ORIGINAL ARTICLE

DNA Isolation from Whole Blood Samples of Endometriosis Patients using the Salting out Method and ITS Scope in Ayurveda

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Abstract

Introduction: As time goes on, more methods and resources for assessing patients' health will become available. Another developing area of science is personalized medicine. Any nucleated cell, including blood, tissue, hair, etc., can have its human DNA retrieved. High protein content and a range of secondary metabolites present in various sources affect DNA purification; highly pure DNA is necessary for molecular study. Here, we extracted a considerable amount of human DNA from endometriosis patients' whole blood using the salting out procedure. This is a cheap, quick, safe, and simple method. Research facilities and medical laboratories may make use of it. **Objectives:** The objectives of the study were (1) to isolate the DNA from the blood of endometriosis patients to conduct a whole-exome sequencing analysis aimed at learning more about the genetic variations connected to the disease and (2) to evaluate the scope of this technique in Ayurveda in endometriosis diseases. **Materials and Methods**: A 5 ml blood sample of diagnosed cases of endometriosis was collected from Sir Sunder Lal Hospital, Banaras Hindu University in a heparinized vial from Obstetrics and Gynaecology OPD. Extraction of DNA will be done in the Centre for Genetic Disorders, Institute of Science, and Banaras Hindu University. **Conclusion**: Using the Salting Out method, good quality DNA samples from human whole blood can be extracted that is enough to perform a Whole Exome Sequencing.

Key words: DNA, endometriosis, prakriti, sequencing

INTRODUCTION

Friedrich Miescher discovered a material termed "nuclein" in 1869, which led to the discovery of DNA. The main source of DNA for human genotype research is blood. One of the most easily published techniques is the salting out approach, which can take three to 4 h to complete. Following this process, a significant number of samples can have their DNA isolated in three to 4 h, with a yield of 5 to 11 ug of high-quality DNA from 300 uL of whole blood.[1] Genetic susceptibility to a certain disease can result from inheritance of one or more genes, and genetic susceptibility to different treatments can vary depending on which genes are inherited. The ~3 billion base pairs in the human genome are 99.9% identical in every individual, according to genome sequencing. This implies that each person on the planet differs genetically from other individuals by an average of 0.1%. The matter of why some people are more likely than others to be healthy or more prone to a specific sickness rests inside that 0.1% of the population. More than 10 million single nucleotide polymorphisms (SNPs) that affect more than 1% of people are found in the human genome. With the development of high throughput SNP genotyping platforms and our growing understanding of genetic variation, we are

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now able to identify the loci responsible for a wide range of common diseases and traits. It is feasible to ascertain whether specific variants, or what is known as association, occur more frequently in those afflicted with a given disease than in the general population. As with the identification of HLA associations in the immune response as the cause of autoimmune illnesses (P = 170), the demonstration of a polymorphic association can imply that the hereditary variation is involved in the etiology of the disorder, It might just indicate that a gene close to linkage disequilibrium (P = 92) is responsible for the disorder's onset.[2]

Inclusion criteria for endometriosis cases

The following criteria were included in the study:

- Females aged >20 years or <45 years, visiting Obstetrics and Gynecology OPD, S.S. Hospital, B.H.U. will be initially screened and evaluated for their eligibility in the study.
- They should be willing to participate in the study.
- Eligible females with a confirmed diagnosis of endometriosis will be recruited as endometriosis cases.

Exclusion criteria for endometriosis cases

The following criteria were excluded from the study:

- Patients with comorbidity affecting mental or physical health.
- Patients with extreme age groups <20 years or >45 years.
- Abuse of drugs and alcohol.
- Patient who are already on treatment for endometriosis.

Informed consent

A consent form will be provided to the patients that include the purpose and procedures of the study and carefully explain the same to the participants in their local language.

Blood sample

After obtaining written informed consent, 5 mL of peripheral blood will be collected by venipuncture 05 mL blood in heparinized tube from diagnosed cases of endometriosis.

