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Influence of storage methods of whole blood samples on DNA integrity in epidemiological studies

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ABSTRACT

Objectives: To investigate the influence of storage methods of whole blood samples on Deoxyribonucleic acid (DNA) quality.

Method and materials: Whole blood samples were collected from 90 patients reported to the Government Tertiary-care Dental College and Hospital, Tamil Nadu, India. The blood samples were stored at three different temperatures −80 °C, −20 °C, 4 °C which were analyzed for DNA quality immediately within 4 h and after a storage period of 60 days. Quantification of DNA in the sample was recorded using a spectrophotometer.

Results: The average concentration of isolated DNA ranged from 0.5 ng/μl to 154.2 ng/μl with purity A_{260}/A_{280} ratio ranging from 0.7 to 2.0. The mean DNA concentration $H = 97.2$ ($p = 0.001$) and purity $H = 80.1$ ($p = 0.001$) obtained from whole blood stored at 4 °C were statistically significant. Thus samples stored at 4 °C did not affect on DNA yield and quality.

Conclusion: We conclude that storage at 4 °C temperature for whole blood does not substantially affect the molecular assay measurements. This study enlightens the influence of storage temperatures and duration in genetic epidemiology.

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1. Introduction

Remarkable advancements in genetics play an indispensable vital role both in Oral health research and Dental practice. To appreciate the genetic aspects of a disease and to create novel methodologies for oral health promotion, the availability of Deoxyribonucleic acid (DNA) is fundamental for epidemiological studies.[1,2] The diagnosis of majority of diseases are nowadays conceivable by direct analysis of DNA and indirectly by investigations of the association between the disease and DNA polymorphisms. Recent advancements are made in our scientific perception of the role of genetics for diagnosis, treatment as well as prevention of oral diseases. Various

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sources of DNA include saliva, buffy coat, buccal cells, sputum, urine, guthrie cards, hair samples, bone material, cultured cells, amniocytes or amniotic fluid, cerebrospinal fluid (CSF). Saliva may contain extensive proportions of DNA and Ribonucleic acid (RNA) from oral bacteria.[3] In spite of this fact and possibility to get DNA from an assortment of sources such as buccal swabs and saliva, they are often constrained in their usefulness as a result of the reduced DNA yields compared with whole blood.

Numerous research centres receives stored blood samples prior to DNA isolation. Hence, the impact of storage time from blood collection to DNA extraction, as well as temperature on DNA yield and quality are imperative. Usually, collected blood samples are processed immediately to avoid any predictable drop in yield and to minimize impending DNA degradation. When processing cannot be undertaken instantly, as in case of multicentre studies, blood samples are usually frozen in deep freezers. The present conviction is that long-term stockpiling of blood at freezing temperatures has a serious and negative effect on the yield and quality of DNA.[4] Freezing blood for even a brief period of time has been reported to show prompt decrease in DNA yield and potential risk of protein contamination.[4,5] Therefore, the study aimed to assess the influence of storage methods of whole blood samples on DNA integrity.

2. Materials and methods

Duplicate 3 ml aliquots of whole blood samples were collected from 90 outpatients reported at Government Tertiary-care Dental College and Hospital, Tamil Nadu, India for the study of Dental fluorosis. Ethical approval for the study was obtained from the Institutional Ethics Committee. Written informed consent was obtained from all the participants after providing them the information sheet of the research. Whole blood was collected from the participants, through venipuncture of the cubital vein in a 5 ml disposable syringe and transferred into 6 ml K_2 EDTA vacutainer (BD Vacutainer®plus# 36789) tube by a trained staff nurse. In the research laboratory the collected blood samples were stored for the period of 60 days in three different storage temperatures −80 °C, −20 °C, 4 °C. DNA was retrieved from the blood sample using the buffer solutions available in the DNA extraction kit (Favorprep® Genomic DNA minikit), immediately within 4 h and after the storage period of 60 days.

2.1. Initial preparation for frozen blood samples

In case of frozen blood samples stored at −80 °C, −20 °C, 30 μl Proteinase K 10 mg/ml was added to the sample. Later, it was mixed with 200 μl GB buffer and incubated in a 70 °C water bath for 15 min to lyse the sample.

