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ORIGINAL ARTICLE

DRIED BLOOD/SERUM SPOT TOTAL CHOLESTEROL ESTIMATION AS AN ALTERNATIVE TO FRESH SERUM TOTAL CHOLESTEROL: AN ANSWER OR A QUESTION IN ITSELF?

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ABSTRACT: INTRODUCTION: Surveillance for risk factors of heart diseases, like increased blood sugar, cholesterol becomes difficult in places with inadequate lab facilities due to difficulty in sample collection, transportation and processing. Feasibility of using dried blood/serum spots in such situations can be thought of as an alternative to fresh serum total cholesterol, as sample collection does not require much expertise. **AIM:** The aim of this study is to determine whether dried blood and serum spots can be used as an alternative to fresh serum for estimation of total cholesterol in field studies. **METHODS:** Fresh serum from 100 subjects selected randomly was used for estimation of total cholesterol. Four spots each of blood and serum from these samples were made on Whatman paper 3, out of which one spot from each were analysed on the day of collection and rest were kept in resealable bags to be analysed on day 7, 15 and 30. The correlation between the dried blood/serum spot values with the fresh serum total cholesterol values was examined. **RESULTS:** A significant correlation with fresh serum total cholesterol values (p value<0.01) was seen for dried serum spots stored up to day 30(0.747 to 0.942) and for dried blood spots stored up to day 15(0.598 to 0.949). **CONCLUSIONS:** Total cholesterol estimated by dried blood spot up to 15 days and dried serum spots stored up to 30 days can be considered as an alternative to fresh serum for estimation of serum total cholesterol.

KEYWORDS: Dried Blood Spot testing, Dried Serum spot, Serum cholesterol.

INTRODUCTION: Non Communicable Diseases (NCDs) comprise a very complex picture of co morbidities with high prevalence of risk factors among the population and the linkage of NCDs with nutrition, smoking, sedentary life. Coronary Heart disease (CHD) among these NCDs is the leading cause of death and a major cause of morbidity worldwide.

'Risk' is defined as a probability of an adverse health outcome, whereas 'risk factor' refers to an attribute or characteristic or exposure of an individual whose presence or absence raises the probability of an adverse outcome.[1] One of the major risk factors of coronary heart disease is increased serum cholesterol. A 10% reduction in serum cholesterol in men aged 40 years results in a 50% reduction in heart disease within five years, while a 20% reduction in heart disease occurs within five years in men aged 70 years.[2]

Hence it becomes essential to control this risk factor in order to bring down the incidence of coronary heart diseases. The first step in bringing down the occurrence or the severity of a risk factor is to know the presence of that risk factor.

It is not possible to improve health status of the people without help from different sectors involved in addressing issues pertaining to health directly or indirectly. A mixture of services not only

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from the medical and non-medical staff but also from the society is required for a wholesome management of risk factors from identification to reduction or control of the same.

The World Health Organization gives the STEPS protocol for risk factor assessment of cardiovascular diseases which recognises three steps, Step 1 being questions on sociodemographic data like age, sex, economic and education status, Step 2 being Physical measurements like Height, weight, Body Mass Index, Step 3 Biochemical measurements like Blood Sugar, Serum Cholesterol, Serum Triglycerides, Serum LDL cholesterol.[3]

Risk factors like sociodemographic history, smoking, alcohol consumption, increased body weight, waist circumference are simple to assess due to the ease in measurement, less economic burden, ease in training non-medical staff for measurement. But in developing nations, the measurement of Biochemical risk factors like increased blood sugar, increased serum lipids like total cholesterol, serum triglycerides, serum LDL cholesterol, serum HDL cholesterol takes a back seat because of reduced coverage areas by laboratories with good quality services, low economic support, limited resources which causes difficulty in collection, transportation and processing of samples. The question here is, if there is any alternative which gives a solution for these shortcomings.

Hence the purpose of this study was to determine whether dried blood/dried serum spots can be used as an alternative to serum total cholesterol estimation in large field studies.