MATERIALS AND METHODS[3]

- Standard chemicals: Science House provided the chemicals we utilized for this approach, which may be bought from major suppliers [Figure 1].
- Human peripheral blood,
- Heparinized syringe,
- Microcentrifuge tube,
- Falcon tube,
- NaCl (0.9%),
- Deionized water,
- Solution "A", Solution "B", Solution "C",
- Sodium dodecyl sulfate (SDS),
- Chloroform,
- Absolute alcohol,
- 70% ethanol,
- TE-buffer

Methods[4]

The salting out method was used to isolate genomic DNA from peripheral blood (Miller *et al*., 1988).

In a 50 mL polypropylene tube, 5 mL heparinized peripheral blood was combined with 20 mL of 0.9% NaCl, then centrifuged at 5000 rpm for 5 min.

The supernatant was removed without disturbing the pellet, and 20 mL of cooled sol A (stored at 4°C) was added to the cell pellet, thoroughly mixed, and centrifuged for 5 min at 5000 rpm.

- The supernatant was discarded once more, and 2 mL of sol B was added to the pellet and thoroughly stirred to break it up [Appendix II]. Without shaking the pellet,
- 200l of SDS (10% w/v) was added after it was broken.
- 500 liters of Sol C were added, and the contents were thoroughly mixed [Appendix II and Figure 2]. 2 mL cooled chloroform was added, mixed, and centrifuged at 5000 rpm for 5 min to separate the layers.
- Three layers were separated, and DNA was found in the upper, clear aqueous layer, which was pipette into a new 15 mL falcon tube [Figure 3].
- DNA was precipitated with cooled absolute ethanol and washed twice with 70% ethanol. DNA was air dried before being dissolved in 200l 1× TE buffer pH8.0 (10 mM Tris-Cl, 1 mM EDTA, pH8.0) (Appendix IV).

For future usage, DNA was kept at 4°C.

Reagent preparation

- Solution "A"
  - Sucrose-109 g, 05 mL of 1M mgCl2 in 1000 mL deionized water was autoclaved then 10 mL of Triton-X 100 was added and stored at 4°C.

- Solution "B"
  - 40 mL of 1M Tris-cl (pH 8.0), 12 mL of 0.5 M EDTA, 15 mL of 1M NaCl, 33 ml of deionized water was autoclaved, and 05 mL of 20% SDS was added.

- Solution "C"
  - 05 M Sodium perchlorate monohydrate
  - TE-Buffer
  - 10 mM Tris Cl (pH 8.0)
  - 01 mM EDTA (pH8.0).

