

Use of dried blood spot as an alternative method to estimate serum triglyceride in field studies

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Abstract

Introduction: Cardiovascular diseases are showing an increasing trend in both developing as well as developed countries. Raised triglycerides is considered as an important risk factor of cardiovascular diseases. Collection, storage and transportation of the blood samples from the field becomes a major limiting factor in areas lacking modern laboratory facilities. In such situations, dry blood or serum spots can become an alternative for the estimation of triglyceride. The objectives of our study was to evaluate the use of dry blood or dry serum spot to estimate triglyceride levels as an alternative method to estimate serum triglyceride and also to study the effect of storage time and storage temperature on the triglyceride content in the dry blood and serum spots.

Methodology: Blood samples were collected from 100 study participants. Seven spots each of blood and serum from these samples were made on Whatman paper 3. After drying, one dried spot each from blood and serum was analysed on same day. Three of blood and three of serum dried spots were kept at room temperature and remaining at a temperature of 2-8° in separate re-sealable bags which were analysed on days 7, 15 and 30. Triglyceride was measured by Glycerol-3-phosphate oxidase-Peroxidase method. For estimating triglycerides in the eluate, 100 microlitre of the extract from the test-tube was added to 1 ml of the commercially available enzymatic reagent kit and incubated at 37°C for 15 min, and the absorbance was measured at 500 nm using a spectrophotometer. The data collected was analysed by Pearson's correlation using SPSSv15.

Results: Study showed a good correlation between the serum triglyceride values and the dry serum or blood spot triglyceride values. Dried serum spot triglyceride was found to be stable for about 15 days at room temperature and 7 days at 2-8°C with good correlation with serum value. The triglyceride concentration in the dry blood spots remained stable for 7 days at both room temperature and at 2-8°C.

Conclusion: Dry blood/serum spots can be used as an alternative to serum for the estimation of triglyceride level which can be used to estimate various biochemical parameters at community level in remote areas which lack modern laboratory facilities.

Key words: Serum triglycerides, Dry blood spot, Dry serum spot, cardiovascular diseases, surveillance.

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Introduction

Cardiovascular diseases are one of the major non communicable diseases in the developing countries and are showing an increasing trend in both developing as well as developed countries. Early detection of cardiovascular disease and proper intervention can reduce the mortality and morbidity caused due to it.^{1,2}

Hypercholesterolemia, raised triglycerides, raised blood sugar are considered as important risk factors of cardiovascular disease. Active surveillance of these cardiovascular risk factors is very much necessary for the early detection and prevention of cardiovascular diseases.³

Screening for these cardiovascular risk factors is essential, needs regular health check-up and routine blood investigation. Providing health care facilities to all

parts of country forms a major goal for active surveillance of cardiovascular risk factors.^{4,5}

Along with the health care personnel, the laboratory facilities should reach all the corners of the country. In the current scenario, few remote corners of the country lack the technology and resources required to provide the adequate health care facilities. In these conditions, with minimal health care professionals, difficulty arises in routine health care and investigation as the blood samples have to be transported to health care centres with laboratory facility for various investigations.⁶

The collection, storage and transportation of the samples from the field becomes a major limiting factor in areas lacking modern laboratory facilities. Dry blood spots can be used in such situations as it can be collected by any health worker with minimum training.

The study was done to know the validity of using dry blood and serum spot to estimate triglyceride levels as an alternative method to estimate serum triglyceride and also to study the effect of storage time and storage temperature on the triglyceride content in the dry blood and serum spots.

Methodology

This study was started after obtaining approval from the Institutional Scientific Committee and the Institutional Ethical Committee of Mandya Institute of Medical Sciences, Mandya.

The cross-sectional study was conducted at the Clinical Biochemistry Section of the Central Diagnostic Laboratory, MIMS, Mandya. 100 fasting blood samples were collected from the patients walking in to Central Diagnostic Lab with a requisition for fasting lipid profile.

Blood samples were collected by venepuncture under aseptic precautions from all the enrolled participants at the collection centre of the central lab in to a non-vacuum plain container.

