Journal of Pakistan Association of Dermatologists. 2024;**34(2)**:556-562.

Case Report

Whole genome sequencing and nested polymerase chain reaction in ocular syphilis: A case report

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Abstract We reported a case of a 22-year-old male that presented with blurry vision in his left eye. He had skin erosion on his testicles over the last year. His presenting visual acuity was 20/200 (left) and 20/200 (right). His right eye funduscopic showed an indistinct margin of papillary cranial nerve II (CN II) and hyperemia. He had reactive VDRL, TPHA, and HIV infection. Nested polymorphism chain reaction (nested PCR) showed *T. pallidum* A2059G mutation, but results from whole genome sequencing (WGS) were engative. He received benzathine penicillin 2.4 million international unit injection (intramuscular) and symptomatic therapy. After three months of treatment, he showed clinical improvement. In conclusion, ocular syphilis can occur at any stage of syphilis and may mimic various ocular diseases. Thus, it is often referred to as "the great masquerader".

Key words
Human immunodeficiency virus; Nested PCR; Ocular syphilis; Whole genome sequencing.

Introduction

Ocular syphilis is a rare manifestation of syphilis with a prevalence of 0.5-1.5%. Patients with ocular syphilis present clinical manifestations such as red eyes, blurry vision, and potential blindness.[1] Serological tests (treponemal and non-treponemal) are commonly used diagnostic tests for syphilis in Indonesia. However, serological tests have limitations as they may yield false positive, false negative, or uncertain results.[2]

Polymerase chain reaction (PCR) has been widely used and recommended by the Centers for Disease Control and Prevention (CDC) to confirm the diagnosis of syphilis, addressing diagnostic challenges.[1] In addition to PCR, Whole Genome Sequencing (WGS) is a promising method for clinical diagnosis and surveillance of several pathogens.[3] In this study, we reported a case of advanced latent syphilis with clinical manifestations of ocular syphilis. This case is reported to further understand ocular syphilis and its management to prevent further complications.

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Case report

A 22-year-old male was referred from the Ophthalmology clinic with a suspected diagnosis of ocular syphilis. Previously, the patient complained of blurry vision in the left eye and had been regularly attending check-ups at the Ophthalmology clinic for the past 2 months. Currently, the patient has no complaints regarding skin or genital areas. However, for the past 2 years, the patient has experienced intermittently appearing and disappearing black

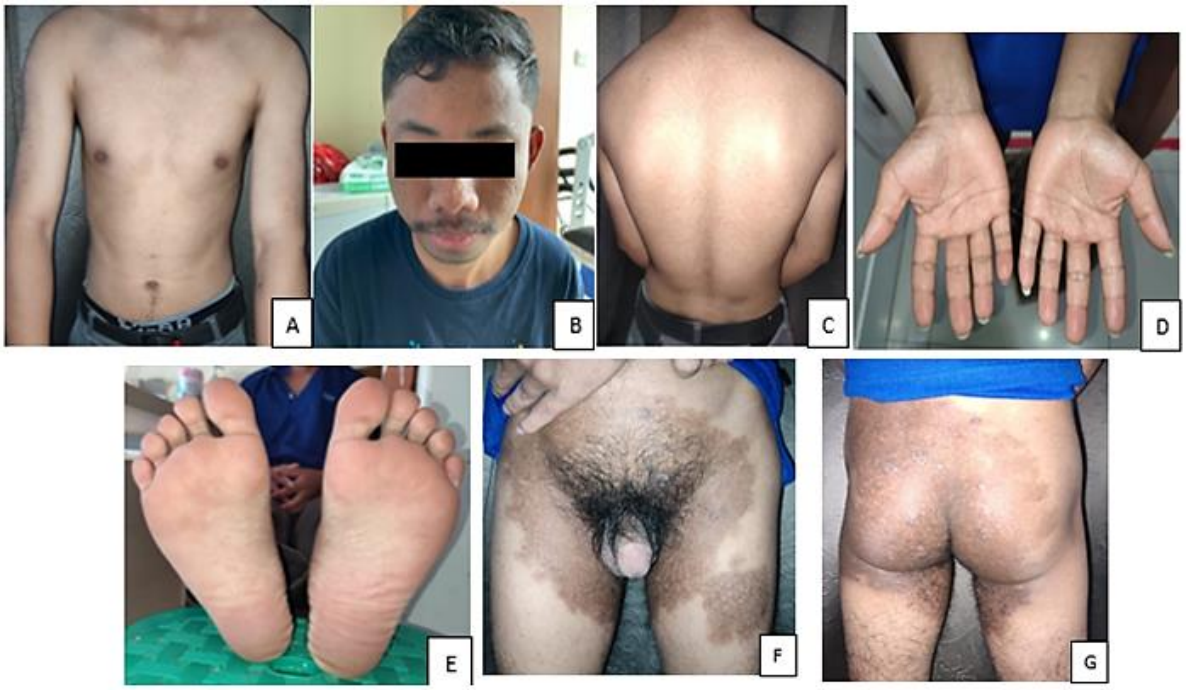

Figure 1 Dermatological status of the inguinal and left femoral regions showing hyperpigmented macules with scales.

