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Original Research Article

Assessment of extracted DNA from whole blood and buffy coat in healthy individuals

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ABSTRACT

Background: Deoxyribonucleic acids (DNA) are the genetic material in all organisms. Extraction of DNA is the initial gait for the field of molecular biotechnology and biomedical research to advance understanding of human illnesses from various diseases and provide practical solutions for diagnosis and treatment. Routine diagnostic techniques in medicine have been greatly enhanced by molecular biology. Due to research on deoxyribose nucleic acid, it frequently finds use in medicine. Efficient isolation of DNA from a sample is the basis for successful forensic DNA profiling. DNA can be extracted from variety of samples such as a whole blood, buffy coat, hair, skin, tissue, urine, buccal swab, saliva etc. Numerous molecular biologic applications require genomic DNA (gDNA) in high quality and in adequate quantity. We extracted genomic DNA from buffy coat and whole blood samples and compared the results of the product obtained in terms of quantity (concentration of DNA extracted and DNA obtained per ml of blood used) and quality (260nm/280nm ratio) of the obtained yield.

Objective: The aim of this study was to extract DNA from whole blood and buffy coat and compare quantity or Concentration (μg/ml) and purity (260nm/280nm) of extracted DNA.

Materials and Methods: The present study was a hospital- based comparative and experimental study. The study was conducted over a period of 1 year on 50 samples. The venous blood sample is taken from the healthy individuals who are willing to donate blood in blood bank. The 5 ml of blood sample taken by venipuncture from healthy individuals were equally dividing into two anticoagulant EDTA tubes by equal volume. The first tube was use as it is, as a whole blood sample and second tube were centrifuged to obtained three phase separation and prepare buffy coat. From both of the samples extraction of DNA is carried out by follow the protocols of modified salting out method. The integrity and purity of the extracted DNA are assessed by Thermo Scientific Nano Drop 2000Spectrophotometers.

Results: Our results show that Mean±SD of concentration of DNA from whole blood and buffy coat was 37.49±25.31 μg/ml and 101.83±31.22 μg/ml ($P < 0.0001$). The Mean±SD of purity (260nm/280nm) of DNA from whole blood and buffy coat was 1.66±0.16 and 1.74±0.15 ($p \leq 0.05$). The both p value was found to be statistically significant.

Conclusion: The present study concluded that DNA extracted from Buffy coat had purity as well as mean concentration significantly higher when compared to DNA extracted from whole blood.

1. Introduction

DNA is the predominant genetic material in the living world. It contains the genetic guidelines used for the growth, development, functioning and reproduction of all known

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living organisms and many viruses.

DNA is the unbranched polymers of nucleotides, composed of two chains of nucleotides that are entwined as a right-handed double helix structure. Both the strands are complementary to each other in the base sequences. Chargaff's confirmed the fundamental discovery of the equivalence of A to T and G to C bases in double-stranded DNA. The genetic functions of DNA are containing the information store encoding the sequences of proteins and also enabling the packaging, accessibility, and replication of the information store. Crucially, both the coding of proteins and RNA molecules, along with the physicochemical properties of the polymer are specified by the base sequence.[1] Genomic DNA is a key component for genomic research. After collection of clinical samples, isolation of DNA is the first step to run molecular diagnostic assays.[2] It is conceivable to extract DNA using both organic and inorganic procedures, with the results varying depending on the type of sample used. DNA can be isolated from any biological material such as living or conserved tissues, cells, virus particles, or other samples for analytical or preparative purposes.[3] DNA can also be acquired from certain biopsies or cell cultures; however it is typically taken from blood leukocytes. In most cases, DNA extraction techniques must be adapted to the type of biological sample, the nature of the genome to be extracted, the number of copies in the sample and the techniques of molecular biology to be used. For DNA extraction, we select buffy coat and whole blood as our sample types. Whole blood is a common biological starting sample for DNA extraction. Compared to other minimally invasive sources of genomic DNA (gDNA), such as saliva or buccal cells, gDNA yield is higher and less fragmented. Red blood cells (RBCs), white blood cells (WBCs), platelets, and plasma are all components of whole blood. WBCs' nuclei contain gDNA. High-quality gDNA from blood is applied in forensics, cancer diagnosis, and several other biological procedures.[4] The buffy coat is simply a concentration of all the white blood cells and platelets in a sample of blood. Following density gradient centrifugation of the blood, the portion of an anticoagulated blood sample that includes the majority of the white blood cells and platelets is known as the buffy coat. A buffy coat suspension is a concentrated leukocyte suspension containing granulocyte, platelets, lymphocytes, monocytes. These cells present in buffy coat play a vital role in human disease pathogenesis. Examination of the leukocyte in buffy coat provides valuable scientific information which has various benefits to the research fields. Buffy coat is common choice of sample used for the DNA extraction from mammalian blood. A large amount of DNA with high quality, integrity and functionality can yield by a reactively small sample of buffy coat. Evidence has shown cell pellets derived from buffy coat can be stored for up to 9 year at -80°C and still maintain high yield of DNA suitable for whole genome sequencing, genome-wide association analysis, and other genetic testing.[5,6]

