CHAPTER-I

INTRODUCTION

Dandruff is a common scalp disorder affecting almost half of the population at the pre-pubertal age and of any gender and ethnicity. Dandruff is produced as a pathological desquamation of the skin and histologically consists of orthokeratotic clumps with minute parakeratotic foci. It is presumed that some nucleated cells could be found in dandruff therefore in some cases sufficient dandruff could be obtained, DNA for the Polymerase chain reaction might be obtained for DNA analysis.

Tape lifting has become a well-established sampling method in forensic biology. Applying adhesive tape, dandruff can be efficiently collected from large areas of fabrics, solid surfaces or skin. EZ tape, an extraction tool for exfoliative cells, developed by Institute of Forensic science, Ministry of Public security is widely used in china. Dandruff was collected by EZ tape together with indefinite particles and DNA was extracted from EZ tape through chelex-100 method directly.

But it still has many drawbacks such as low utilisation rate of sample, easily obtaining mixture genotypes and failing to recover alleles. Results show that sufficient quantities of DNA can be obtained from as little as 1 to 1.5mg of dandruff. The most widely used extraction techniques are Chelex and Organic method are applicable. Both methods yield DNA, although organic procedure seems to yield more DNA.

The major bacterial and fungal species inhabiting the scalp subjects were identified by cloning and sequencing of the conserved ribosomal unit regions(16S for bacterial and 28S for fungal) and were further quantified by qualitative PCR.

The two main bacterial species found on the scalp surface were Propionibacterium acnes and Staphylococcus epidermis, while Malassezia restricta was the main fungal inhabitant. Dandruff was correlated with a higher incidence of M. restricta and S.epidermidis and lower incidence of P.acnes compared to the control population. These results suggested for the first time using molecular methods that dandruffs linked to the balance between bacteria and fungi of the host scalp surface.

Figure1.1

STRATUM CORNEUM AND DANDRUFF

The stratum corneum acts as a barrier against pathogenic invasion by microorganisms, toxic agents, Oxidants and ultraviolet radiations. However, the most fundamental requirement of the stratum corneum is to act a barrier to water loss, the so called epidermal permeability barrier. An essential mechanism that helps maintain water balance within the stratum corneum is the natural moisturing factor. The stratum corneum is a multilayered composed of a nucleated, flattened corneocytes surrounded by multiple lamellar sheets of lipids.

SIGNS AND SYMPTOMS

The signs and symptoms of dandruff are itchy scalp and flakiness. Red and greasy patches of skin and a tingly feeling on the skin are also symptoms. The cause is unclear but believed to involve a number of genetic and environmental factors.

As the skin layers continually replace themselves, cells are pushed outward where they die and flake off. It is hypothesized that for people with dandruff, skin cells may mature and be shed in 2-7 days, as opposed to around a month in people without dandruff. The result is that dead skins are shed in large,oily clumps, which appear as white or greyish flakes on the scalp, skin and clothes.

According to one study,dandruff has been shown to be possibly the result of three factors.

1.Skin oil commonly referred to as sebum sebaceous secretions.

2.The metabolic by-products of skin microorganisms (most specifically Malassezia yeasts).

3.Individual susceptibility and allergy sensitivity.

Dandruff scale is a cluster of corneocytes, which have retained a large degree of cohesion with one another and detach as such from the surface of the stratum corneum. A corneocyte is a protein complex that is made up of tiny threads of keratin in an organised matrix. Parakeratotic cells often make up part of dandruff

FORENSIC SIGNIFICANCE OF DANDRUFF

1. A Flake of dandruff or a hair will soon be all that is needed to depict the colour of the suspect's eyes, hair and skin ,and whether they have a full or a long face, and even the shape of their earlobes.
2. Dandruff particles contain a considerable portion of nucleated cells within the aggregates of nuclei-free corneocytes. Dandruff can identify the usual wearer of a garment or a person who has recently worn it.
3. Dandruff may also found around the collars and shoulders of upper body garments.
4. DNA extracted from dandruff can be used for criminal profiling by creating a database.

CHAPTER-II

LITERATURE REVIEW

2.1 Palanivel Sathishkumar,Johnson preethi,Fuad Ameen, 2016

Research topic was “Anti acne, antidandruff and anti-breast cancer efficacy of green synthesised silver nanoparticles using coriandrum sativum leaf extract”.

They found that the AgNPs synthesised by Coriandrum sativum were assessed against P.acnesMTCC 1951 and Malassezia.Flavanoids involved in the green synthesis of AgNP’s using Coriandrum sativum.AgNPs showed remarkable cytotoxicity on human breast adenocarcinoma(MCF-7) cell.The size of phyto-synthesised AgNPs was found to be 37 nm.

