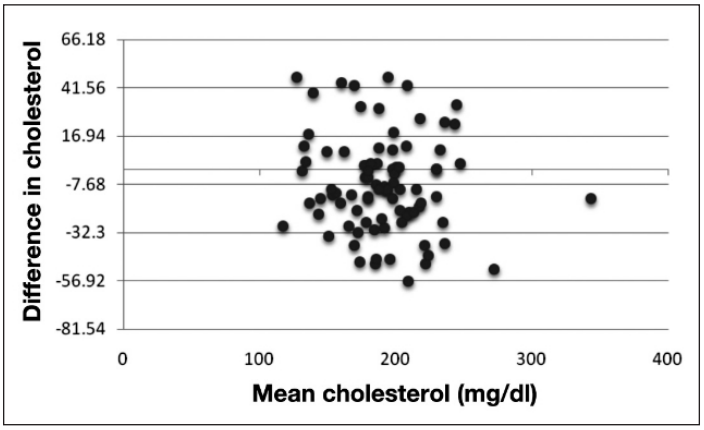

Figure 2. Bland–Altman plot of differences in cholesterol values between dried blood and serum.

of dried blood for the screening of cholesterol and triglycerides needs evaluation under field conditions. Blood collection in the field is difficult in a developing country such as India where technical expertise in remote places may be limiting. Dried blood would be of great utility in remote locations where facilities

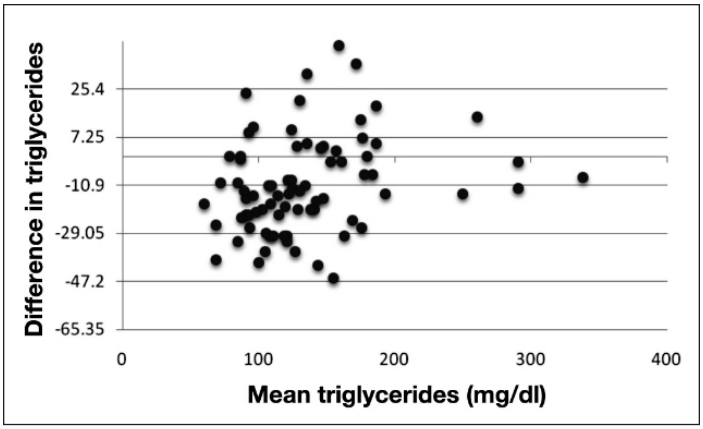

Figure 3. Bland–Altman plot of differences in triglyceride values between dried blood and serum.

for centrifugation are not accessible. The use of dried blood for the measurement of total cholesterol and triglycerides in epidemiological studies has not been described before. Starck and Lovegren[18] used 4°C stored blood specimens for 5-14 years for the analysis of sterols. They did not look at sterol levels in fresh samples and therefore could not evaluate the degree of degradation with storage. Kapur and colleagues[19] reported the usage of dried blood for cardiometabolic risk factors, including triglyceride screening in patients at high risk. The dried blood collection in this study was also under controlled laboratory conditions.

We found a reasonably good correlation between dried blood and serum for total cholesterol and triglyceride measurement. The extraction and analysis of cholesterol and triglycerides from filter paper depended on the quality of blood spots prepared. Unlike in controlled laboratory conditions, the collection and transportation of blood on filter paper in the field were beset with several caveats. The following shortcomings were noted in the collection of blood spots: spots were too small in some instances and disks could not be punched out for extraction. Blood was spotted twice at the same spot in some samples. Filters were not dried properly prior to putting inside the resealable bags. The Ziploc bags were not closed tightly; as a result, moisture entered inside. Filter papers were not labeled properly. Because of the aforementioned factors, the coefficient of variation in dried blood measurement was high, which is likely to affect the value of the assay in screening. For the dried blood approach to be successful in field conditions, variations in measurements due to these factors would need to be minimized. A more rigorous training of field staff and preparation and circulation of an instruction manual with pictorial representations of do's and don'ts may help in minimizing these preanalytical variations.

We have previously evaluated the stability of dried blood for the measurement of cholesterol and triglycerides and found stability for up to 1 month at room temperature and up to 2 months at 4°C.[9] Some of the dried blood spots were stored for 6 months at 4°C prior to analysis. Because temperature and humidity conditions vary in different parts of the country, these may also have an important bearing on the quality of blood spots. Minimization of preanalytical variations with proper collection and storage of blood would be an important determinant for the success of mass screening of total cholesterol and triglycerides using dried blood for risk factor assessment.

Conclusion

We have demonstrated a positive correlation in levels of both cholesterol and triglycerides as determined in serum and dried blood spots collected under field conditions. The utility of dried blood for the measurement of cholesterol and triglycerides from remote places in epidemiologic study needs to be assessed with more studies, as temperature and humidity conditions vary in different parts of the country.

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