Overall, this study shows that the DNA extraction from whole blood and buffy coat sample by following the manual protocol of modified salting out method.

2. Aim and Objectives

The aim of the present study was to extract DNA from whole blood and buffy coat as well as to measure the concentration and absorbance ratio (260nm/280nm) of the extracted DNA samples by using Thermo Scientific NanoDrop 2000Spectrophotometers to compare quantity and purity (260nm/280nm) and was statistically analyzed.

3. Materials and Methods

3.1. Sample collection

The present work was an experimental study carried out in the laboratory of Molecular Biology of the department of biochemistry in Guru Gobind Singh Medical College, Faridkot. 50 blood samples were collected in the anticoagulant EDTA tubes. The samples had been drawn from healthy volunteers after the informed consent had been obtained.

3.2. Sample processing

5 ml of blood were collected in a tube with EDTA from the vein in the fold of the elbow. Each blood sample was aliquoted into two tubes of 2.5ml, in tube A, buffy coat preparation was done while another tube B was used for the whole blood.

3.3. Preparation of buffy coat

The blood samples in tube-B were centrifuged at 3000 rpm for 10 minutes to obtain a three-phase separation: plasma, cell pellet and Buffy coat (the white ring between the supernatant and the pellet) Figure 1. For collection of buffy coat the upper phase of plasma was removed without touching the leukocytes Five hundred microlitre (500μl) of the buffy coat was collected.

DNA extraction protocols

DNA extracted from 500μl of whole blood and buffy coat samples by using modified salting out method.[7]

1. 500μl of sample was suspended in 1.5 ml of lysis buffer 1 (three times the volume of blood sample) in micro-centrifuge tube briefly vortexed and then centrifuged (REMI Cooling microfuge CM-12) at 12000 rpm for 10 minute. The supernatant containing the lysed RBCs was discarded and the pellet was resuspended in 1.5 ml of RBCs lysis buffer.
2. These above two steps were repeated till a clear white pellet of WBCs was obtained (Figure 2).