2.2 Seul-ong ohk,Chung-Ang University,2015

Research topic was “Heterologous expression and characterisation of CYP61A1”.

From dandruff-causing Malassezia globosa”.This study provide new insight into the biosynthesis of fungal sterols in M.globosa and a basis for the development of antifungal as potential therapeutic agents to treat dandruff.CYP61A1 is a sterolC-22 desaturase in dandruff causing M.globosa. CYP61A1 is a sterol C-22 desaturase in dandruff causing M.globosa. Recombinant CYP61A1 was heterologously expressed and purified.The tight binding to antifungal azoles suggests that CYP61A1 may be a therapeutic target. Study of CYP61A1 leads to a better understanding P450 for fungal sterols biosynthesis.

2.3 Alan.M.Magee,Michelle Breathnach,Stephen Doak,2018

Research topic was “Wearer and non-wearer DNA on the collars and cuffs of upper garments of worn clothing”.

This study shows that interpretable DNA profiles are more likely to be obtained from collars.Relevant when the issue is whether a person has worn or simply touched a garment, Investigates DNA transfers to collars and cuffs of worn clothing, demonstrates that DNA quantities assist in the interpretation of results, shows how the generated data can be applied to a casework.

2.4 P. Honnovar,A.K Gosh,S.Dogra,2018

Research topic was “Identification of Malassezia species by MALDI-TOF MS”.

After expansion of database”The present study was aimed to improve Matrix-assisted laser desorption ionization time flight mass spectrometry based identification of Malassezia species.

2.5 Teun Boekhout phD,Dawson jrPhD,2004

Research topic was “Skim diseases associated with Malassezia species”ee

This review focuses on the clinical, mycologic and immunologic aspects of the various skin disease associatedwith Malassezia.It also highlights the importance of individual Malassezia species in the different dermatologic disorders related to these yeasts. The yeast of the genus Malassezia have been associated with a number of diseases affecting the human skin such as Seborrheic dermatitis and dandruff.Although Malassezia yeasts are a part of the normal microflora,under certain conditions they can cause superficial skin infection.

2.6 Christina M. Gemmer,Yvonne M De Angelis,Bart Theelen,Teun Boekhout,2002.

Research topic was “Fast,Noninvasive Method for Molecular detection and Differentiation of Malassezia Yeast Species and A pplication of the method to Dandruff Microbiology’’

The method described here is expected to be useful in the clinical assessment of the Malassezia species associated with other fungal infections.

2.7 Georgios Gaitanis, Chrysoula Petrokilidou, Eleftherios Pavlou,2019.

Research topic was " The lipid profile of three Malassezia species assessed by Raman spectroscopy and discriminant analysis''. Raman spectroscopy is a sensitive and specific approach to adequately differentiate Malassezia cultures at species. The varied uptake and metabolic conversion of fatty acids by Malassezia spp was probed by Raman spectroscopy. Raman spectroscopy and partial least squares-discriminant analysis can classify Malassezia yeasts in culture media.

CHAPTER-III

AIM AND OBJECTIVES

3.1. AIM

The aim of the study is to extract DNA from dandruff for identification of individuals by amplifying the individual DNA by creating a database in the Criminal justice system.

3.2. OBJECTIVES

1. Identification of dandruff found on objects such as hats, collars etc. and linking to a crime.
2. Identification of dandruff cells from the scalp surface for disease findings.
3. Identification of dandruff cells for DNA profiling for creating a database in the criminal justice system.

CHAPTER-IV

MATERIALS AND METHODOLOGY

4.1. MATERIALS REQUIRED

1. QIA amp genomic DNA kits

2. Extraction kits

3. Amplification kits

4. EZtape

5. Sterile gloves

QIA amp genomic DNA kits

Qiagen's extensive range of QIA amp kits sets the the standard for DNA purification. QIA amp kits utilize the selective binding properties of the unique QIA amp silica membrane to isolate pure DNA. After lysis in an optimised buffer and adjustment of DNA binding conditions the sample is loaded directly onto a QIA amp spin column. DNA is bound to the silica membrane and contaminants are completely removed in 2 wash steps. Pure DNA is eluted in small volumes of a low-salt buffer or water, ready for use in downstream applications.

QIA amp genomic DNA Kits provide:

- Reliable DNA purification.
- Fast procedure and easy handling.

- Pure, ready-to-use DNA free of contaminants and enzyme inhibitors.
- High DNA recovery from a wide range of samples
- No phenol-chloroform extraction or time consuming alcohol precipitation.

EZ tape

Dandruff collected from EZ tape extract DNA directly using chelex-100 method with oscillation. Applying adhesive tape dandruff can be efficiently collected from rather large areas of fabrics, solid surfaces or skins.EZ tape an extraction tool for exfoliative cells was collected by EZ tape with other indefinite particles and DNA is extracted from it. But it still has many drawbacks such as low utilisation rate of sample, failing to recover alleles and easily obtaining mixture genotypes etc.