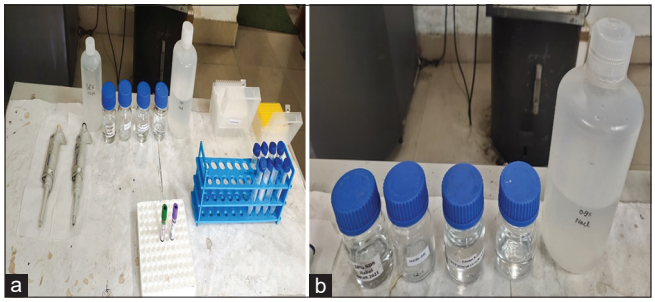

Figure 1: (a and b) Chemical required for DNA extraction

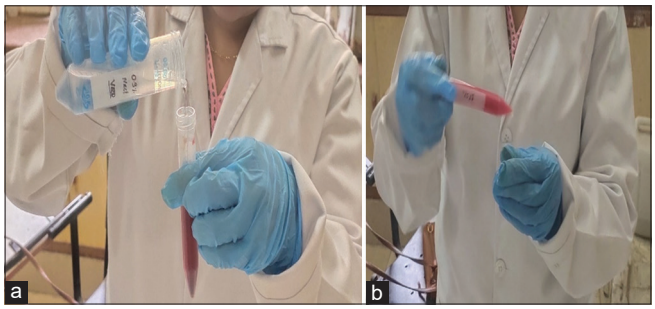

Figure 2: (a and b) Mixing of the solution

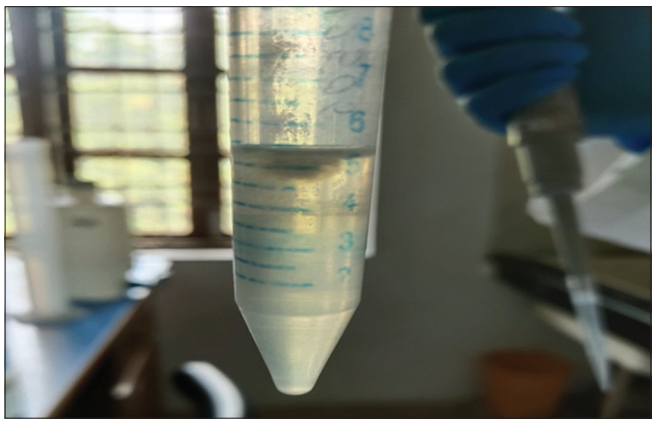

Figure 3: Extracted DNA from blood samples

Quality and quantity assessment of DNA-spectrophotometer (Nanodrop®, Thermo, USA)[5]

A spectrophotometer (NanoDrop ND-2000, Thermo Fisher Scientific, USA) [Figure 4] was used to test the quality of genomic DNA. Furthermore, the amount of DNA was determined using the optical density (OD) of OD260. The OD260:OD280 ratio was used to assess the quality (protein contamination) of the DNA. A graph for linearity was plotted using the mean values of absorbance and the concentration of each point. Integrity of DNA and no-contamination of RNA was assessed by 0.8% agarose gel electrophoresis in 1× Tris-Acetate-EDTA (TAE) buffer [Appendix III].

Protocol

The program to which the Spectrophotometer (Nanodropâ, Thermo, USA) was connected was launched. Our preferred

Figure 4: Spectrophotometer (Nanodrop®, Thermo, USA)

application (DNA, RNA, or protein) was chosen. The instrument's hinged arm, which hangs horizontally, was gently raised. A little black patch, the pedestal on which our samples were put, could be seen. A lint-free wipe was used to carefully clean the measuring pedestal. We pipetted 1–2 µL of our blanking solution, that is, TE buffer, onto the measuring pedestal. The sampling arm was lowered gradually. The "Measure blank" tab was selected. The arm was raised and a lint-free wipe was used to clean it. A sample of 1 µL of DNA was pipetted onto the measuring pedestal. The sampling arm was lowered, and the spectrum measurement was started using the PC program. The DNA sample concentrations were recorded and kept in a file folder [Appendix I]. Following the completion of the measurements, the sampling arm was lifted and the sample was cleaned off both the top and lower pedestals using a lint-free laboratory wipe. Simple wiping avoids sample persistence in successive tests for samples changing the concentration.

Agarose gel electrophoresis of genomic DNA

The genomic DNA sample was pipetted into the sample wells; then electric current was applied to cause the negatively charged DNA to migrate. To assess RNA contamination and DNA integrity, a 0.8 % agarose gel in IX Tris-Acetate-EDTA (TAE) buffer was used [Figure 5].

RESULTS

The purity 260/280 ratios ranged consistently from 1.7 to 1.8 demonstrating good deproteinization.

Samples OD and concentration details

[Table 1] Yield and Purity of 10 DNA samples of Endometriosis patients extracted by salting-out method.

Table 1: Concentration of endometriosis patient DNA samples

S. No	Sample ID	OD260:OD280	Concentration (ng/μL)
1.	EM-01	1.8	338
2.	EM-02	1.8	590
3.	EM-03	1.8	805
4.	EM04	1.8	300
5.	EM-05	1.8	240
6.	EM-06	1.8	930
7.	EM-07	1.8	720
8.	EM-08	1.8	855
9.	EM-09	1.8	725
10.	EM-10	1.8	450