Immediately after collecting each blood sample, seven blood spots corresponding to 10 microlitres were spotted on Whatman filter paper no 3. The sample in vacutainer was then centrifuged at 3500 rpm for 15 minutes to get serum. Fresh serum was then tested for serum triglyceride by auto analyser ERBA XL 300 (transasia) by Glycerol-3-phosphate oxidase-peroxidase method. Remaining serum was then used for spotting the serum spots each corresponding to 10 microlitres on Whatman filter paper no 3. The Whatman filter papers were then kept on a non-absorbent surface for drying at room temperature for about 1 hour.

After drying, one dried spot each from blood and serum was taken to analyse triglyceride content after elution, on the day of collection. The remaining discs of dried blood and dried serum were kept in separate resealable bags. Three of blood and three of serum dried spots were kept at room temperature and remaining at a temperature of 2-8°C. These dried spot samples were then eluted and analysed for triglyceride content on day 7, day 15 and day 30.

To elute and analyse the triglyceride in blood spot, Lakshmy R et.al method was used with slight modifications. After the standardization of the method with serial standard sample spots, estimation of triglyceride was done.⁷

Normal saline and standard solution from the kit were used to make dry spot for the blank and standard respectively for the dry serum spot. Washed blood cells with normal saline and washed blood cells with standard were used respectively for blank and standard for dry blood spot triglyceride estimation. Along with test blank, the serial standards were estimated on day 1, day 7, day 15 and day 30.

For triglyceride measurement, one disc corresponding to 10 microlitres of blood and one disc corresponding to 10 microlitre of serum was cut and put into individual stoppered test tubes and 400 microlitre of methanol was added to each test tube. The tubes were incubated at 37°C for 2 hours on a shaker.

Triglyceride was measured by Glycerol-3-phosphate oxidase-peroxidase method. For estimating triglycerides in the eluate, 100 microlitre of the extract

from the test-tube was added to 1 ml of the working reagent from the commercially available enzymatic reagent kit. The reaction mixture was stirred and incubated at 37°C for 15 min, and the absorbance was measured at 500 nm using a spectrophotometer. From the absorbance obtained by blank, standard and the test the triglyceride value in the sample was calculated.

The collected data was entered in Microsoft Excel sheet and was analysed using SPSSv15 software. Pearson correlation was applied for analysing the data.

Results

The triglyceride values obtained from dry serum spots and dry blood spots were compared with corresponding serum triglyceride value estimated in Central Diagnostic Laboratory using the autoanalyser on day 0.

To assess stability of triglyceride concentrations in the dry spots based on time, samples were analysed at various time intervals like day 7, day 15 and day 30. Stability of these spots were also assessed at various storage temperatures, at 2-8°C in fridge and at room temperature.

The correlation coefficient or r value is given in **Table 1**. The r value from 1 to 0.5 is considered as strong correlation, 0.3 to 0.5 as moderate and 0.1 to 0.3 as weak correlation. p value <0.05 is considered as significant in the study.

Effect of time and temperature on dry serum spots

The relationship between serum triglyceride and dried serum spot triglyceride level analysed on the day of collection was linear and strongly correlated with r value of 0.62 and p value less than 0.01 as graphically represented in **graph 1**.

The triglyceride concentration in the dry serum spots remained stable for 15 days at room temperature and for 7 days at 2-8°C, as the r value which showed strong correlation at day 7 dropped down to moderate and low correlation values after that. **Graph 3** represents the correlation of dry serum spots with the serum triglyceride values on day 7, day 15 and day 30 for samples stored in both fridge and room temperature.

Effect of time and temperature on dry blood spots

The relationship between serum triglyceride and dried blood spot analysed on the day of collection is shown in **graph 2** with correlation coefficient or r value of 0.79 and p value less than 0.01, showing strong correlation between them.