2.2. DNA extraction

Three hundred microlitres of fresh blood/frozen blood (after initial preparation) was transferred from the K_2 EDTA tube into a 1.5 ml micro-centrifuge tube and was mixed with 900 μl of red blood cell lysis buffer and spun at 3000 rpm for 5 min. The resulting pellet was rewashed and vortexed in 200 μL of Gel DNA Binding GB buffer, and the mixture was then incubated at room temperature for 10 min until the sample lysate cleared. Two hundred μL of ethanol 96–100% was added and mixed immediately. Resuspended with wash buffer solution and spun at 13,000 rpm for 30 s and flow through was discarded. Then, 100 μL of preheated elution buffer 10 mM Tris–HCl, pH 8.5 at 25 °C was added and spun at 13,000 rpm for 30 s to elute the purified DNA from subsequent storage at 4 °C. The yield quantity and purity of the DNA was calculated by Nanodrop spectrophotometer which depicted the concentration of DNA in Nanogram/microliter (ng/μl) and the purity of the sample in A_{260}/A_{280} ratio. A UV absorbance ratio A_{260}/A_{280} ratio of 1.6–1.9 was considered to be good quality DNA.

2.3. DNA amplification

DNA was amplified by the conventional polymerase chain reaction (PCR) method which constituted an initial denaturation cycle of 94 °C for 3 min, followed by 30 cycles for denaturation at 94 °C for 30 s, annealing at 56 °C for 30 s, extension at 72 °C for 45 s, final extension at 72 °C for 5 min and 72 °C for 30 s to assess the quality of DNA for use in molecular techniques.

The PCR was performed in the total volume of 20 μl reaction mixture for samples which constituted varying amounts of genomic DNA (ranging from 2 ng/μl to 25 ng/μl), 10 μl Master mix Thermo scientific Pfu DNA polymerase# EP0501, 2 μl each of a primer pair and distilled water to a final volume of 20 μl. The primers COL1A2 F 5′-GGG ATA TAA GGA TAC ACT AGA GG-3′[6] COL1A2 R 5′-GAA ATA TCG CCG CTG GAA-3′[6] (Shrimpex®) were used to amplify the 771-bp fragment region of COL1A2 PVUII site suspected to exhibit polymorphism. PCR products were run on 2% agarose gel and visualized in the presence of ethidium bromide DNA intercalator. As a quality control measure among six DNA samples, two at each storage temperatures underwent a standard PCR reaction. Gel electrophoresis of the PCR product revealed that a 771-bp DNA product of the COL1A2 gene was successfully amplified from all six samples using between 50 and 120 ng/μl of DNA.

2.4. Statistical analysis

Statistical analyses were performed using Statistical Package for the Social Sciences SPSS, ver. 18.0; SPSS Inc., Chicago, IL, USA. According to Shapiro–Wilks test of normality, the sample was found to be in non normal distribution. Thus, Kruskal–Wallis H test was applied to estimate p values for comparing the mean DNA concentration and purity between three storage temperatures and time. Pearson's product-moment correlation coefficient was employed to find correlation between DNA concentration and age.

3. Results

A total of 90 persons with mean age of 34 ± 7.6 years were enrolled into this study. The mean DNA concentration and purity (A_{260}/A_{280} ratio) was obtained from the aforesaid samples ranged from 0.5 ng/μl to 154.2 ng/μl and 0.7 to 3.0 respectively. Among the three methods of storage group

Table 1 – Comparison of DNA concentration between storage temperature groups.

Storage temperature group	DNA concentration Mean concentration (ng/μl)	*H* value	*p* value
4 °C	62.18 (33.31)	97.2	0.001*
−80 °C	23.94 (17.13)		
−20 °C	11.85 (9.66)		

Note. *$p \leq .05$. Standard deviations appear in parentheses below means. Means with differing DNA concentration within rows are significantly different at the $p < .05$ based on Kruskal–Wallis *H* test.

Table 2 – Comparison of DNA purity between storage temperature groups.

Storage temperature group	DNA purity UV absorbance ratio A_{260}/A_{280} ratio	*H* value	*p* value
4 °C	1.6	80.1	0.001*
−80 °C	2.4		
−20 °C	2.2		

Note. *$p \leq .05$. Means with differing DNA purity within rows are significantly different at the $p < .05$ based on Kruskal–Wallis *H* test.

