Fungal microbiome (mycobiome) and virome of the lacrimal sac in patients with PANDO: the lacriome paper 5

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ABSTRACT

Purpose To study the fungal microbiome (mycobiome) and the virome of the lacrimal sacs in patients with primary acquired nasolacrimal duct obstruction (PANDO).

Methods A prospective study was performed on 10 consecutive samples of the lacrimal sac contents obtained from patients with PANDO. The samples were obtained from the lacrimal sacs under endoscopy guidance and immediately transported on ice to the laboratory. Following DNA extraction and library preparation, a whole shotgun metagenome sequencing was performed on the Illumina platform (NOVASEQ 6000). The fungal internal transcript spacer analysis was performed using the PIPITS v2.7 . The viral taxonomy profiling was performed using Kraken2 against the virus database.

Results The taxonomic hit distribution across the lacrimal sac samples showed rich fungal diversity (4 phyla, 12 classed, 21 families and 26 genera). The major phyla were *Ascomycota* and *Basidiomycota,* and the key genera identified were *Alternaria, Hyphopichia, Malassezia, Aspergillus* and *Epicoccum*. The virome analysis identified 13 phyla, 15 classes and 27 families. The viruses were commonly from the families *Poxviridae, Retroviridae, Siphoviridae* and *Myoviridae*, *Poxviridae* being the most prevalent family. The BeAn 58058 virus, a member of the *Poxviridae* family, was the most abundant in all the samples.

Conclusion The present study is the first whole metagenome sequencing exclusively of the fungal microbiome and virome from the lacrimal sacs of patients with PANDO. The lacrimal sacs harbour diverse fungal and viral communities with distinct ecosystem dynamics. Further studies of their functions and interactions with the hosts would provide valuable insights.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The Lacriome is an integrated approach to study cellular and molecular profiles within specific lacrimal ecosystems.

⇒ Metagenomic studies of the lacrimal drainage have elucidated the bacterial taxa and their involvement in different metabolisms and environmental information processing within the lacrimal ecosystem.

WHAT THIS STUDY ADDS

⇒ The study elaborates on the rich diversity of the fungal and the viral taxa within the lacrimal drainage.

⇒ The key fungal genera were Alternaria, Hyphopichia and Malassezia. The viruses were mostly from the family Poxviridae.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE, OR POLICY

⇒ The study is an incremental step forward in the efforts to understand the functional roles and interactions of fungi and viruses with the bacteria and the host tissues in promoting disease pathogenesis in primary acquired nasolacrimal duct obstruction.

INTRODUCTION

The lacrimal drainage system harbours several commensals and potentially pathogenic organisms. Primary acquired nasolacrimal duct obstruction or PANDO is a common lacrimal drainage disorder.[1] Several factors are implicated in its aetiopathogenesis, and microbial influence is one of them.[2 3] It is important to characterise the microbial world of the lacrimal drainage at a genome level and assess their functions and interactions with the host tissues to understand the disease pathogenesis better. One step in such a quest is to study metagenomics in the context of the Lacriome, which is an integrated approach to assess molecular and cellular profiles within a specific lacrimal ecosystem.[4]

Very few studies assessed the metagenomics of the lacrimal drainage and found a rich diversity of bacterial species.[5–7] Assessment of the microbial functions demonstrated their involvement in several metabolisms and environmental and genetic information processes.[6–8] The taxonomic hit distribution from the lacrimal sac samples of earlier studies demonstrated, as expected, an overwhelming presence of the bacteria (95.12%–98.94%).[5] The fungal and viral components are often ignored since they are comparatively a minority and do not aggressively register their presence. Besides, their analysis would need a more detailed and specific sequencing. The evolution of high-throughput sequencing of specific fungal internal transcribed spacer regions 1 and 2 (ITS1 and ITS2) has revolutionised the assessment of fungal taxonomy and its functional diversity.[9] Similarly, many of the virus particles (10 to the power of 13) per human body are unknown and dark matter. Metagenomics is shedding light on this aspect to enhance our understanding of these microbes.[10]

The present study, a first of its kind, has attempted to assess the metagenomics of the fungi and viruses

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present in the lacrimal sac, with a long-term aim to understand their functional roles and interactions with bacteria in promoting disease pathogenesis in PANDO.

METHODS

Sample collection

The samples were collected immediately after a full-length lacrimal sac marsupialisation during the dacryocystorhinostomy. None of the patients had a history of recent (2 weeks) respiratory or acute lacrimal infections or topical or systemic antibiotics use. Sterile flocked swabs were directly guided into the opened lacrimal sac endoscopically. The swabs were rotated back and forth within the lumen and on the surfaces of the luminal mucosa to obtain the contents adequately. Care was taken to avoid contact with the septum or lateral wall during the retrieval to minimise contamination. The Eppendorf tubes were immediately placed in ice containers and transported to the laboratory in a secured cold chain.

Fungal and viral metagenome sequencing

The whole shotgun metagenome sequencing technique was partly like the one published earlier.[5 6] In brief, following the DNA extraction and quality assessment, the Qubit 4.0 fluorometer and DNA HS assay kit (Thermo Fischer Scientific, Massachusetts, USA) were used to quantify the final libraries. The sequencing was performed on the Illumina NOVASEQ 6000 platform (Illumina, California, USA). Analysis of fungal internal transcribed spacer (ITS) sequences from the Illumina sequencing platform was performed using PIPITS v2.7 (https://github.com/hsgweon/pipits).[11] The Illumina fastq sequences were assembled with VSEARCH.[12] The classic tabular OTU table was converted into a BIOM format, and taxonomy was assigned with UNITE (RDP Classifier). Heatmaps and Taxonomic bar plots based on percent frequency were plotted using the microeco R packages (https://chiliubio.github.io/microeco/). Krona chart was plotted using psadd R packages. The viral taxonomy profiling was done using Kraken2 against the Virus database.[13] The Kraken2 output was converted to BIOM files using kraken-biom[14] and analysed using Microeco R package.[15]

RESULTS

The mean age was 52.7 years (range: 33–69 years). There was a female gender predominance (70%, 7/10). Epiphora with the discharge was the universal complaint. None of the patients had acute dacryocystitis. All 10 patients had successful surgical outcomes with endoscopic dacryocystorhinostomy at the sixth month follow-up.