MATERIALS & METHODS: A cross sectional study was initiated after approval from institutional ethics committee.100 samples were selected from subjects walking into central laboratory with requisition for serum total cholesterol. Four blood spots from each sample, corresponding to 10 microlitre were made on whatman filter paper no 3. The rest of the sample was centrifuged. Fresh serum was tested for total cholesterol and four serum spots each corresponding to 10 microlitres were made on whatman filter paper no 3 and dried. After drying for 3-4 hours, one dried spot each of blood and serum were analyzed after elution, on the day of collection. The three filter discs of dried blood and dried serum were kept in separate resealable bags at room temperature. These dried spots were eluted for estimation on day 7, day 15 and day 30. For dried blood total cholesterol measurement, one disc corresponding to 10 µl of blood based standard, sample and blank were cut and put in test tube with screw caps and 1ml methanol (Analytical grade) was added and incubated at 37^0C for two hours coupled with centrifugation at 3500 rpm for 15 minutes. 500 µl of the extract was added to 1 ml of the commercially available enzymatic reagent kit. The colour development was read after 30 minutes at 500nm.

MEASUREMENTS: Total cholesterol was measured by the cholesterol oxidase/p-aminophenazone method using enzymatic kits from Randox Laboratories Ltd., United Kingdom. Total cholesterol in serum was estimated in the laboratory on a autoanalyzer (Erba 300 XL).

STATISTICAL ANALYSIS: Pearson correlations were computed using SPSS version 15.0 (SPSS Inc., Chicago, IL).

RESULTS: Dried blood and serum spots were collected from 100 samples. In case of dried blood spots at room temperature it was seen that there was strong positive correlation between those values and original values on Day 1 but this correlation comes down moderate positive correlation on

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day 7 and day 15. On day 30 the correlation coefficient dips down to 0.300 giving a weak positive correlation. In case of serum spots there was strong positive correlation on day 1, 7, 15 which comes down to moderate positive correlation on day 30(0.747). Hence to summarize, a significant correlation with fresh serum total cholesterol values (p value<0.01) was seen for dried serum spots stored at room temperature up to day 30(0.747 to 0.942) and for dried blood spots stored at room temperature up to day 15(0.598 to 0.949).