−80 °C, −20 °C, 4 °C, the maximal mean concentration of 62.18 ± 33.31 ng/μl was observed at 4 °C with highly significant *p* value ($p = 0.001$) as shown in Tables 1 and 2.

There is no statistically significant difference in DNA concentration and purity among DNA isolated from samples immediately within 4 h of collection and after storage period of 60 days in all the three temperature groups. In addition, no correlation was established between age and DNA concentration.

4. Discussion

This study was initiated to determine the expected total DNA yield and its suitability for its viable employment of pharmacogenomics and genetic-based discerning tests in dentistry.[7] In this context, the recuperation of DNA from long-term stored whole blood in diverse storage temperature is a developing field in genetic epidemiology studies.[8] Blood samples are the primary DNA source for many genomic and epidemiologic studies. Even though it is possible to obtain DNA from various sources such as saliva, buffy coat, bone material, buccal cells, cultured cells, amniotic fluid, sputum, urine, CSF hair samples, they are limited in their utility because of the lesser DNA yields compared with whole blood samples.[9] Whole blood not only offers a readily accessible resource but also provides relatively extensive quantities of high-quality DNA.[10] DNA recovered from whole blood performed significantly superior to saliva which may contain significant proportions of DNA and RNA from oral bacteria.[11] One of the basic challenges for bio-banking is to maintain a storage temperature for sample indicating that in bio-banking, cost efficiency ought to be rehearsed while storing sufficient samples for future use.[12]

The present findings strongly suggest that the mean concentration of DNA was higher at storage temperature at 4° C. Likewise, Lahiri and Schnabel found that fresh blood stored at 4 °C and 37 °C temperature resulted in higher yield of undegraded quality of DNA than from samples frozen at −20 °C, or −70 °C temperatures. Since DNA from blood samples that had undergone more freeze–thaw cycles was found to be partially degraded.[13] Hence the concentration of DNA was higher when blood stored at 4 °C comparative to frozen samples this finding was consistent with the present study.

In addition, the quality of the isolated DNA did not seem to be affected when blood stored at short storage length for 60 days, this finding was similar with observation of Madisen et al.[14]

Several studies have also demonstrated a decrease in DNA yield with frozen storage temperature (−20 °C, −80 °C). To a certain extent, our observation was in concurrence with the previous studies.[12,15] The effects of frozen storage temperature on the total yield of DNA may be due to increased risk of protein denaturation of blood or enzymatic DNA degradation released through erythrocyte lysis.[12]

The quality of genomic DNA was analyzed by measurements of the 260/280 nm ratio by NanoDrop analysis (Nano Drop 8000 Spectrophotometer, Thermo Scientific Inc). The peak of UV light absorption for DNA is 260 nm, the peak of UV light for proteins is 280 nm. The 260/280 nm ratios of DNA from blood stored at 4 °C was on average 1.6, whereas the average ratios from DNA from frozen blood samples at −20 °C, −80 °C were 2.2 and 2.4 respectively. Our results support the explanations by Hansen et al.[16], Udtha et al.[17] and Rogers et al.[18]

However, storage of frozen blood, results in cell lysis on thawing, with a resultant misfortune in DNA yield. Studies have shown that DNA purified from buccal cells of mouth swabs, was contaminated with carbohydrates, peptides, or other aromatic compounds, which could be undesirable for certain molecular techniques, including PCR.[16,18]

5. Conclusion

Short-term storage of blood up to 60 days at 4 °C may serve as an alternative strategy to current storage options for research

laboratories to perform molecular genetics from clinical samples in epidemiological settings.

Thus, given the crucial role played by DNA in bio-medical research and in its related applications, storage methods of human specimens for DNA extraction is essential. Owing to dramatic progress in genetic approaches to risk assessment and diagnosis, prevention, early intercession, and management of craniofacial conditions, it is imperative to intensify genetic studies in Dental research. Blood collection from geographically dispersed population in feasibility of deep freezer and immediate blood processing is a common occurrence in epidemiologic studies. Hence, this study recommends a feasible blood storage temperature strategy for genetic analysis which minimizes the freezer storage cost in outsized research programmes. Further studies are warranted to find out the long-term storage of biological samples for obtaining a large amount of information from limited precious biological samples.

Conflicts of interest

The authors have none to declare.

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