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The Effect of Sample Storage on Total Cholesterol and Hdl-cholesterol Assays

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Abstract: The result of sample analysis is said to be as good as the sample collection and preparation. Without adequate sample handling, accuracy and precision of sample analysis will be greatly affected. The effect of sample storage temperature and length of storage time on serum total cholesterol (TC) and HDL-cholesterol (HDL-C) assays was studied using twenty different samples, pooled into three. Each pool was split into three and stored at different temperatures- room temperature (16 - 25 ˚C), refrigerator (4 ˚C) and freezer temperature (-20 ˚C), for 6, 30 ad 51 days respectively. The observations from the results obtained using day zero as control showed that the TC concentration in the samples stored at room temperature increased with the length of storage time. However, these increases were not statistically significant (P>0.05), for the duration of the study. HDL-C concentration was found to be significantly increased (p=0.0211 and 0.0117) from day 2 and 10, at room and refrigerator temperatures respectively. However, there was no statistically significant difference (P>0.05) in the samples stored at freezer temperature in both TC and HDL-C concentrations. Except for the TC concentration in the sample stored at freezer temperature, the samples showed a significant correlation at room, refrigerator and freezer temperatures respectively with the duration of storage. From this study, it can be concluded that the concentrations of TC and HDL-C in stored samples are both time and temperature dependent. Also, the concentration is mostly affected at room temperature and least affected at freezer temperature. Hence, if samples for TC and HDL-C assays are to be stored, freezer temperature is highly preferred.

Key words: Sample, storage, total cholesterol, HDL-Cholesterol, room temperature, refrigerator, freezer, assay, duration, effect,

INTRODUCTION

Cholesterol is found in virtually all animal cells and is primarily a membrane component as well as one of the building blocks of stress and sex hormones, and bile acids (Burtis *et al*., 2001; Hu *et al*., 2001). Cholesterol is stored in the adrenal glands, testes and ovaries where it is available for conversion to steroid hormones (Tall, 1990), and also transported in the plasma predominantly as cholesteryl esters, associated with plasma proteins (Gwyne, 1989). Being insoluble in blood, cholesterol is attached to certain protein complexes called lipoproteins (Betteridge, 1989; Ockrene *et al*., 2004) in order to be transported through the blood stream. Low density lipoprotein (LDL) transports cholesterol from its site of synthesis in the liver to the various tissues, whereas high density lipoprotein (HDL) transports excess or unused cholesterol from the tissue back to the liver (Shaefer, 1985; Betteridge, 1989; Stryer, 1998), where it is broken down to bile acids and excreted. Both total cholesterol (TC) and HDL-cholesterol (HDL-C) are components of the lipid profile, a test which shows the body lipid and lipoprotein status (Kiwiterovich, 1998; Burtis *et al*., 2001). Serum or plasma, free of haemolysis is the ideal specimen for the analysis of TC and HDL-C (Myers *et al*., 2000). All samples must be stored appropriately, as rough handling, bacterial contamination or inadequate refrigeration of serum samples can lead to inaccurate test results (Ono *et al*., 1981; Evans *et al*., 1995). According to the WHO, MONICA project (1988), isolation of HDL-C should be done on fresh aliquots on the day of blood collection, if impossible, the serum or plasma for HDL-C

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determination should be frozen at -20 ˚C and isolation should be performed within 14 days. A study by Shih *et al* (2000) recommended that the TC and HDL-C levels should be assessed on the day of sample collection. Progressive changes in measured HDL-C values under all conditions of storage with highly significant correlation was reported by Bachorick *et al* (1980), as samples with low HDL-C concentration tend to increase with storage and those with high HDL-C concentration tend to decrease. Values changed most rapidly during storage at 4 ˚C and were accompanied by changes in precipitability.

MATERIALS AND METHOD

Subjects: The subjects in this study were apparently healthy volunteers at the University of Nigeria teaching Hospital, UNTH Enugu. A total of 20 apparently healthy volunteers (male and female) were recruited for the study after receiving the institution's ethical committee approval for the study to proceed. Informed consent was obtained from each subject before participating in the study.