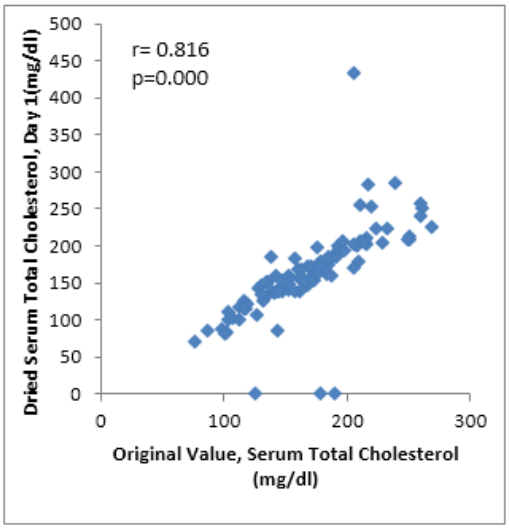

Fig. 1: Scatter plot of Total Cholesterol from dried serum on day 1

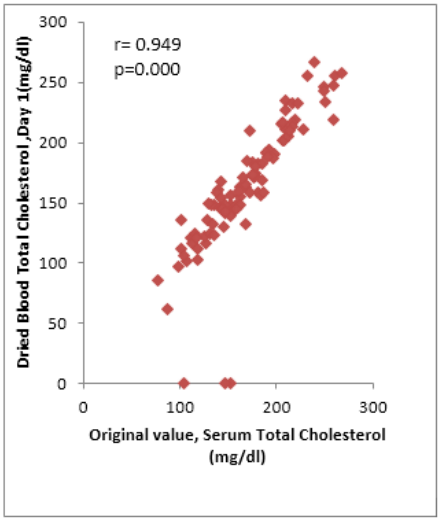

Fig. 2: Scatter plot of Total Cholesterol from Dried Blood on day 1

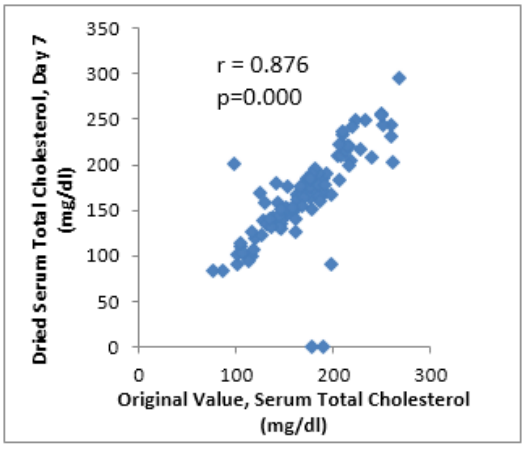

Fig. 3: Scatter plot of Total Cholesterol from Dried Serum on day 7

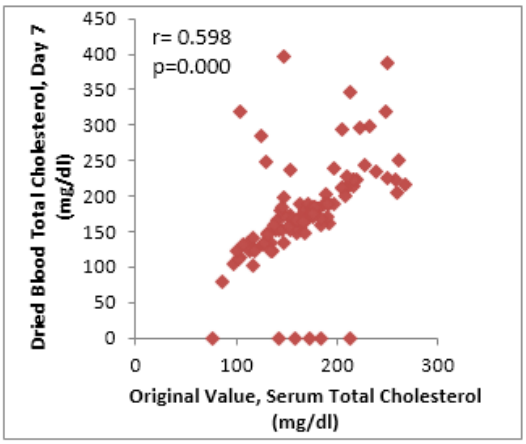

Fig. 4: Scatter plot of Total Cholesterol from Dried Blood on day 7

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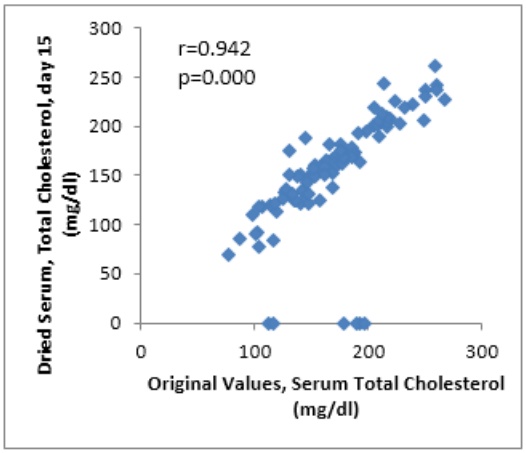

Fig. 5: Scatter plot of Total Cholesterol from Dried Serum on day 15

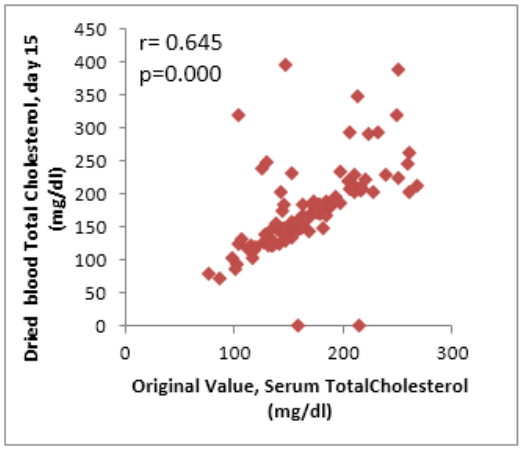

Fig. 6: Scatter plot of Total Cholesterol from Dried Blood on day 15

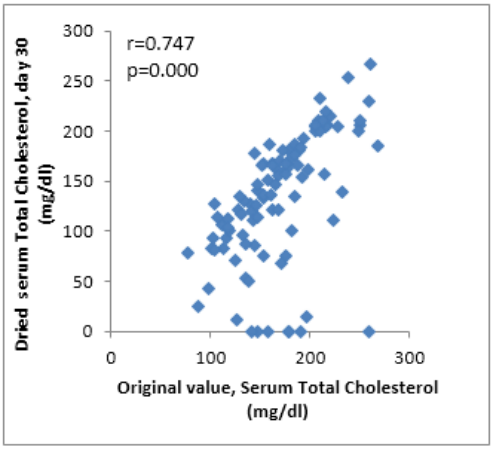

Fig. 7: Scatter plot of Total Cholesterol from Dried Serum on day 30

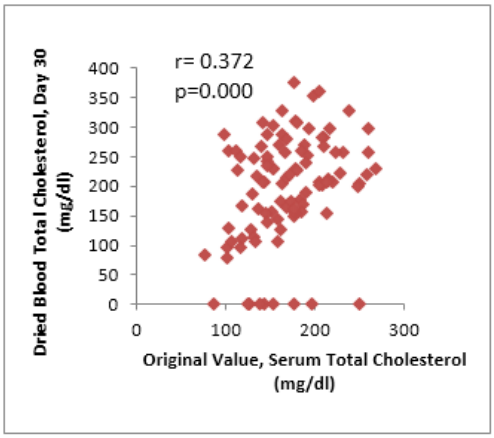

Fig. 8: Scatter plot of Total Cholesterol from Dried Blood on day 30

Original Vs	Correlation	P value
Day 1	0.816(**)	0.000
Day 7	0.876(**)	0.000
Day 15	0.942(**)	0.000
Day 30	0.747(**)	0.000