Fig. 1: Showing buffy coat between two layers

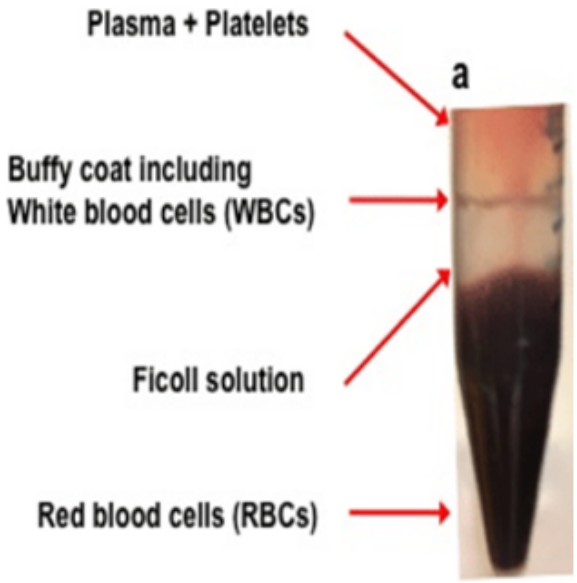

Fig. 2: White pellet of WBC's

Fig. 3: Extracted DNA Sample for quantification

3. The WBCs pellet was suspended in 500μl WBCs lysis buffer.
4. Then 35μl of 10% SDS was added and the mixture was incubated overnight at 37°C in water bath.
5. After overnight incubation, the lysate was brought to the room temperature, then 250μl of 7.5M Ammonium acetate was added and vortex thoroughly.
6. The contents of tube were centrifuged at 12000 rpm for 15 minutes. The supernatant was collected in a labeled fresh sterile microcentrifuge tube.
7. Two and half times volume of chilled absolute ethanol was added to the supernatant along the wall of the tube and swirled gently to spool the DNA.
8. The DNA pellet was obtained by spinning at 8000 rpm for 10 minutes.
9. The supernatant was discarded and 500μl of 70% ethanol was added.
10. The contents of tube were centrifuged at 8000 rpm for 3 minutes.
11. The alcohol was decanted and the pellet was air dried at room temperature for 5-10 minutes.
12. The pellet was dissolved in 70μl of TE buffer.
13. DNA was stored at -20°C till further analysis.
14. Quantification of DNA was done on Thermo Scientific Nano Drop 2000 Spectrophotometers (Figure 3).

3.4. Spectrophotometry of DNA

For quantification of extracted DNA, the concentration of the double stranded DNA was measured from a Ultra-Violet spectrophotometer (Thermo Scientific Nano Drop 2000Spectrophotometers) by an absorbance at 260nm. The quality and purity of the extracted DNA is evaluated by the ratio A260/A280. A ratio between 1.8 and 2 indicates generally as pure form of DNA. The presence of proteins in a sample increases the value at A280. The low ratio indicates the presence of protein, phenol or other contaminants that absorb strongly at or near 280nm.[8,9]

3.5. Study design and statistical analysis

The present study was a hospital- based comparative and experimental study. The study was conducted over a period of 1 year on 50 samples. All graphs were performed with the Excel program. The numerical values represented in the graphics correspond to the mean and the bars to the standard deviation (SD). In the numerical charts, the values are represented as Mean±SD. For the contrast of independent continuous variables (normal distributions), one-way repeated-measures analysis of variance (ANOVA)

test or Student's t test was used.

4. Results

Table 1: Mean±SD of the age and age distribution of participants

Age(year)	Total(N)	Percentage
20-25	15	30%
25-30	18	36%
30-35	10	20%
35-40	6	12%
40-45	1	2%
Total	50	100%
Mean±SD (year)	28.8±5.22	
Range (year)	20-43	

Table 1 shows the age distribution of participants. Maximum number (36%) of donor were from the age group of 25-30 year followed by (30%) in the age group of 20-25year. Mean± SD and range of age are 28.8±5.22 and 20-43 years respectively.

Sex distribution of 50 participants enrolled in the study. 25 males and 25 females were enrolled in this study (Figure 4).

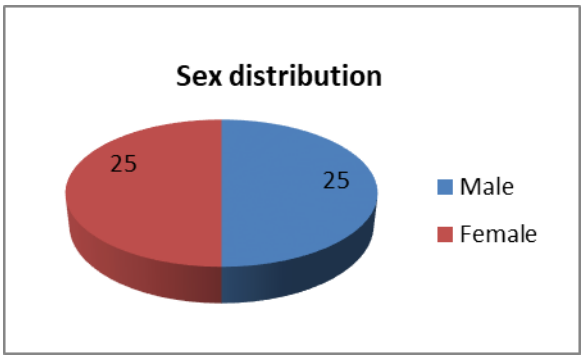

Fig. 4: Sex distribution data

Table 2: Mean±SD of concentration (μg/ml) of DNA from whole blood and buffy coat

Type of sample	Mean±SD	P Value
Whole blood	37.49±25.31	P < 0.0001
Buffy coat	101.83±31.22	

Showing Mean±SD of concentration of DNA from whole blood was 37.49±25.31μg/ml and Mean±SD of concentration of DNA from buffy coat was 101.83±31.22 μg/ml (Figure 5). This difference was found to be statistically highly significant for concentration of both samples with p value ≤0.005.