The taxonomic hit distribution across the lacrimal sac samples showed rich fungal diversity (4 phyla, 12 classes, 21 families and 26 genera). The major phyla identified were *Ascomycota* and *Basidiomycota*. Several genera were recognised; the key ones were *Alternaria, Hyphopichia, Malassezia, Aspergillus* and *Epicoccum* (figure 1). Numerous species and several operational taxonomic units were identified and included *Alternaria_OTU109, K_fungi OTU6, Malassezia globosa, Hyphopichia_OTU170, 176, and 228, Epicoccum_OTU118, Capnodiales_OTU147, Rhizopus_OTU90, Debaromyces_OTU123, Aspergillus_OTU 101, 107, and 119, Wallemia mellicola, Xeromyces bisporus* and *Saccharomycopsis malanga* (figures 2 and 3).

The virome analysis identified 13 phyla, 15 classes and 27 families. The top four abundant phyla were the *Nucelocytoviricota, Artverviricota, Uroviricota* and *Peploviricota* (figure 4). The viruses were commonly from the families *Poxviridae, Retroviridae, Siphoviridae and Myoviridae* (figure 5). The BeAn 58058 virus, a member of the Poxviridae family, was the most abundant in all the samples (figures 5 and 6). The other common species identified *include human endogenous retrovirus K, proteus virus Isfahan, Shamonda orthobunyavirus, Simbu orthobunyavirus* and *Choristoneura fumiferana granulovirus* (figure 6).

DISCUSSION

The current study performed the first whole metagenome sequencing and analysis of fungi and viruses isolated from lacrimal sacs of patients diagnosed with primary acquired nasolacrimal duct obstruction. The major fungal phyla identified were *Ascomycota* and *Basidiomycota*, and the key genera were *Alternaria, Hyphopichia, Malassezia, Aspergillus* and *Epicoccum*. The key viral families identified were *Poxviridae, Retroviridae,*

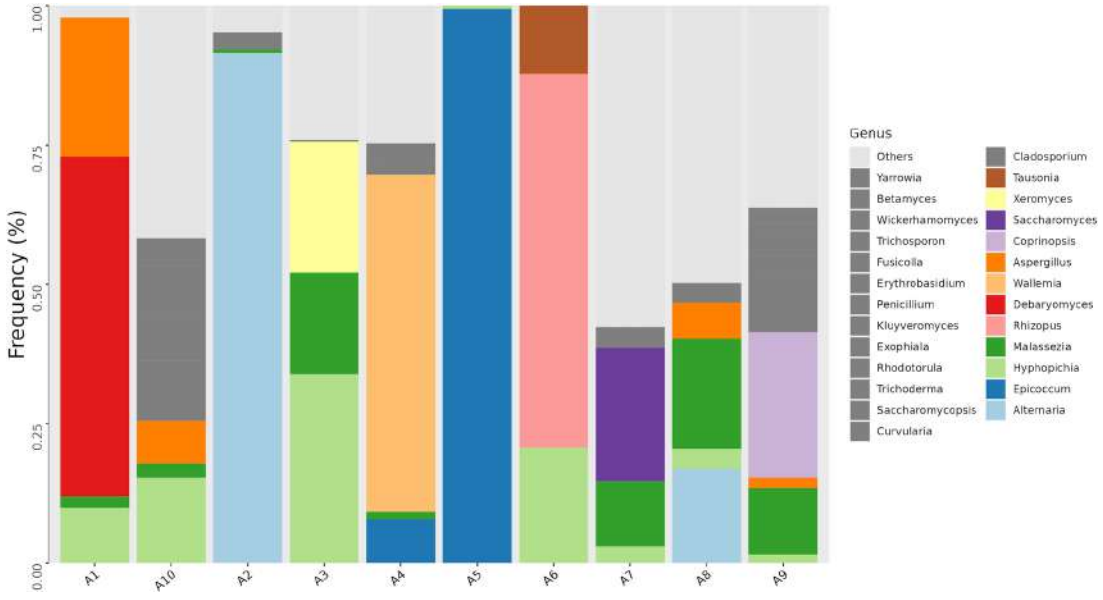

Figure 1 Taxonomic bar plot of the lacrimal sac samples showing key fungal genera.

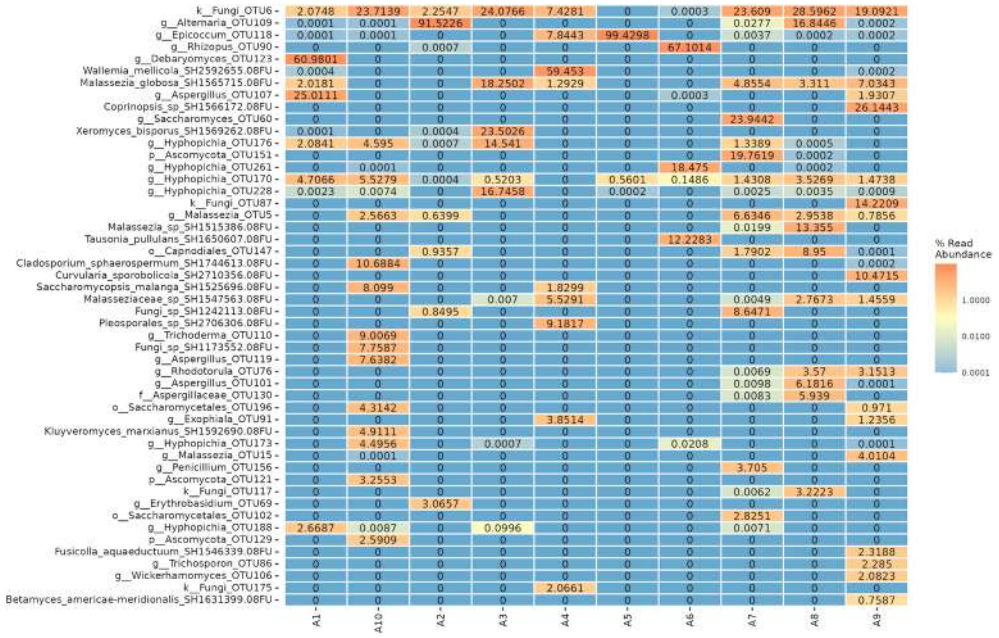

Figure 2 Hierarchically clustered heatmap showing relative abundance of fungal taxonomic composition of the samples at species level. Relative abundance is shown in coloured log scale.

