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Effects of Storage Duration and Temperature on Some Most Frequent Routine Biochemical Parameters

WANKASI, M.M[1*] AGORO, E.S[2] TOINPRE K.T[1] OPUGBENE, E.D[1] JACKTO, H[1]
ADIGHIJE, A.C[3]
1.The Department Of Medical Laboratory Science, College of Health Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria
2.Enis Biomedicals (eBm) LTD, Igbogene Epie, Yenagoa, Bayelsa State, Nigeria
3.Department of Pediatric, Federal Medical Centre Yenagoa, Bayelsa State, Nigeria

Abstract
Storage of samples at a given temperature for future Laboratory investigation is a routine due to several technical constraints. This study was designed to determine the effect, reliability and accuracy of studied biochemical parameters upon prolonged sample storage at a given temperature. A total of hundred apparently healthy subjects of equal sex difference were recruited for the study. As case may be, either plasma or serum extracted from the blood of the subjects were stored at -20^{o}C and laboratory analysis carried out on day 1, day 2, day 20 and day 30 respectively for total protein, albumin, globulin, total cholesterol, triacylglycerol, HDL, aminotransferases and alkaline phosphatase using WHO approved methods. The results of the biochemical parameters showed that the mean concentrations of total protein and albumin were significantly higher ($P<0.05$) after storage for 30days.The baseline value of the aminotransferases and alkaline phosphatase activity exhibited a significant decrease ($P <0.05$) after day 30. Also a significant decrease ($P < 0.05$) was observed in concentrations of total cholesterol, triglyceride and HDL on day 30. In conclusion samples for clinical purpose should be analyzed within two days, whereas that for research should not exceed 20 days to ensure reliability and accuracy.
Keywords: Storage time, temperature, biochemicals, proteins, lipids, enzymes

INTRODUCTION

Biochemical investigations have central function in providing biochemical information for the clinicians in the diagnosis, prognosis, monitoring and screening of diseases. It is widely accepted that the majority of medical decisions are made using laboratory data (1). The diagnosis, treatment and follow up of patients rely heavily on the accurate measurement of blood analytes in the laboratory (2). Such information will be of value only if it is accurate and relevant and if its significance is appreciated by the clinician so that it can be used appropriately to guide clinical decision making (3).

The laboratory investigations of interest in this study include liver enzymes (aminotransferases and alkaline phosphatase), proteins and lipid profiles. These parameters are usually utilized in the diagnosis of liver derangement, circulatory compromise and homeostatic status.

The procedures and factors involved in laboratory analysis must be guided appropriately for the generation of a reliable result. Such procedure involved the pre-analytical, analytical and post-analytical stages of laboratory investigations. The pre-analytical stage of laboratory analysis is more pivotal as a derail cannot be corrected, except on a demand for a fresh sample.

This study is focused on the effect of pre-analyticals such as storage and temperature on laboratory results. The volume of samples received in some laboratories are large, hence the samples are stored and subsequently analyzed as individual analysis is economically not viable coupled with the laborious nature. Also, some laboratories lack the expertise and equipment for the analysis of some biochemical parameters. These laboratories usually froze the samples and transport it to other laboratories with such an expertise and equipment (4).

A lot of authors have stated explicitly that preanalytical errors may markedly affect the concentrations of many biochemical variables (5). Studies carried out on stability of biochemical parameters on both humans and animals revealed that temperature and duration of the storage are important factors which may impact on the results of biochemical analysis 6).

Pre-analytical variables such as specimen-storage time and temperature constituted the most common causes of error in the laboratory (7). This could be avoided by ensuring a stable temperature that is not deleterious to the biochemical parameter of choice. Samples are usually stored in the door of a refrigerator (4-8^{o} C) for short durations or in a freezer (-20^{o} C) for longer durations. Thus the temperature at which the samples are stored constitutes an important pre-analytical variable that may affect analysis results in a laboratory (8).

Standard guidelines for blood sample handling states that plasma or serum should be separated (within 20-30 min) from cells as soon as possible after clot formation is complete to avoid clot-induced changes in the concentration of serum analytes (9).

Various schools of thought on the suitability of samples subjected to storage and temperature difference abound. This study is designed to answer questions basically on the time duration of storage that could be detrimental to the reliability and precision of laboratory results for clinical and research decisions.

MATERIALS AND METHODS

Study Area

This research was carried out at Amassoma community in southern Ijaw local government area of Bayelsa state. Amassoma with a population of 49,730 as recorded by the National Population commission in 2006, have a geographical coordinates of 4.97 North Latitude, 6.110 East Longitude and an elevation of 79 meter above sea level.

Study Population

A total number of 100 individuals (50 females and 50 males) between the ages of 18-35 years were recruited for this study. The randomly selected subjected were apparently healthy with no known disease or sickness as established by the research clinician.

Ethical Approval

The ethical clearance was approved by the Ethics Committee of the Niger Delta University, Amassoma, Bayelsa State. Also, the individuals consent was granted before sample collection.