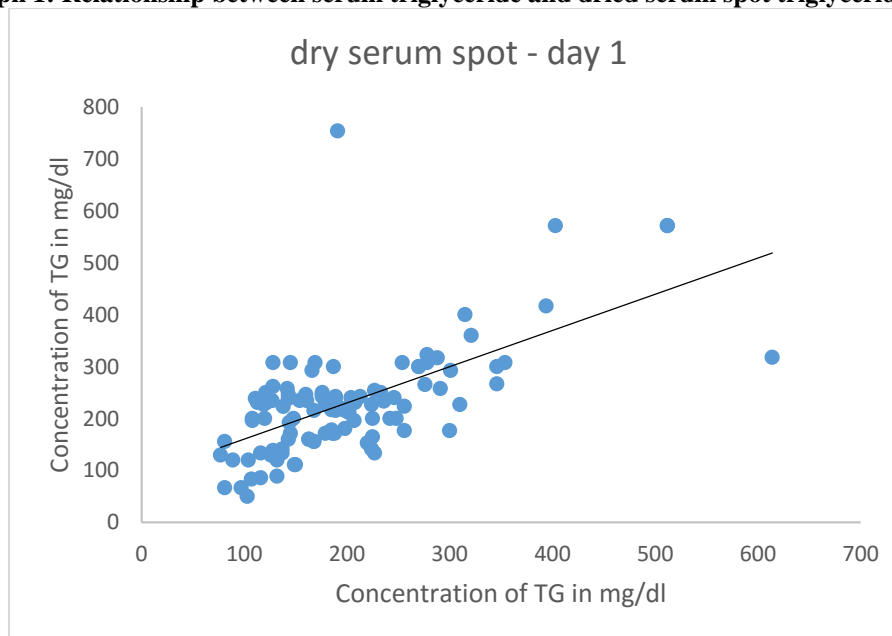
The triglyceride concentration in the dry blood spots remained stable for 7 days at both room temperature and at 2-8°C, as the r value which showed strong correlation dropped down to moderate to low correlation values after that. **Graph 4** represents the relation of dry blood spots with the serum triglyceride values on day 7, day 15 and day 30 for samples stored in both fridge and room temperature.

Table 1: Correlation value (r value) of triglyceride value of dry spot compared to serum value obtained by autoanalyzer

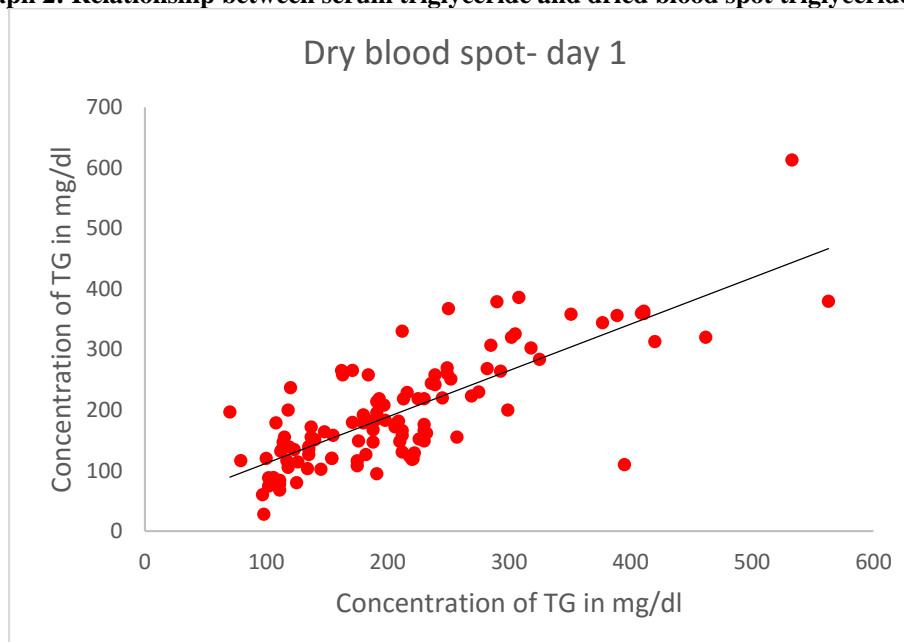
	Dry serum spot room temp (n=100)	Dry serum spot Fridge (2-8° C) (n=100)	Dry blood spot room temp (n=100)	Dry bloodspot fridge (2-8° C) (n=100)
Day 1	0.617**	0.617**	0.785**	0.785**
Day 7	0.750**	0.882**	0.679**	0.514**
Day 15	0.586**	0.183	0.471**	0.212*
Day 30	0.309**	0.043	0.248**	0.186