Siphoviridae and Myoviridae. BeAn 58058 virus of the poxviridae family was the most abundant across all the lacrimal sac samples.

The fungal kingdom is composed of approximately 3.8 million species and is known to influence ecosystems and the overall food chain.[9] The evolution of high-throughput sequencing is significantly helping in our understanding of their taxonomic and functional diversity. It is now possible to deep sequence the fungal internal transcription spacer regions 1 and 2 (ITS1 and ITS2) fully.[9] The human mycobiota has been studied in the oral cavity, lungs, gut and vagina in health and disease and is known to exhibit rich diversity.[9 16–20] Key genera in these areas include *Candida, Malesezzia, Cladosporium, Penicillium, Alternaria, Saccharomyces, Eurotium, Aspergillus, Cryptococcus, Galactomyces, Debaryomyces, Mucor, Trichosporon and Schizophyllum*.[9 16–20] The gut mycobiome is comparatively less stable than others and significantly influenced by environmental factors.[16] The lung mycobiome is known to be altered in patients with asthma and cystic fibrosis.[17 18] Dysbiosis of oral mycobiome is implicated in the causation of gingivitis and caries.[19] Occupational exposure to fungal bioaerosols is being explored for their roles as a biohazard.[20] The key genera found in the present study from the lacrimal sacs include *Alternaria, Hyphopichia, Malassezia, Aspergillus* and *Epicoccum*. The lacrimal drainage systems traverse the lateral wall of the nose up to the inferior meatus; hence, some overlap with the sinonasal microbiome would not be surprising.[21]

The human virome largely remains uncharacterised, although advanced metagenomic techniques are slowly helping unravel the viral dark matter. Based on the existing knowledge, the healthy human virome is composed of three components: first, those which enter the body through food or air and do not replicate;

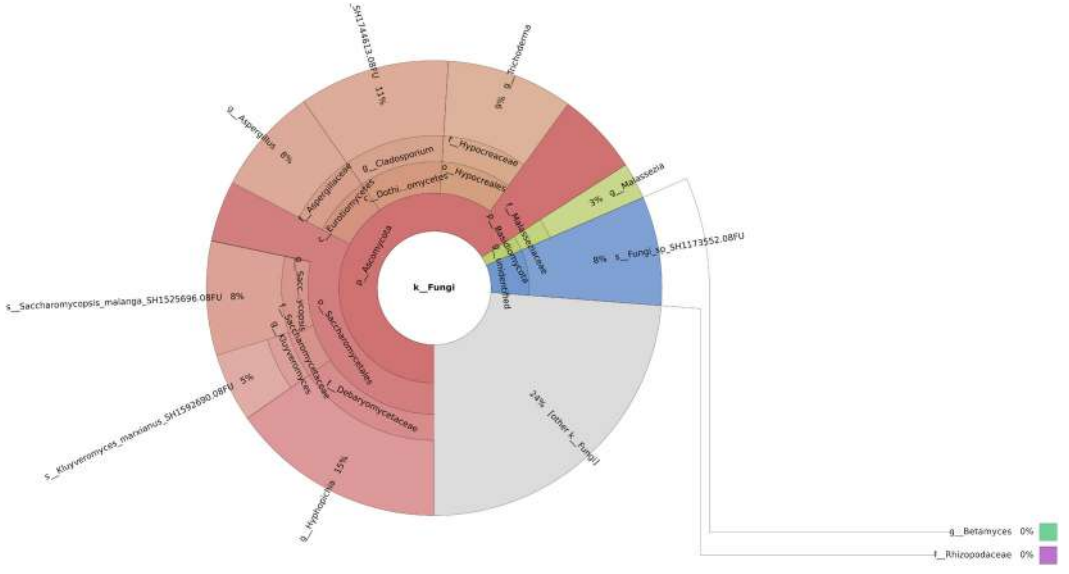

Figure 3 Krona plot demonstrating fungal taxonomic levels from the superkingdom to the species level in a sample from the lacrimal sac.