4.2. METHODOLOGY

1. Dandruff samples were collected from one individual for isolation of DNA. If the quantity of sample is more only then you will get more nucleated cells.

2. From the dandruff the no of nucleated cells were identified. From 1 n.g good DNA profiling of epithelial cells is identified.

3. Nucleated cells are extracted by using Organic extraction, or by using chelex-100 method.

4. Quantification of DNA using Real time PCR. From the real time PCR then you will get the degradation index and quantifiable amplified DNA. Real time PCR is commonly used to measure gene expression. The first step in real-time PCR is the conversion of RNA to complementary DNA. The next step uses fluorescent reporters and a PCR reaction to amplify and detect specific genes.

5. Amplification using different amplification kits. Primers and Buffers have forensically validated kit. Taq Polymerase enzyme is used for amplification. DNA was amplified using AGCU ex22 kit. In this the sample is heated so that the DNA denatures or separates into two pieces of single stranded DNA. Next an enzyme called Taq Polymerase builds two new strands of DNA using the original strand as template.

6. By using Capillary Electrophoresis using genetic analyser 3500 we can identify the no of repeats in individuals. The system's main components are a sample vial, source, destination vial, and capillary filled with electrolyte such as aqueous buffer, electrodes, high voltage power supply, detector, data output and handling device. Sample is introduced to the capillary via capillary action. The migration of the analytes is initiated by an electric field applied between source and destination. In Capillary Electrophoresis all ions positive or negative are pulled through the capillary in the same direction by electro osmotic flow. The output of the detector is sent to a data output and a computer.

7. The results were analysed with Gene mapper ID v3.2 software. This software improves workflow by streamlining the transition between data collection and analysis. They are presented in a table format that you can easily customize to meet your individual laboratory requirements. It helps to identify the cause of low quality allele calls.

4.3. EXTRACTION METHODS

1. Organic extraction

2. Chelex extraction

3. FTA paper extraction

4. Qiagen extraction

5. Automated extraction using different types of machines

4.3.1.ORGANIC EXTRACTION

In this extraction the key step is the removal of proteins, can often be carried out simply by extracting aqueous solutions of nucleic acids with phenol or chloroform.

PROCEDURE

1. Cell lysis buffer-lyses cell membrane, nucleic acid are intact, pellet nucleic.

2. Resuspended nucleic add SDS, Proteinase K. Lyse nuclear membrane and digest protein.

3. DNA released into solution is extracted with phenol-chloroform to remove proteinaceous material.

4. DNA is precipitated from the aqueous layer by the addition of ice cold 95% ethanol and salt.

5. Precipitated DNA is washed with 70 % ethanol, dried under vacuum.

4.3.2. ORGANIC EXTRACTION REAGENTS

1. Cell lysis buffer- Non-ionic detergent, salt, buffer, EDTA designed to lyse outer cell membrane of blood and epithelial cells but will not break down nuclear membrane.

2. EDTA- is used as a chelating agent of divalent cations such as Mg2+

3.Proteinase K- It is used to remove most of the protein by digesting with proteolytic enzymes such as proteinase K, which are active against a broad spectrum of native proteins before extracting with organic solvents. Proteins can be denatured by SDS or by heat.

4. Phenol-Chloroform- The standard way to remove proteins from nucleic acid solutions is to extract once with a 1:1 mixture of phenol and chloroform and once with chloroform

PROS

1. Yields relatively pure, high molecular weight DNA

2. DNA is double stranded good for RFLP.

CONS

1. Time consuming

2. Requires sample to be transferred to multiple tubes,increases risk of contamination.

3. Involves use of hazardous chemicals.

4.3.3. CHELEX EXTRACTION

Chelex 100 is an ion exchange resin composed of styrene divinylbenzene copolymers containing paired iminodiacetate ions that act as chelating groups in binding polyvalent metal ions such as Mg2+. The Chelex extraction process denatures double stranded DNA and yields single stranded DNA. A 5% solution of chelex is added to the sample and incubated at 56 degreesCelsius for 30 minutes. This step is used to lyse cells and remove contaminants and

inhibitors. The sample is heated at 100 degrees Celsius for 8 minutes causing the DNA to be denatured and destroying cellular proteins. After centrifugation the supernatant containing DNA is removed.

PROS

1. Relatively fast

2. Can extract directly from cloth

3. Removes PCR inhibitors

CONS

Results in single- stranded DNA not useful for RFLP

4.3.4. FTA PAPER EXTRACTION

It is a unique mixture of strong buffers, protein denaturants, chelating agents and a UV absorbing free radical trap. The reagents are impregnated into a cellulose-based filter matrix such as Whatman BFC180 OR 31ET paper.