Table 1: Correlation Coefficients Dried Serum spots with original values

** Correlation is significant at the 0.01 level

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Original Vs	Correlation	P value
Day 1	0.949(**)	0.000
Day 7	0.598(**)	0.000
Day 15	0.645(**)	0.000
Day 30	0.372(**)	0.000

Table 2: Correlation Coefficients Dried Blood spots with original values

** Correlation is significant at the 0.01 level

DISCUSSION: The most prominent use of dried blood samples for clinical laboratory purposes was when Dr. Robert Guthrie developed an assay for the detection of phenylketonuria. His application for collecting blood on filter paper led to the population screening of new-borns for the detection of inherited metabolic diseases.[4] Today, collection of blood onto paper has become a significant tool helpful in the diagnosis of a number of common medical conditions.

Simplicity of the technique for collection, storage and transport of the samples has led to increased adoption of this methodology. Reduced complications in sample collection, storage, and transportation make blood spot a good option for large population-based surveys.

Fairly good correlation between dried blood and serum for total cholesterol and triglyceride measurement have been found in previous studies.[5,6] But, in these studies micro titre plates, shakers have been used which have been replaced by use of vortex and centrifuge. In this study it was seen that dried blood and serum spot could be used as an alternative to fresh serum for total cholesterol estimation. Dried blood spots could be used up to 15 days at room temperature and dried serum could be used up to 30 days according to this methodology. The limitations of the study was that the effects of moisture, humidity, hematocrit could not be studied which will be investigated in further endeavours.

It was intended in this study to standardize a protocol for processing sample spots to get the results. Hence serum and blood spots corresponding to 10 micro litres were taken which will not be possible practically because of requirement of a technical expertise in drawing blood, spotting. Hence it is proposed to extend this study by standardizing protocols for directly collecting blood spots onto the filter paper following finger pricks where local population volunteers can be trained to do the same. But in this case various measures will have to be taken to prevent erroneous results due to double spotting and inadequate spotting. This may be done by spotting blood on a particular diameter circular area. One more disadvantage of this method is the manual work required which in turn might increase the pre analytical errors and also the turnaround time.

This method has an advantage of being cost effective and also reducing bio hazardous wastes.

The use of dried blood specimens also presents significant economic advantages. Collection and processing of a dried blood sample is considerably cheaper than samples collected by venipuncture into vacuitainers. Lancets are available at a one third of the price of sterile disposable needles and syringe. The collection of capillary blood for laboratory testing has significant advantages over venipuncture; it is simple to perform, requires minimal training, and does not involve the risks associated with the use and disposal of needles and syringes. A dried blood specimen also represents

a low infectious hazard as some viruses such as HIV-1 and -2, human T cell leukemia/lymphoma virus (HTLV)-I and -II, and hepatitis C virus (HCV) that are known to be present in serum or plasma lose infectivity owing to disruption of their envelope on drying. The Centres for Disease Control and Prevention (CDC) maintain an independent quality control program for blood spots, and as per CDC reports, the filter paper blood collection device is as good as any standard method for collecting blood, such as vacuum tubes and capillary pipettes.[4] Also most analytes are stable at room temperature on drying for at least a week, which makes use of cold transport chain redundant.

But problems like effect of moisture, humidity, spot size need to be addressed for a better accuracy. All these problems do raise the question if dried blood/serum spots can be used extensively in field studies given the possibilities of increased pre analytical and analytical errors. In an age of automation wherein tests are becoming faster and less error prone, dried blood spot testing has a long way to go in proving its usefulness in large field studies. Bioengineering tools which will bring down the manual work and hence errors and also by keeping in check the effects of moisture, humidity along with rigorous training of the local population for the same will definitely tilt the balance towards the positive answer for the use of dried blood/serum in field studies.

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