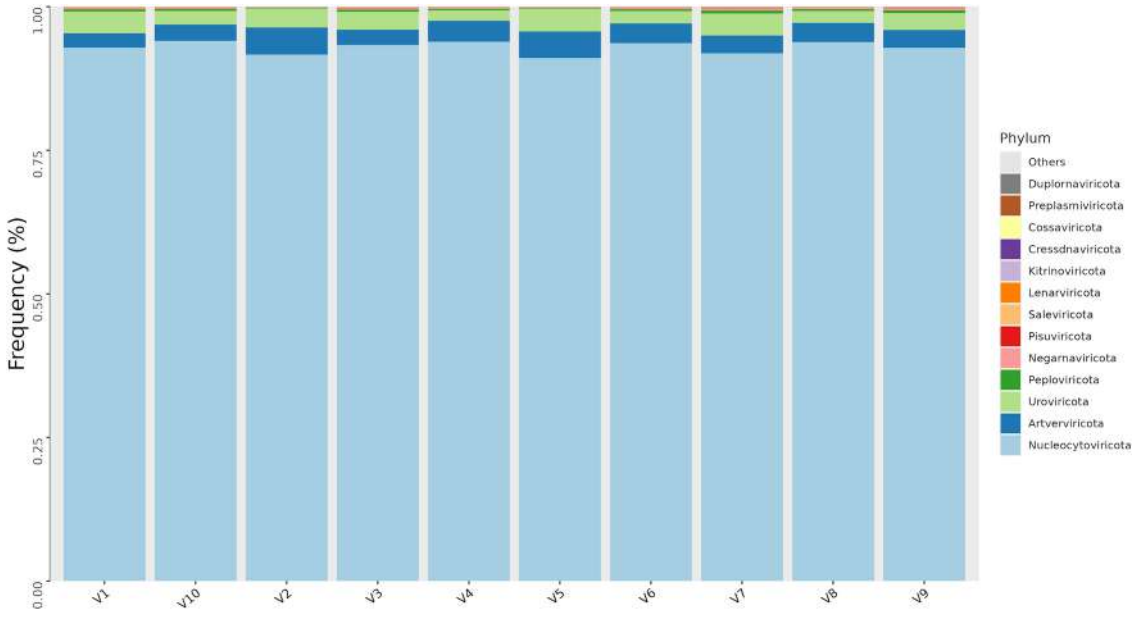

Figure 4 Taxonomic bar plot showing percentage relative reads abundance of major viral phyla across the lacrimal sac samples.

second, those which infect other microbes (eg bacteriophages); and finally, those that replicate and persist in humans. Several metagenomic surveys from various locations like the gut, lungs, oral cavity, vagina, skin and urinary system have documented the presence of several families, including *Herpesviridae, Papillomaviridae, Anelloviridae, Adenoviridae, Picornaviridae, Calciviridae, Redondoviridae, Circoviridae* and *Polyomaviridae*.[10 22–26] Several factors like anatomical location, diet and age can influence the virome. Interestingly, alterations in virome have been documented in conditions like diabetes, hypertension, inflammatory bowel disease and malignancies.[26–29]

The major components of a virome are believed to be phages. The major taxa of phages are Caudovirales (tailed phages), and several phages (Siphoviridae, Myoviridae, Microviridae, Podoviridae and Inoviridae) have been documented from different locations of the human body.[10] The present study also identified several members of the taxa Caudovirales, Siphoviridae and Myoviridae. It is important to understand this finding in the context of PANDO, where tear stasis and subsequent bacterial proliferation are common. Phages can have a variable relationship with the hosts and show an increase in their numbers in response to bacterial proliferation. They are known to modify and regulate bacterial colonies and contribute to microbiota homeostasis.[30] The question to answer here in the context of PANDO is twofold. First, is this relationship mutual where both are proliferating, and second, are the viruses infecting bacteria and trying to help the host by preventing an acute infection? Suppose the latter is found to be true. In that case, it may be possible to use the phage cocktail (eg, in bacterial infections and Crohn's disease)[31 32] at least in patients with recurrent and recalcitrant acute dacryocystitis secondary to multidrug resistant or host immunodeficiencies.

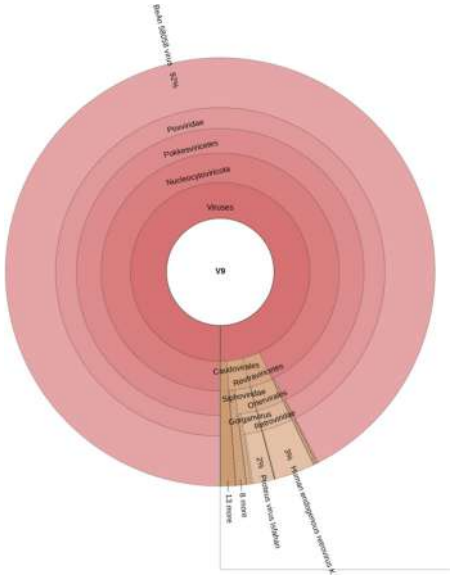

Figure 5 Krona plot demonstrating viral taxonomic levels from the superkingdom to the species level in a sample from the lacrimal sac.

An exciting aspect of the present study was the abundant presence of BeAn 58058 virus, a member of the *Poxviridae* family, across all the lacrimal sac samples. Poxviruses are complex double-stranded DNA viruses and are primarily zoonotic. *Variola* virus, which caused smallpox, is an example of a poxvirus. BeAn 58058 was first isolated from rodents in 1963, which are the natural hosts.[33] Very few studies detected BeAn 58058 from humans. An earlier study on sputum from patients with COPD showed BeAn 58058 virus abundance in all the samples,[34] like in the present study. Several theories have been proposed to explain this finding.[34] First, BeAn 58058 is an ancient virus that could have integrated into the human genome over time. However, there is no concrete evidence for this possibility. Second, since BeAn 58058 is closely related to the *Vaccinia* virus (used for the smallpox vaccine), it could be a DNA artefact of the vaccine.[34] However, three patients in the present series were not vaccinated, and the theory does not entirely explain this. Third, since rodents are ubiquitous urban pests, humans could acquire the virus via environmental exposure. A rare possibility of it being a contaminant cannot be ruled out. It would be interesting to ascertain the origins and the role of BeAn 58058 within the lacrimal ecosystem.