Experimental design and Sample Preparation: Whole blood samples, (10ml each) were collected through clean venepuncture, after a 12 hour fast, while avoiding stasis or haemolysis. Blood samples were collected with the subjects in the sitting position ad the samples were dispensed into sterile plain tubes and allowed to clot. The clotted samples were centrifuged at 3000rpm for 5 minutes and the separated clear serum supernatants were transferred into sterile tubes. These freshly drawn serum supernatants are the specimen of choice for TC and HDL-C assays.

The samples were pooled together to obtain 3 pools of different concentrations and analysis was carried out on the 3 different pools immediately after separation at room temperature. After analysis the sample were divided into 3 different containers, (aliquoted) in small bottles and stored at room, refrigerator and freezer temperatures.

TC and HDL-C assays were carried out on all the samples after a period of 6, 30 and 51 days at room, refrigerator and freezer temperatures respectively, to determine the effect of storage on the samples.

Only an aliquot for the day's assay is removed from the freezer, to avoid thawing and freezing of the samples.

Analytical Methods: Total cholesterol (TC) assay was done by enzymatic- spectrophotometric method (Allain *et al*., 1974), while HDL- cholesterol (HDL-C) estimation was carried out using precipitation / enzymatic – spectrophotometric method (Grove, 1979).

Statistical Methods: The statistical analysis (students t-test and correlation analysis) were done using graph pad prism computer software package. Results are presented as mean ± standard deviation (±SD)

Table 1: Mean ± SD (mmol /L) of TC and HDL-C at day zero (control) and at subsequent days of assay at room temperature.

Days	TC	P-Value	HDL-C	P-Value
Day zero	4.56 ± 0.62	--	1.37 ± 0.08	--
Day1	4.63 ± 0.83	0.9080	1.28 ± 0.14	0.3739
Day 2	5.18 ± 0.95	0.3982	1.62 ± 0.08	0.0211
Day 3	5.73 ± 0.98	0.1548	2.04 ± 0.46	0.0500
Day 6	6.49 ±1.07	0.0533	2.47 ± 0.46	0.0152

Table 2: Mean ± SD (mmol/L) of TC and HDL-C at day zero (control) and at subsequent days of assay at refrigerator temperature.

Days	TC	P-Value	HDL-C	P-Value
Day zero	4.56 ± 0.62	--	1.37 ± 0.08	--
Day 2	4.68 ± 0.43	0.7697	1.29 ± 0.08	0.3234
Day 5	4.78 ± 0.42	0.6564	1.47 ± 0.17	0.4043
Day 10	4.83 ± 0.52	0.5865	1.66 ± 0.08	0.0117
Day 16	4.97 ± 0.42	0.3916	1.85 ± 0.14	0.0069
Day 23	5.39 ± 0.75	0.2129	1.90 ± 0.08	0.0014
Day 30	5.80 ± 0.90	0.1209	2.04 ± 0.09	0.0006

Table 3: Mean ± SD (mmol/L) of TC and HDL-C at day zero (control) and at subsequent days of assay at freezer temperature.

Days	TC	P-Value	HDL-C	P-Value
Day zero	4.56 ± 0.62	--	1.37 ± 0.08	--
Day 2	4.63 ± 0.52	0.8937	1.33 ± 0.08	0.5185
Day 5	4.63 ± 0.52	0.8937	1.33 ± 0.08	0.5185
Day 10	4.42 ± 0.32	0.7392	1.33 ± 0.16	0.6779
Day 16	4.63 ± 0.32	0.8762	1.28 ± 0.14	0.3739
Day 23	4.83 ± 0.66	0.6297	1.28 ± 0.14	0.3739
Day 30	4.48 ± 0.43	0.8746	1.28 ± 0.14	0.3739
Day 37	4.49 ± 0.44	0.8869	1.28 ± 0.14	0.3739
Day 44	4.49 ± 0.44	0.8869	1.23 ± 0.08	0.1012
Day 51	4.49 ± 0.44	0.8869	1.23 ± 0.08	0.1012

RESULTS

Tables 1-3 show the results (mean SD) of total cholesterol (TC) and HDL cholesterol (HDL-C) concentrations (mmol/L) at day zero, and at subsequent days of assay at room, refrigerator and freezer temperatures respectively.