*p value <0.05, ** p value <0.01

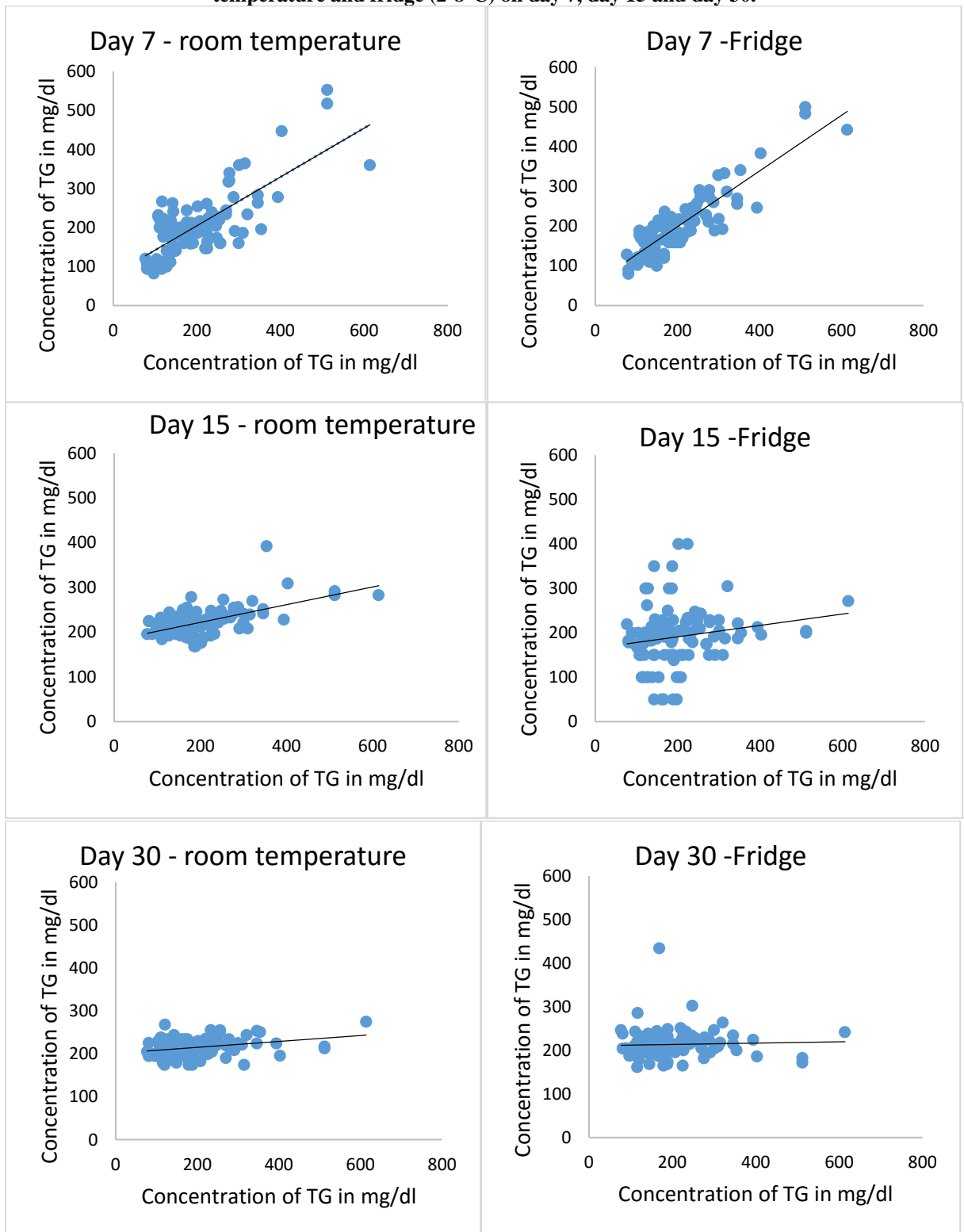
Graph 1: Relationship between serum triglyceride and dried serum spot triglyceride level