Collection of Sample

Blood was collected from the ante-cupital vein using needle and syringe into a plain and K_3EDTA containers. 7ml of blood was collected from each subject into the two containers as stated. The blood samples in the plain containers were allowed to clot at room temperature for 20 minutes and then centrifuged at 4000rpm for 5minutes, whereas blood in the K_3EDTA containers were spoon and the plasma extracted into plain sterile contains. Haemolysed samples were excluded. The Serum samples obtained from the plain containers was used for the assay of proteins and enzymes, whereas plasma of K_3EDTA for the lipid profile.

Laboratory analysis

Serum total protein and albumin were measured quantitatively using biuret and bromocresol green (BCG) methods as modified by Randox Laboratories (United Kingdom) (Randox kit leaflet) respectively. Serum globulin concentration was derived by subtracting serum albumin from that of the total protein (10).

Total Protein = Albumin + Globulin

Hence, Globulin = Total Protein – Albumin.

Plasma total cholesterol, triglyceride and HDL were estimated quantitatively using Agappe kit as specified by Agappe Diagnostics (Switzerland) (Agappe Kit Leaflet). Enzyme end-point method as postulated by Reitman *et al*., (11) was used for the estimation of serum aspartate and alanine aminotransferases and alkaline phosphatase.

Statistical Analysis

The statistical analysis of the data was done using SPSS (18-20) software application. One way ANOVA (LSD-Post Hoc) and Pearson's correlation statistical tools were used for the statistical analysis. P-values of <0.05 (or 95% confidence level) were considered to be statistically significant.

RESULTS

Table 1: A Multiple Comparisons of Serum Liver Function Tests (LFTs) and Lipid profiles on the Basis of Durations of storage

Parameters	Duration in Days				F-Value	P- value
	DAY 1	DAY 2	DAY20	DAY 30		
TP	67 ± 5	71 ± 7	70 ± 5	77 ± 7αδ	10.904	0.035
ALB	53 ± 3	52 ± 3	51 ± 4	54 ± 5δ	3.187	0.553
GLO	14 ± 2	19 ± 4	19 ± 1	23 ± 2α	8.113	0.009
AST (U/L)	9.9 ± 2.2	10.4 ± 2.1	9.2± 2.2	8.3 ± 1.7α β	3.594	0.017
ALT	5.3 ± 3.1	6.4 ± 3.2	7.7 ± 4.1 α	1.9 ± 1.7 αβδ	12.065	0.000
ALP	26.2 ± 4.9	24.7 ± 4.9	25.8 ± 4.8	22.1 ± 4.9αδ	2.795	0.046
TC	3.5±0.7	3.7±0.7	3.8±0.4	3.1±0.7βδ	5.075	0.003
TG	0.9±0.13	0.92±0.16	0.79±0.24	0.73±0.24αβ	4.39	0.006
HDL	0.96±0.21	0.95±0.22	0.89±0.23	0.79±0.21αβ	3.09	0.031

Legend

Day 1 =α Vs; Day 2=β Vs; Day 20=δ Vs

TP= Total Protein; ALB= Albumin; GLO= Globulin; AST= Aspartate aminotransferase; ALT= Alanine aminotransferase; ALP= Alkaline phosphatase; TC= Total cholesterol; TG= Triacylglycerol; HDL= High Density Lipoprotein

Table 1 Shows that there was significant increase ($p<0.05$) in the mean concentration of total protein, albumin and globulin after 30 days of storage at -20^o C. Furthermore, the concentrations of total cholesterol, triglyceride and high density lipoproteins cholesterol decrease significantly ($P<0.05$) on storage at -20^{o}C for 30 days. Also, a significant decrease ($p>0.05$) was seen on day 30 for the aminotransferases and alkaline phosphatases.

Table 2. The observed Sex Mean ± SD concentrations of Studied Biochemical Parameters due to storage for Day 1 & 2.

Parameters	DAY 1		DAY 2	
	Male	Female	Male	Female
TP	65 ± 5	62 ± 5	70 ± 6	71 ± 9
ALB	50 ± 3	53 ± 3	55 ± 5	56 ± 6
GLO	15 ± 2	11 ± 2	15 ± 3	15 ± 4
AST (U/L)	10.1 ± 3.1	9.7 ± 1.9	10.3 ± 3.3	9.9 ± 2.3
ALT	6.3 ± 2.1	5.8 ± 3.0	6.6 ± 3.3	6.2 ± 3.4
ALP	27.7 ± 5.2	27.3 ± 6.0	25.5 ± 4.7	24.3 ± 3.8
TC	3.7±1.2	4.0±0.9	3.8±0.6	3.9±0.7
TG	1.0±0.11	0.99±0.20	0.96±0.15	0.97±0.11
HDL	0.99±0.19	0.97±0.23	0.94±0.22	0.91±0.22