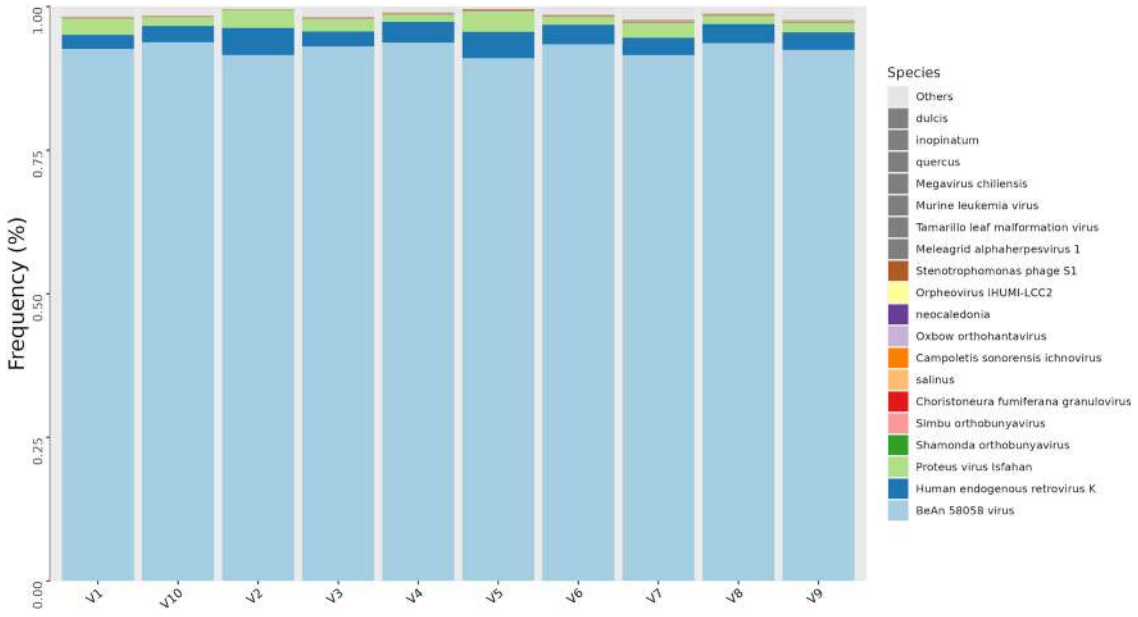

Figure 6 Taxonomic bar plot showing percentage relative reads abundance of lacrimal sac virome at the species level.

Human endogenous retroviruses (HERV) belong to the kingdom Pararnavira and employ reverse transcriptase for their replication. About 3000 HERVs are integrated into the human genome and constitute about 8% of it.[35] The present study found that 2%–5% of the studied virome of the lacrimal sac was contributed by the HERV-K, a bonafide member of the healthy human virome. HERV-K is known to perform important biological functions, including the modulation of innate immunity.[22 36] It would be interesting to delve deep into this to understand their role in the possible pathogenesis of PANDO.

The current study's limitations include smaller sample size, limitations of the ITS sequencing, lack of data from normal lacrimal sacs, microbial variations among samples and the rare possibility of contamination during sample collection. However, the strengths are detailed metagenome assessment of the fungal and viral microbiota.

In conclusion, the current study demonstrated metagenomics of the fungi and viruses isolated from the lacrimal sacs. Further focused work is needed to ascertain their functions and interactions with the bacteria and the host tissues.

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Competing interests None declared.

Patient consent for publication Not applicable.

Ethics approval Institutional review board and ethics committee approval of the L.V. Prasad Eye Institute was obtained (Ref LEC-03-2015-028). The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all the participants.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request.

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OPEN

Nasolacrimal Duct Coronary Stent Recanalization (NCR): First Cadaver Experience and Its Potential as an Alternative to DCR

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Purpose: To investigate the feasibility of implanting a drug-eluting cobalt-chromium alloy coronary stent in the nasolacrimal ducts (NLDs) of human cadavers.

Methods: The pilot study was carried out in 5 NLDs of 4 adult human cadavers. Sirolimus-eluting coronary stents of 2 mm in width and lengths of 8 and 12 mm, which were mounted on balloon catheters, were used. Following dilatation of the NLDs, the balloon catheters were introduced into the NLDs under direct endoscopy guidance. The stents were delivered following dilatation of the balloon to 12 ATMs and secured in a locked (spring out) position. The balloon is then deflated and securely extubated. The dacryoendoscopy confirmed the stent position. The lacrimal system was then dissected to assess several key parameters like the uniformity of the NLD expansion, anatomical interactions of the NLD mucosa with the stent rings and struts, integrity of the soft and bony NLD, stent movement on mechanical push and pull, and ease of manual removal.

Results: The cobalt-chromium alloy coronary stents could be delivered with ease and secured in the cadaveric NLDs. Its position was confirmed by a dacryoendoscopy and later by the direct NLD dissection. The NLD was uniformly dilated 360° with a wide and uniform lumen. NLD mucosa was noted to be uniformly distributed in spaces between the stent rings without influencing the expanded lumen. Following the lacrimal sac's dissection, the NLD stent showed significant resistance to downward movement but could be easily retrieved with forceps. The 12-mm stents could reach the near total length of the NLD with good luminal expansion. The integrity of the bony and soft-tissue NLD was maintained. The learning curve is shallow if the surgeon is adept with the techniques of balloon dacryoplasty.

Conclusion: Drug-eluting cobalt-chromium alloy coronary stents can be precisely deployed and secured within the human NLDs. The study is the first of its kind to demonstrate the technique of NLD coronary stent recanalization in human cadavers. It is a step forward in the journey to evaluate their use in patients with primary acquired NLD obstructions and other NLD disorders.

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The quest to develop minimally invasive lacrimal procedure for several lacrimal drainage disorders continues, and primary acquired nasolacrimal duct (NLD) obstructions (PANDO) are not an exception. PANDO is a common disorder and accounted for nearly half of all the lacrimal drainage disorders in a large series of 20102 patients.[1] Although precise etiopathogenesis is still elusive,[2–5] dacryocystorhinostomy via external or endoscopic approaches remains the mainstay of management, with success rates beyond 90%.[6–8] Several attempts with minimally invasive alternatives have been tried over the last 2 decades for recanalization of the NLD followed by intubation but much remains to be desired for them to come close to the success rates that DCR surgeries offer.[9,10]

The use of coronary angioplasty balloons in the management of congenital NLD obstructions, punctocanaliculoplasty, and revision dacryocystorhinostomy has the potential to significantly impact the health economics of lacrimal drainage disorders in developing countries like India.[11–14] Following a good experience with coronary balloon-assisted dacryoplasty, it is logical to explore the use of coronary stents for NLDs. Daigle et al[15] used coronary stents in the canaliculi of a single human cadaver and found them to be precisely deliverable with seamless integration of the stent with the canalicular tissues.