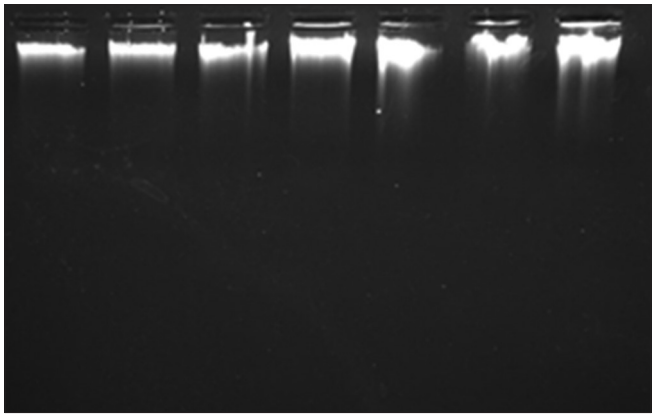

Figure 5: 0.8% agarose gelelectrophoresis of genomic DNA

DISCUSSION

Endometriosis is a disease whose pathophysiology is unclear. Genomic DNA collected from human whole blood samples will be utilized for next-generation sequencing (NGS), which will be further linked at the *Prakriti* level to offer novel possibilities for illness prevention, diagnosis, and treatment. It can also be helpful for genotype studies, which will support personalized medicine. Furthermore, there is not much genome-level researches integrated with *Ayurveda* on endometriosis patients conducted in India.

CONCLUSION

As per *Ayurveda* general etiology of *Yonivyapad* or gynecological disorders is commonly occur by abnormal dietetics and mode of life, abnormalities of *artava* and *bija* (either ovum or sperms and ovum both) and curses or anger of god (in the absence of apparent cause, the diseases are said to develop due to curses of god) are the causative factors of all these twenty disorders of *yoni*, is the opinion of *Caraka*.[6]

Ayurveda contains numerous verses that describe genetics. The disease endometriosis is classified under the term *yonivyapad*. Based on the verse above, we can conclude that genetic variations, which have been known to cause endometriosis in the past, are among its causes. However, in the modern era, it is necessary to explain these variations through research ranging from the macro to the micro level. There is enough DNA left over to carry out additional NGS research. High-quality DNA samples from human whole blood were extracted using the aforementioned technique to investigate gene polymorphisms in the human population for numerous additional diseases and to investigate the idea of personalized therapy. *Ayurvedic* diseases do not all have pathologies and symptoms the same as endometriosis. However, Ayurveda has its system of classification and recommendations for diagnosing diseases. *Charak Samhita* states that if a physician knows the *Dosha*, place of manifestation, and *nidana* (causes) of a disease, then his attempts to treat it will always be successful, even if he is unable to name a particular illness. We can draw comparisons between endometriosis and *vatik yoni vyapad, Udavarta, Granthi, Gulma,* and *Visarpa,* among other things, based on the different symptoms that patients experience.[7]

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Conflicts of Interest: None declared.

APPENDIX I

Sucrose – 109.5g

1M MgCl2 – 5 mL

Triton-X 100 – 10 mL

Maintained the volume upto 1 litre, autoclaved and stored at 4ºC.

Solution B (For 100 mL)

1M Tris-Cl – 4 0mL

0.5M EDTA – 12 mL

(pH 8.0)

1M NaCl – 15 mL

Maintained the volume to 95 mL, autoclaved and 5 mL of 20% SDS added to make volume 100 mL after cooling. Stored at room temperature.

Solution C

5M Sodium perchlorate, autoclaved and stored at room temp.

APPENDIX II

1X TE buffer pH-8.0

10mM Tris-Cl pH8.0

1mM EDTA pH8.0

APPENDIX III

50× Tris-Acetate-EDTA (TAE) buffer (For 1 litre)

Tris base – 242 g

Glacial acetic acid - 57.1 mL

0.5M EDTA pH8.0 – 100 mL

APPENDIX III

DNA was precipitated with cooled absolute ethanol and washed twice with 70% ethanol. DNA was air dried before being dissolved in 200l 1× TE buffer pH8.0 (10 mM Tris-Cl, 1 mM EDTA, pH8.0).

APPENDIX IV

DNA was precipitated with cooled absolute ethanol and washed twice with 70% ethanol. DNA was air dried before being dissolved in 200l 1× TE buffer pH8.0 (10 mM Tris-Cl, 1 mM EDTA, pH8.0).