Showing the Mean±SD of purity (260nm/280nm) of DNA from whole blood was 1.66±0.16 and Mean±SD of purity (260nm/280nm) of DNA from buffy coat was

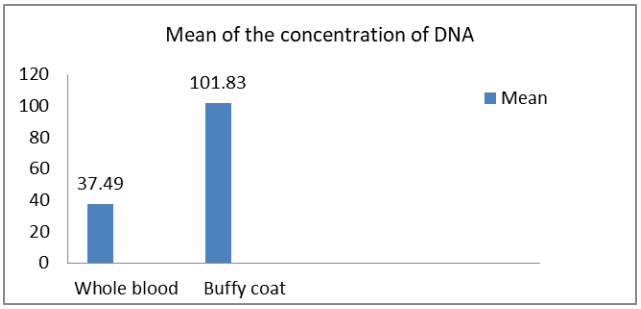

Fig. 5: Mean concentration of DNA at 260nm

Table 3: Mean±SD of purity (260nm/280nm) of DNA from both samples

Type of sample	Mean±SD	P Value
Whole blood	1.66±0.16	P = 0.0184
Buffy coat	1.74±0.15	

1.74±0.15 which was found to be statistically significant (p≤0.05) (Figure 6).

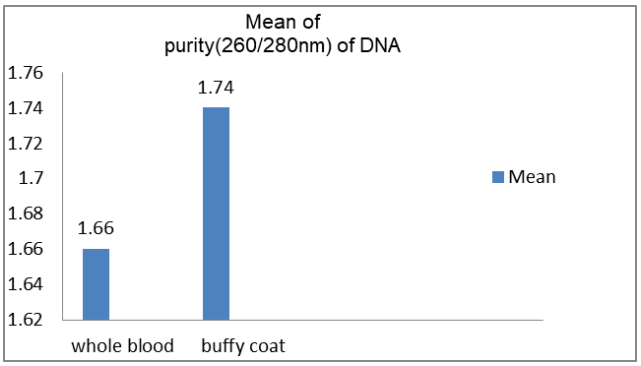

Fig. 6: Showing the mean of purity (260nm/280nm) of DNA from whole

5. Discussion

The present study was conducted in the Department of Biochemistry at GGS Medical College and Hospital, Faridkot. For this we enrolled 50 'healthy individuals and their blood sample were taken for DNA extraction. We extracted the DNA from whole blood and buffy coat by using modified salting out method and the aim of the study was to compare the outcome by these samples in terms of quantity and purity at 260nm and 280nm.

After DNA extraction the DNA concentration of each sample was determined by Thermo Scientific Nano Drop 2000Spectrophotometers. For quantitating DNA reading were taken at wavelength of 260nm and 280nm. Ratio of absorbance at 260nm/280nm was use to asses' purity of DNA. Our results showed the Mean±SD of concentration of DNA from whole blood and buffy coat which was

37.49±25.31 μg/ml and 101.83±31.22 μg/ml and this difference was statistically highly significant with P < 0.0001(Table 2) whereas the results of the mean of purity of DNA from whole blood and buffy coat which was 1.66 and 1.74 (Table 3) and this difference was comparable. The results of the study are consistent with the results of other studies conducted Kamangu et al (2019) the Buffy coat produces more DNA than whole blood because the Buffy coat is the enriched fraction of Leukocytes in a collection of whole blood. Hence, DNA extraction on Buffy coat should logically yield 5 to 10 times more DNA than its equivalent in blood volume[10] and another study Mychaleckyj et al (2011) demonstrate that buffy coats can be used as a long term clinical trial or biobank specimen for DNA extraction significantly higher than the, in lieu of immediately isolating the DNA at collection.[5] Similar results are seen in study conducted by ZHOU et al. (2015) found that DNA yields from the buffy coat would be sufficient for the downstream experiments. The quality of the DNA is more important than the quantity. The DNA extracted from the buffy coat is adequate and good in quality for the downstream experiments for most research. The quality of the DNA extracted from the buffy coat is capable of meeting the requirements of other demanding methods. Storage of buffy coat, instead of whole blood and ACP, is a better approach for the epidemiologic researchers who need sufficient quantities of high-quality DNA while controlling the costs and labor.[11]

6. Conclusion

It was concluded that DNA extracted from Buffy coat had purity as well as mean concentration significantly higher when compared to DNA extracted from whole blood. The use of buffy coat will reduce the cost and labor. Also, hence DNA extraction from buffy coat remains the method of choice.

7. Ethics Declaration

This study was approved by the Ethics Committee of Clinical Research from GGSMC, Faridkot.

8. Consent

Written informed consent was obtained from all participants for the use of their blood sample for this research and the publication of any findings in the scientific literature.

9. Source of Funding

None.

10. Conflict of Interest

None.

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