The present study is the first to describe a surgical technique of NLD coronary stent recanalization (NCR) to deliver drug-eluting cobalt-chromium alloy coronary stent into the NLDs of human cadavers. The purpose of the present work was to assess the feasibility and precision of delivering metallic coronary stents by the technique of NCR to develop more promising minimally invasive modalities for the management of PANDO.

METHODS

The study was approved by the Institutional ethics committee and adhered to the tenets of the Declarations of Helsinki. The pilot study was carried out in 5 NLDs of 4 adult human cadavers. Sirolimus-eluting coronary stents of 2 mm in width and lengths of 8 and 12 mm, which were crimped and mounted on balloon catheters, were used.

Characteristics of the Coronary Stent. The coronary stent used was Yukon Choice Flex^R (Translumina Therapeutics LLP, Uttarakhand, India). The delivery system is a rapid exchange catheter with a balloon at its distal tip. The stent material was L 605 cobalt-chromium alloy mounted on a semicompliant polyamide balloon and is 1 mm longer (0.5 mm on either side) than the stent. The catheter shaft has two platinum-iridium radiopaque markers to indicate the balloon position. The strut thickness was 68 μm, and the strut width was 78 μm. The outer diameter of the catheter shaft was 0.825 mm. The nominal balloon inflation pressure was 11×10^5 Pascals or 10.8 ATM or 11 Bar. The rate burst pressure was 16×10^5 Pa or 15.79 ATM or 16 Bar. The drug coating of

the stent was a combination of biodegradable resomer and shellac resin mixed with sirolimus. The nominal sirolimus dosage for the 2 × 8 mm and 2 × 12 mm stents used were 100 and 150 µg, respectively. The metallic platform of the stent was MR-conditional and can be scanned safely postimplantation in a 3-Tesla magnetic field with a gradient field of 500 Gauss/cm (Fig. 1A and B).

Surgical Technique of NCR. The puncta and canaliculi were dilated, followed by the passing of Bowman's probe into the NLD under endoscopy guidance. A 2.75 × 10 mm coronary angioplasty balloon (SPALNO, Cardiomac, Haryana, India) was used to dilate the NLD as per standard protocols of balloon dacryoplasty, as described earlier.[11] Following dilatation of the NLDs, the coronary stent mounted on the balloon catheter was introduced into the NLD under direct endoscopy guidance (Fig. 1C). The catheter should be positioned in a way that the stent should not be visible at the NLD opening of the inferior meatus but instead 2–3 mms proximal to it within the duct (Fig. 1D). Once the location is secured, the balloon is inflated to a pressure just beyond the nominal pressure but much below the rate burst pressure. In the present series, it was at 12 ATMs. Once inflation is performed, along with the balloon, the stent expands to 2 mms and springs out into a locked position. The balloon is then gently deflated, and the whole delivery system sans the stent is retrieved. Following the successful delivery of the stent, the delivering balloon becomes bare (Fig. 2A). A 0.8-mm dacryoendoscope (Karl Storz, Tuttlingen, Germany) is then used to ascertain the location and security of the delivered stent (Fig. 2B).

CADAVERIC DISSECTION

Following the stent delivery, the lacrimal system was dissected to expose the NLD and to study several key parameters such as the ease of delivery, security of the delivered stent, uniformity of the NLD expansion, anatomical interactions of the NLD mucosa with the stent rings and struts, the integrity of the soft and bony NLD, stent movement on mechanical push and pull, and ease of manual removal and complications during the procedure.

RESULTS

The cobalt-chromium alloy coronary stents could be easily delivered and secured in the NLDs of all the cadavers and its position was confirmed by a dacryoendoscopy and later by the direct NLD dissection (Fig. 2B). The NLD was uniformly dilated 360° with a wide, uniform, and consistently circular lumen throughout its length (Fig. 2C). NLD mucosa was noted to be uniformly distributed in spaces between the stent rings without influencing the expanded lumen (Fig. 2D). The anatomical integration of the NLD wall with the stent rings was excellent without any evidence of a recoil. The integrity of the bony and soft-tissue NLD was maintained (Fig. 3A and B). The bony NLD had no cracks or fractures, and it could comfortably house the uniformly dilated NLD (Fig. 3A). Following the lacrimal sac's dissection, the NLD stent showed significant resistance to downward movement but could be easily retrieved with forceps (Fig. 3C and D). The 12-mm stents could reach the near total length of the NLD with good luminal expansion (Fig. 2D). No untoward complications were noted. The learning curve is shallow if the surgeon is adept with the techniques of balloon dacryoplasty.

DISCUSSION

The current study is the first proof of principle in human cadavers of the feasibility and precision in delivering the drug-eluting cobalt-chromium alloy coronary stents in the NLDs by