There is a continuous increase in TC concentrations from day 1 to 6 of the study at room temperature. However, the increase is not statistically significant (P>0.05). HDL-C showed a continuous statistically significant increase (P= 0.0211 to P= 0.0152) from day 2 to day 6 of the study (Table 1).

At refrigerator temperature, there was a gradual increase in TC concentrations from day 2 to day 30 of storage. The level of increase was however not statistically significant (P>0.05), while HDL-C showed a continuous significant increase (P= 0.0117 to 0.0006) from day 10 to day 30 of the assay (Table 2).

At freezer temperature, there was no pattern of change in TC concentrations. Also, both TC and HDL-C showed no statistically significant difference (P>0.05) between day zero and the subsequent days of the study (Table 3).

Figures 1-3 are bar charts representing the results (mean SD) of TC and HDL-C concentrations (mmol/L) at day zero and at subsequent days of assay at room, refrigerator and freezer temperatures respectively.

There is a continuous increase in both TC and HDL-C from day 1 to day 6 of study at room temperature (Fig. 1). Fig. 2 shows a gradual increase in both TC and HDL-C concentrations from day 2 to day 30 of assay at refrigerator temperature.

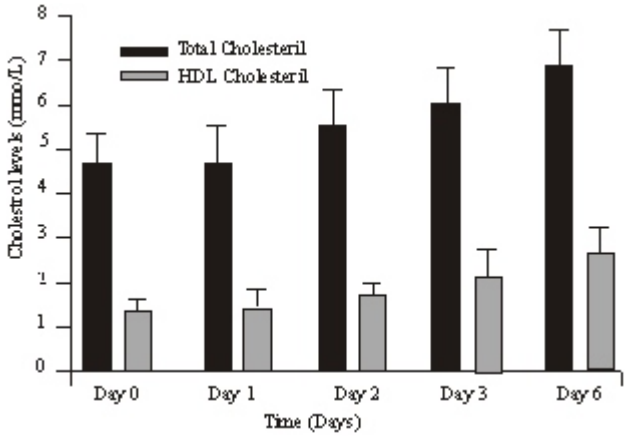

Fig. 1: Bar Chart (Mean ± SD) of TC and HDL-C Concentrations with Duration of Storage at Room Temperature.

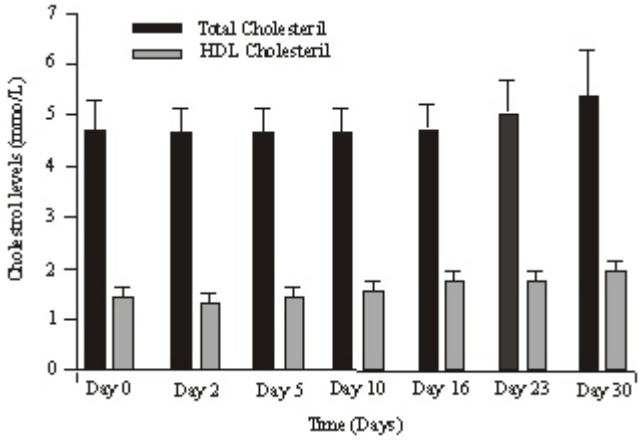

Fig. 2: Bar Chart (Mean ± SD) of TC and HDL-C Concentrations with Duration of Storage at Refrigerator Temperature.