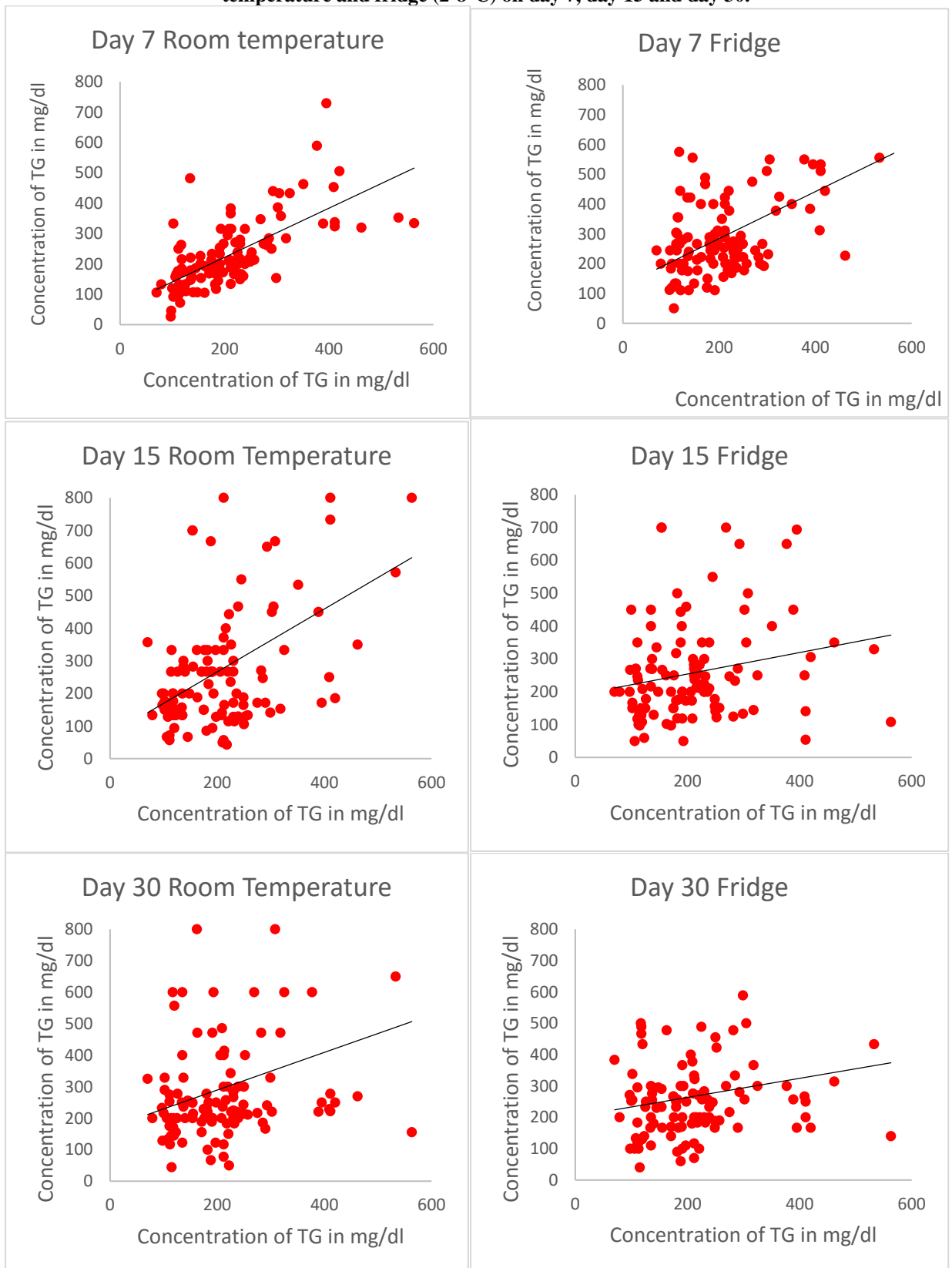
Graph 2: Relationship between serum triglyceride and dried blood spot triglyceride level



Graph 3: Graphical representation of the triglyceride values of dry serum spot at room temperature and fridge (2-8°C) on day 7, day 15 and day 30.