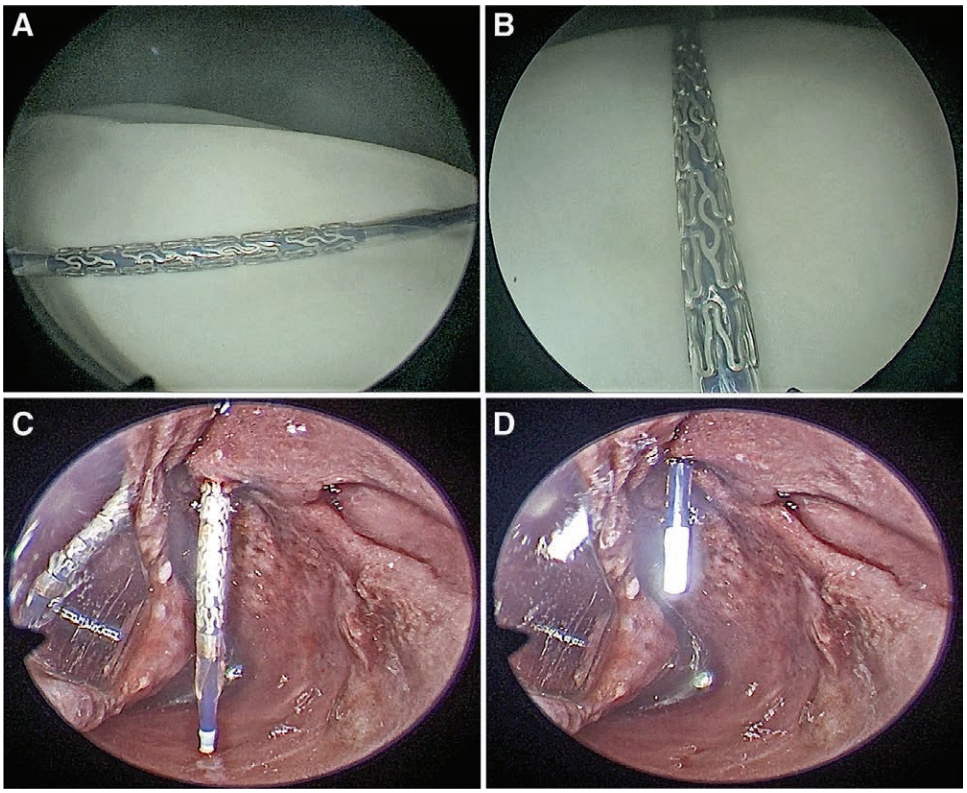

FIG. 1. Technique of NCR. **A,** External image demonstrating a coronary stent mounted on the coronary balloon catheter. Higher magnification image of the coronary stent. **B,** Note the crimped cobalt-chromium alloy stent with its rings, struts and connectors. **C,** Endoscopy image of the left inferior meatus showing the passage of stent through the NLD and (**D**) the final position of the catheter before delivering the stent into the NLD.

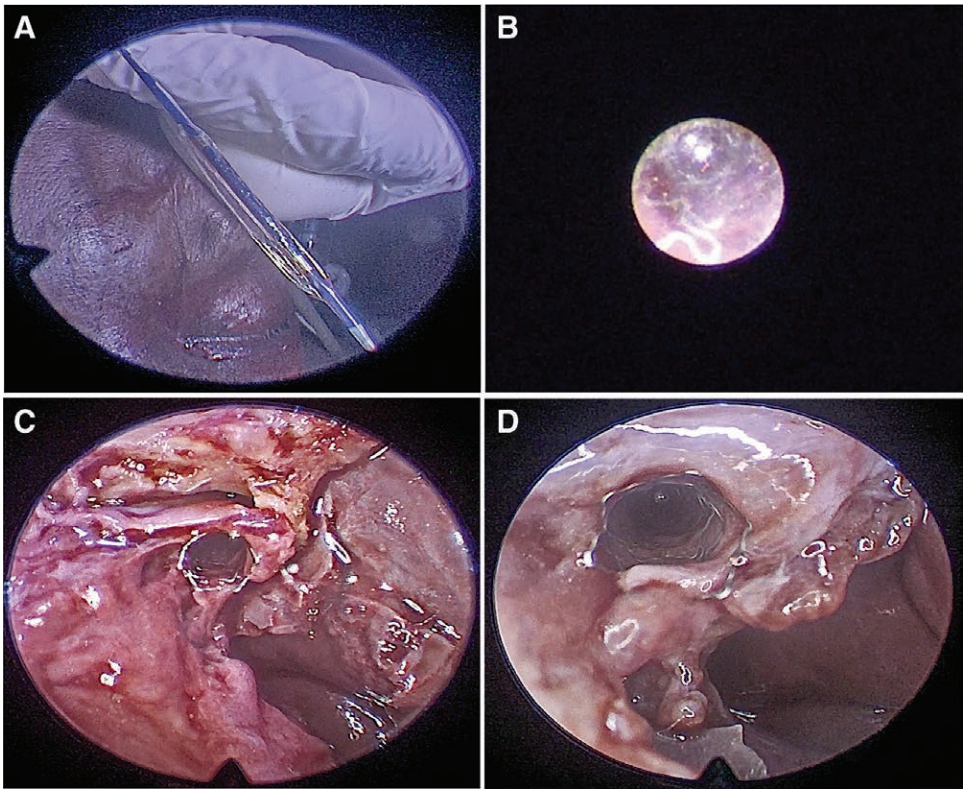

FIG. 2. Technique of NCR. **A,** External image demonstrating the deflated bare balloon following delivery of the stent into the NLD. **B,** Dacryoendoscopy image showing the springed out and locked stent within the NLD. Cadveric dissection image showing an end-on view of the proximal NLD opening with locked stent within the entire NLD. **C,** Note the uniformly maintained circular shape of the NLD. Higher magnification image of the stent within the NLD. **D,** Note the excellent integration of the stent with the NLD lumen.

the herein-described technique of NCR. The location and security could be well assessed following the procedure. Cadaveric dissections of the lacrimal sac and NLDs confirmed several safety parameters that were studied. The NCR technique could have potential implications in the management of PANDO, syndromic NLD stenosis, and functional NLD obstructions.

Percutaneous transluminal coronary angioplasty using the plain old balloon angioplasty was first introduced by Andreas Gruentzig in 1977.[16] The high rates of recurrence and vessel remodeling with plain old balloon angioplasty were big disappointments.[17] This led to the development of stents, the first of which was deployed by the French surgeon Jacques Puel in 1986.[18] Till the turn of the century, several bare metal stents were developed.[17,19,20] The modern drug-eluting stents used in the present study have designs that maintain a consistent circular lumen and prevent the acute recoil that otherwise happens with a plain balloon procedure. The in-stent restenosis has also been reduced with newer designs, drugs, and delivery agents. Coronary stenting remains the most performed medical procedure globally and its indications have significantly expanded for several peripheral vessels.[17,21]

Bothra et al evaluated modern coronary balloon catheters for dacryoplasty as a low-cost alternative to traditional balloon dacryoplasty catheters.[11] They found that using coronary balloons reduced the cost by two-third and had several other advantages over the traditional balloons, such as a large range of diameters and lengths, compliant and semicompliant variants, high rate burst pressure, and an overall better profile.[11] The same group showed its efficacy and safety in congenital NLD obstructions and cases of revision DCR's.[11,12,14] With this experience with coronary balloons, the next logical step was to evaluate the feasibility and develop surgical techniques to precisely deliver the drug-eluting cobalt-chromium stents into the NLD.