PROS

1. Very quick

2. Useful for both storage and extraction

CONS

1. Not useful for RFLP

4.3.5. QIAGEN EXTRACTION

Purification using Qiagen magnetic particles technology is based on a simple bind-wash-elute procedure.Nucleicacids are isolated from lysates through binding to the magnetic particles in the presence of chaotropic salts which removes water from hydrated molecules in solution. In this procedure buffer conditions are allowing adsorption of DNA specific to the silica-gel membrane.

4.3.6. AUTOMATED EXTRACTION USING DIFFERENT TYPES OF MACHINES

Robotic liquid handling technology in automated DNA extraction systems can streamline the tasks involved in extracting DNA from a sample such as serial dilution and cherry picking. Systems typically also include functions such as shaking, temperature control and PCR protocols.

INSTRUMENTS

1. Real time PCR- also called RT-PCR OR Quantitative PCR or QPCR. The key feature in RT-PCR is the amplification of DNA is detected in real time PCR is in progress by the use of fluorescent reporter. The fluorescent reporter signal strength is directly proportional to the number of amplified DNA molecules. It is used to measure gene expression. It is best suited for studies of small subsets of genes. The principle of real time PCR is the amount of the nucleic acid present into the sample is quantified using the fluorescent dye.

2. Capillary Electrophoresis using Genetic analyser 3500- is the separation of molecules using electricity and a very small tube called capillary. It is a technique that can be used to analyse and separate proteins. It has a high resolving power that exceeds other electrophoretic techniques and is capable of distinguishing between proteins that differ only slightly in amino acid composition or glycosylation.

3. Gene Mapper is a flexible genotyping software package that provides DNA sizing and quality allele calls for all life technologieselectrophoresis-based genotyping systems. The software uses Process Quality Values for automated identification that reduces data review time for high throughput genotyping.

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CHAPTER-V

RESULTS AND CONCLUSIONS

5.1. RESULT

In this study the DNA is being identified, extracted, quantified and amplified by capillary electrophoresis using genetic analyser.

The following result is being obtained by using gene mapper software.

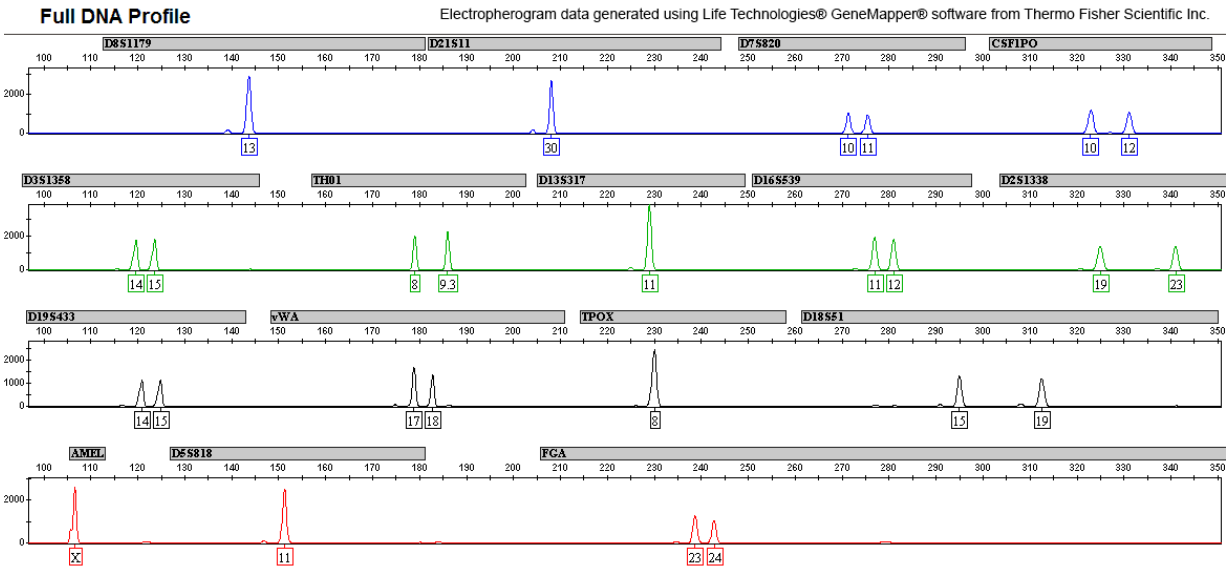

Figure 5.1.

5.2. CONCLUSION

From the present study I have concluded that DNA can be extracted from dandruff by identifying the no of nucleated cells present in it. By identifying the no of repeats present in an individual we can differentiate the homozygous and heterozygous loci of an individual.

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