Table 2 *Shows that there was non-significant difference ($p<0.05$) between male and female on the study parameters upon exposure temperature durations.*

Table 3. The observed Sex Mean ± SD concentrations of Studied Biochemical Parameters due to storage for Day 20 & 30

Parameters	DAY 20		DAY 30	
	MALE	FEMALE	MALE	FEMALE
TP	72 ± 3	69 ± 4	76 ± 6	79 ± 7
ALB	52 ± 5	49 ± 3	53 ± 4	53 ± 4
GLO	20 ±1	20 ± 1	23 ± 4	26 ± 5
AST (U/L)	9.7± 2.3	9.3± 1.9	8.2 ± 1.6	7.7 ± 2.0
ALT	7.2 ± 2.1	7.8 ± 3.3	2.0 ± 1.6	1.9 ± 1.6
ALP	24.7 ± 3.9	26.7 ± 3.0	23.2 ± 4.0	20.3 ± 3.8
TC	3.7±0.2	3.6±0.4	3.2±0.8	3.2±0.6
TG	0.80±0.21	0.79±0.20	0.77±0.23	0.78±0.25
HDL	0.88±0.22	0.90±0.21	0.80±0.22	0.78±0.22

Table 3 *Shows that there was non-significant difference ($p<0.05$) between male and female on the study parameters upon exposure temperature durations.*

DISCUSSION

The results of serum proteins (total protein, albumin and globulin) in this study measured before and after storage showed that the mean concentrations of total protein and albumin were significantly higher ($p<0.05$) after storage for 30days (Table 1). Hence, prolonged storage of serum total protein, albumin and globulin up to 30 days may inadvertently affect the accuracy and precision expected of any laboratory analysis. This increase in concentration could be as a result of protein denaturation, which is the disruption of the three-dimensional structure (native or tertiary structure) of proteins causing unfolding of polypeptide chains. This denaturation could have been caused by agitation, handling techniques or time outside of the freezer before testing. Other factors which could have caused denaturation include the presence of protease enzymes. These enzymes are usually known to denature proteins. Their activities are reduced during lower temperatures but are not completely inactivated thus causing denaturation of proteins at a slower rate. The later point could be the main reason for the increase observed.

The theory of cold denaturation of protein also comes into play. Under normal storage conditions, serum analytes (protein, albumin and globulin) are supposed to be stable when stored at -20 degree celsius and below but it has been discovered that denaturation still occurs at such temperatures (12). This is due to negative changes in the free gibbs energy as temperature decreases. As a result of this change, the polypeptide chains tightly packed in a compact native structure, unfolds at a sufficiently low temperature, exposing the internal non polar groups to water. This study agrees with previous research work that was done by Manju *et al*., (12) on the effect of storage time and temperature on serum biochemistry of proteins.

Moreover, the study revealed that the concentrations of total cholesterol, triglyceride and high density lipoproteins decreases significantly on storage at -20^{o}C for 30 days. Day two of storage shows no significant differences ($P<0.05$) when compared with the fresh sample, this is consistent with previous report (13) that there was significant decrease in concentration of HDL cholesterol in serum after storing for 1 week at -20^{o}C, and other lipids also shows some variable changes after storing at 4^{o}C and -20^{o}C for 1 week .

This findings may be due to structural changes in lipoprotein caused by freezing and thawing that may have affected the density characterize of particles and caused the aggregation of particle (14). Similarly, other possible causes for the observed differences in the metabolites resulting from freezing thaw are enzymatic hydrolysis and synthesis, enzymatic transfer of lipid between lipoprotein and non-enzymatic oxidation.

Furthermore, the findings of the study also showed that the serum enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase) measured during the study durations indicated that the mean activity of all the enzymes was significantly decreased ($p<0.05$) by the 30th day of storage at -20°C (Table 1). This means that long time storage at -20°C for up to 30days is not suitable for serum AST, ALT and ALP assay. This finding is consistent with previous report by Nwosu *et al.*,(15) that showed the time related effects and different temperatures on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase in serum and plasma. This study revealed that reliable values for ALT, AST and ALT estimations can be obtained from refrigerated samples if analyzed within 30 hours (2days) of blood collection and separation. During this period, values obtained were not significantly different from the baseline values.

In conclusion, the effect of gender on storage of the study parameters showed no significant difference ($P > 0.05$) as indicated in table 2 & 3. The patterns of proteins, lipids and liver enzymes variation were similar as the duration of storage increases. Hence, gender should disregard in the chemistry of storage of the studied parameters.

CONCLUSION

Prolonged storage of samples in the refrigerator or freezer may cause significant changes in the serum studied parameters. Although, serum or plasma was stored at -20^{o} C, significant changes were found in the concentrations of most of the studied biochemical parameters on the 30th day. This result indicates that freezing serum or plasma for more than 20 days may affect the studied analytes concentrations; hence timely laboratory analysis is apt so as to avoid inaccurate results.

Conflict of Interest: Non Observed

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