Sirolimus is a macrolide compound produced by the bacteria *Streptomyces hygroscopicus* and is known to have anti-inflammatory and antifibrotic properties. It binds to immunophilin, FK binding protein-12, and subsequently inhibits the activation of "mammalian target of rapamycin" and prevents the progression of the cell cycle from G1 to the S phase.[22] Sirolimus inhibits T lymphocyte activation and vascular smooth muscle cell proliferation. Sirolimus has been used in several childhood disorders and orbital lymphatic malformations. Sirolimus-eluting stents have been extensively used in coronary and peripheral vascular disease and have shown promise as urinary tract stents.[17,23,24]

The coronary stent used in the present study had a coating of biodegradable Resomer and shellac resin mixed with sirolimus. The abluminal surface of the stent has the coating, and nominal dosages of sirolimus vary with the diameter and length of the stent. The nominal sirolimus dosage for the 2×8 mm and 2×12 mm stents used were 100 and 150 µg, respectively.

The current techniques of NLD recanalization have several limitations that could be improved.[10] One, microdrills, probes, or similar techniques are used to trephine or mechanically dislodge the fibrous tissue or overcome the fibrous obstruction. This potentially risks creating trauma, false passages, and a proinflammatory environment that can lead to restenosis or reobstructions. Using balloon dacryoplasty alone can have the risk of acute recoil which may not be preventable by a silicone stent alone. The silicone stents in the NLDs may also affect the proximal lacrimal drainage (ocular irritation, punctal cheese wiring, premature prolapse, or extrusion), where it may not be needed as in cases of PANDO.[25] The data on the development

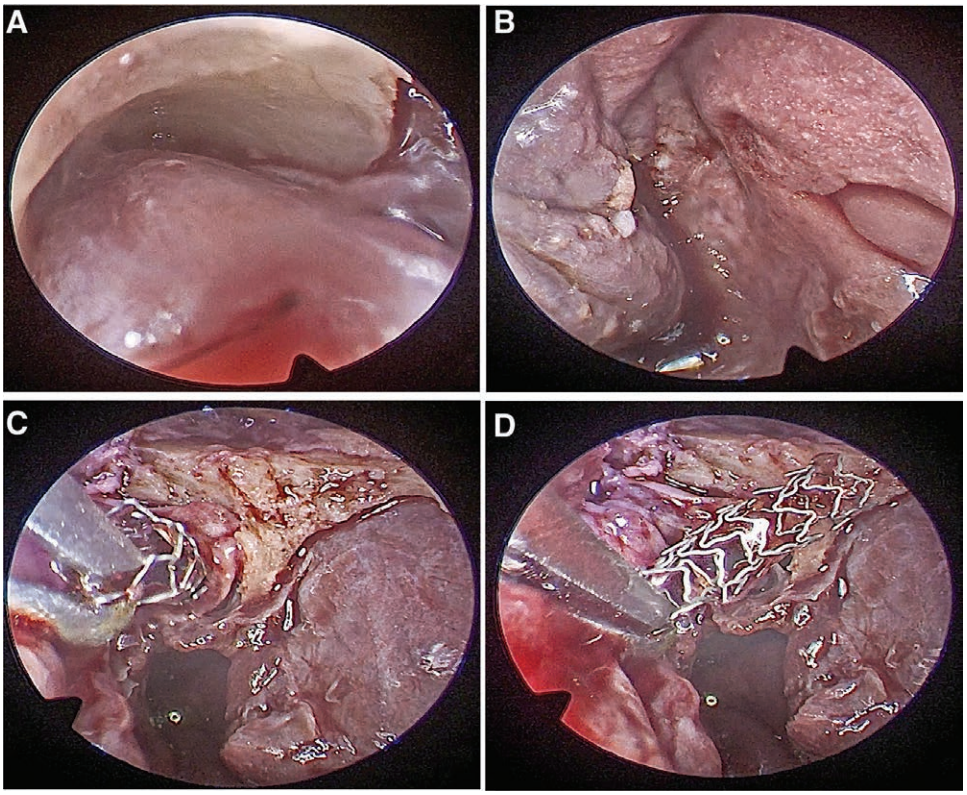

FIG. 3. Technique of NCR. **A,** End-on endoscopy view of the bony NLD. Note its integrity and that of the soft-tissue NLD that it houses. Endoscopy image of the left inferior meatus with the stent in situ. **B,** Note the normal appearance of the NLD and its opening reflecting minimal interference with the normal anatomy. **C,** Cadaveric dissection image demonstrating the removal of the stent from the NLD with the help of forceps and following its complete removal (**D**). Note the design of the expanded locked stent and compare it the pre-expansion stage in Figure 1A and B.

of biofilms on the silicone stents and the possible mounting of inflammation on the host tissues make them less desirable if alternative options are available.[26,27]

There are several limitations of the present study. This cadaveric study has a small sample size, which is common in a pilot study. The possibility of false passages, failure to completely recanalize the tough fibrosed segments in PANDO and unknown precise indications need to be further explored. The actual effects in living humans, long-term effects on the NLD mucosa, and long-term outcomes are unknown. Nevertheless, this study provides a proof of the principle of coronary stent implantation with precision in the NLD and is a step forward toward its implantation in living humans.

In conclusion, drug-eluting cobalt-chromium alloy coronary stents can be precisely deployed and secured within the human NLDs. The study is the first of its kind to demonstrate the technique of NCR in human cadavers. Coronary stents are one of the commonest medical procedures performed globally. They are extensively utilized in the peripheral vessels across the human body, and the author is optimistic about their potential in managing patients with PANDO and other NLD disorders.

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