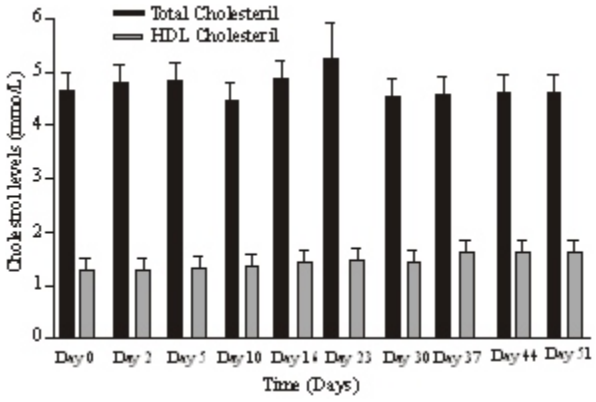

Fig. 3: Bar Chart (Mean ± SD) of TC and HDL-C Concentrations with Duration of Storage at Freezer Temperature

At freezer temperature, TC concentration shows no pattern of change while HDL-C concentrations tend to decrease gradually from day 2 to day 51 of the study (Fig. 3).

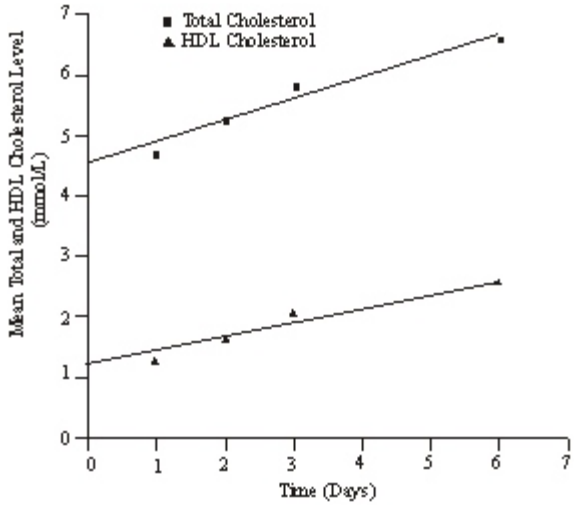

Fig. 4: Plot of Mean TC and HDL-C Concentrations with Duration of Storage at Room Temperature.

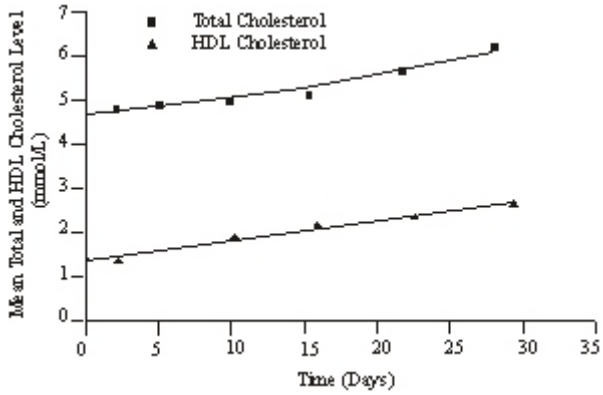

Fig. 5: Plot of Mean TC and HDL-C Concentrations with Duration of Storage at Refrigerator Temperature.

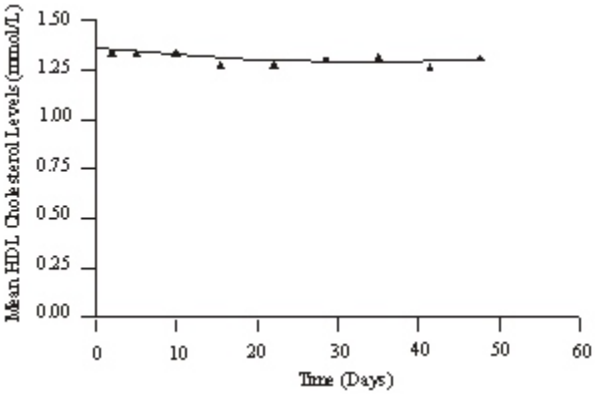

Fig. 6: .Plot of Mean HDL-C Concentrations with Duration of Storage at Freezer Temperature.