Graph 4: Graphical representation of the triglyceride values of dry blood spot at room temperature and fridge (2-8°C) on day 7, day 15 and day 30.



Discussion

Raised triglyceride values are one of the important risk factors of cardiovascular diseases. Early detection and intervention is necessary to prevent the morbidity.⁸ Studies have shown that dry blood spot analysis can be used in estimation of triglyceride.⁹⁻¹²

The use of dry blood spot for the estimation of various biochemical parameters is becoming a part of analysis in recent days especially in neonates. Collection and transportation of dry blood spots would be comparatively easier and hence it would help in analysis of samples collected in remote areas lacking modern laboratory facilities with good quality assurance programs.¹⁴

Dry blood spot analysis can be an alternative tool for the measurement of biochemical parameters due to its precision and reproducibility when collected on filter paper which is same as standard blood collection methods as reported by Mei J V et al.¹⁵

Previous studies have shown good correlation between dried blood and serum for total cholesterol and triglyceride measurements. In a study done by Lakshmy R, there was a good correlation with r value of 0.94 for triglycerides between dried blood spots and serum samples. It also stated that dried blood on filter paper can be a successful option for screening population in remote areas with well controlled preparation and storage method of dry blood spot.¹¹

In a study done by Quraishi R et al, dried blood triglyceride concentrations were found to be stable for one month when stored at room temperature, and for 3 months when stored at 4 degree centigrade.¹³ In a study by Kapur S, there was a positive correlation between serum triglyceride and dry blood spot triglyceride with r value of 0.95 and 0.94 in fasting and non-fasting samples respectively.¹⁶

In a systematic review and meta-analyses conducted by Affan E, the value of serum triglyceride and dry blood spot triglyceride were directly comparable, dry blood spot triglycerides were stable up to 1 month at room temperature and up to 2 months at 4°.¹⁷

Lakshmy R evaluated the feasibility of dried blood spot in measurement of cholesterol and triglycerides in a multicentre surveillance study for Non-communicable diseases. In this study there was a good correlation between serum triglyceride values and triglyceride in dried blood spot ranging from 0.756 to 0.880.¹⁸

The current study showed that there was good correlation between dry serum and blood spot with the serum triglyceride value. A good correlation between dry blood spot and serum suggests that the method is valid and has potential to be used for the screening of cardiovascular risk factors.

In our study, triglyceride level obtained from dry serum spot was found to be stable for about 15 days at room temperature and for 7 days at 2-8°C with good correlation with serum triglyceride value. The triglyceride concentration in the dry blood spots

remained stable for 7 days at both room temperature and at 2-8°C.

Transportation of dry blood spot samples packed at room temperature from the remote areas to the diagnostic centre would not take more than a week and also require less man power with basic skill set and resources. At community level, this method could be used for mass screening of cardiovascular risk factors in large populations. Irrespective of sample size, the collection and transportation is easier in this method.

Collecting blood spots onto the filter paper following finger pricks would reduce the necessity of venepuncture and collection of samples in vacutainers which would require trained technicians. Volunteers can be trained to collect samples using finger prick and care to be taken to reduce double spotting or inadequate spotting while collection of samples. It also reduces the risk of biohazard as it does not involve the risks associated with the use and disposal of needles and syringes.¹⁹

Major limitation of this study is that the effect of haematocrit, moisture and humidity on the triglyceride values have not been studied.

Even though this method would require manual work which would eventually increase the turnaround time, it can also lead to some pre-analytical errors which may not be acceptable in the current trend of automation. But the estimation of biochemical parameters using dry blood spots would help in providing medical care and facilities in peripheral and remote corners of the country. As laboratory facilities in these places are negligible and also the possibility that even when the facilities are available, the quality of the report provided by these laboratories due to its lack of regular quality control checks is questionable, the dry blood spot analysis could be used as an alternative tool in remote areas.

Conclusion

Dry blood and serum spots can be used as an alternative to serum for the estimation of serum triglyceride in remote areas which lack modern laboratory facilities. It can be used in community level for analysing bulk samples as estimation of biochemical parameters by dry blood spot is cost effective by use of modern technology and microtiter plates. It can also be used as a screening tool for the diagnosis of various metabolic disorders.

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