Figures 4-6 represent the graphs of mean TC and HDL-C (mmol/L) at room, refrigerator and freezer temperatures against their various days of storage.

Fig. 4. shows a significant correlation (r = 0.0982, p= 0.0029; r = 0.9605, p= 0.0094 respectively) with time of storage in mean TC and HDLC at room temperature.

There was a positive correlation (r = 0.9733, p= 0.0002; r = 0.9690, p= 0.0003 respectively) with time of storage in mean TC and HDL-C at refrigerator temperature (Fig. 5).

Fig. 6 shows no correlation with storage time in mean TC. However, mean HDL-C shows a significant correlation (r = -0.9347, p=0.0001) with storage time at freezer temperature.

DISCUSSION

Serum is usually recommended for lipid measurement because of the diluting effect of the anticoagulants which are contained in plasma samples. Sample storage at various temperatures are likely to be affected by prolonged power failure (for samples stored at refrigerator and freezer temperatures) and bacterial contamination (for samples stored at room temperature).

Absence of primary reference method for separation of HDL-C and also differences in precipitation procedure affects the concentration of HDL-C. This can alter the population of particles precipitated, since all methods do not give the same result for HDL-C measurement and therefore standardization of HDL-C is difficult (WHO, MONICA project, 1988).

Results obtained from this study, show that storage temperature and duration affects the concentration of TC and HDL-C in samples. The number of days which the samples were stored also has an effect on the levels of the analytes obtained in the study.

At room temperature, a continuous increase in TC and HDL-C was observed while a gradual increase was seen from day 2 to day 30 at refrigerator temperature. The samples stored at freezer temperature showed no pattern of change in TC concentration. However, HDL-C concentration was slightly reduced from day 2 to day 51 of the assay at freezer temperature. This corresponds with the findings of Ferrario (1999) which states that, the storage of frozen samples for more than 14 days at -20 ˚C leads to a decrease in HDL-C concentration.

From tables 1-3, as well as figs 1-3, it could be seen that both TC and HDL-C concentrations are mostly affected at room temperature, followed by refrigerator temperature. Although there were continuous increases in TC of samples stored at room temperature (for 6 days) and at refrigerator temperature (for 30 days), they were not statistically (P>0.05) when compared with the base line (day zero). Ferrario (1999) also pointed out that there are no crucial changes in samples stored for 4 days at room temperature for TC assay, provided that bacterial contamination was avoided.

Samples stored at freezer temperature for up to 51 days, showed no significant difference (P>0.05) in both TC and HDL-C when compared to day zero of assay (Table 3).

There was statistically significant positive correlation (p=0.0029, 0.0094; p=0.0002, 0.0003) respectively between the mean TC and HDL-C of the samples stored at room and refrigerator temperatures, and the length of storage time (Fig. 4-5).

Also, samples stored at freezer temperature (Fig. 6) shows a statistically significant negative correlation (p=0.0001) between the mean HDL-C and the length of storage. The samples at various storage temperatures tend to increased or decreased with length of storage. This implies that the concentrations of TC and HDL-C in stored samples are both temperature and time dependent.

CONCLUSION

From the study, it is obvious that freezer temperature is the best storage temperature for samples for TC and HDL-C analysis, rather than refrigerator or room temperature. However, the continuous decline observed in the mean HDL-C concentration of samples, at freezer temperature, shows that assays should be carried out as soon as possible.

Prolonged storage for more than 51 days may lead to a significant decrease in concentration.

Also samples should not be stored for more than a day at room temperature and 5 days at refrigerator temperature as shown by this study. Samples for TC estimation, on the other hand, should not be stored for more than 3 days at room temperature and 30 days at refrigerator temperature.

As shown by the study, scientists and analysts are advised to stop uncontrolled and indiscriminate storage of samples for TC and HDL-C assay as this leads to inconsistent results. Also if storage is necessary, freezer temperature is recommended as the best storage temperature for samples for TC and HDL-C assays.

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