patches in the groin and buttocks. These patches are sometimes accompanied by itching, especially during sweating. After treatment, the condition temporarily improved, but the patches reappeared along with itching, several times.

Approximately 1 year ago, the patient had an ulcerative lesion in the scrotal area along with red patches on both palms and soles. The patient did not receive Benzathine Penicillin G injections. The patient also complained of frequent fevers accompanied by weight loss and loss of appetite. The patient's last sexual encounter was in June 2023 with three different partners (both male and female) engaging in oral and anal sex without using condoms.

Approximately one month ago, the patient was diagnosed with left optic neuritis and was hospitalized for 3 days. During the hospital stay, the patient received intravenous methylprednisolone therapy at a dosage of 1 gram/ 24 hours intravenously and ranitidine at a dosage of 50 mg/12 hours intravenously. The patient underwent several examinations, subsequently being diagnosed with syphilis and human immunodeficiency virus (HIV) infection. The patient is currently undergoing antiretroviral therapy (ART) with oral citicoline tablets at a dosage of 500 mg/ 24 hours, oral cotrimoxazole tablets at a dosage of 960 mg/ 24 hours, oral dolutegravir at a dosage of 1 tablet/ 24 hours, and oral isoniazid at a dosage of 1 tablet/ 24 hours.

Physical examinations showed hyperpigmented macules with scales in the inguinal region and the left femoral trunk. No lymph node enlargement was detected. Ophthalmic examinations showed no signs of anemia or jaundice, and the visual acuity for the right eye (OD) was 20/20 with no improvement with a pinhole, and 20/200 for the left eye (OS) with no improvement (-0.50) at 20/80. Fundoscopy results for the right eye showed a positive fundus reflex; optic nerve (N.II) with clear boundaries; cup-to-disc ratio (CDR) of 0.3; A:V ratio of 2/3; positive foveal reflex in the macula;

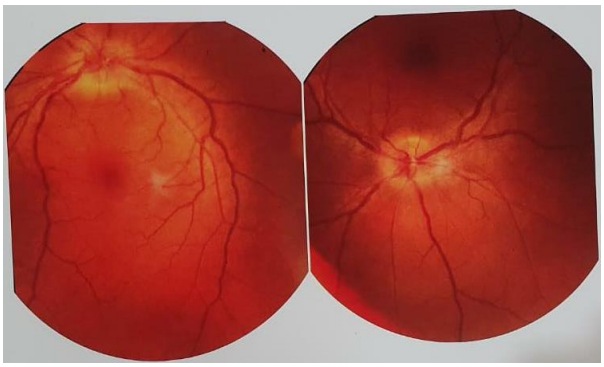

Figure 2 Left ocular examination showing unclear margins of the optic nerve, while the right ocular examination was within normal limits.

and normal peripheral retina. Fundoscopy results for the left eye showed a positive fundus reflex; optic nerve (N.II) with unclear boundaries; CDR of 0.3; suggestive of hyperemia; A:V ratio of 2/3; positive foveal reflex in the macula; and normal peripheral retina.

The VDRL test result was reactive with a titer of 1:128, and the TPHA test was reactive with a titer of 1:5120. The anti-HIV-3 test showed a reactive result with a $CD4^+$ count of 236 cells/L. The polymorphism chain reaction (PCR) revealed a positive presence of Treponema pallidum with the A2059G gene mutation **(Figure 3a)**. Further examination through whole genomic sequencing did not detect Treponema pallidum **(Figure 3b)**. The patient was diagnosed with advanced latent syphilis with HIV and tinea cruris. Additionally, the patient was diagnosed with optic neuritis secondary to syphilis.

The patient was treated with intramuscular injections of Benzathine Penicillin G, 2.4 million international units, administered once a week for a total of three doses. Before administering the injections, the patient underwent a skin test, followed by observation after the injection. The patient experienced fever and body aches several hours post-injection, which improved after taking oral paracetamol 500 mg.

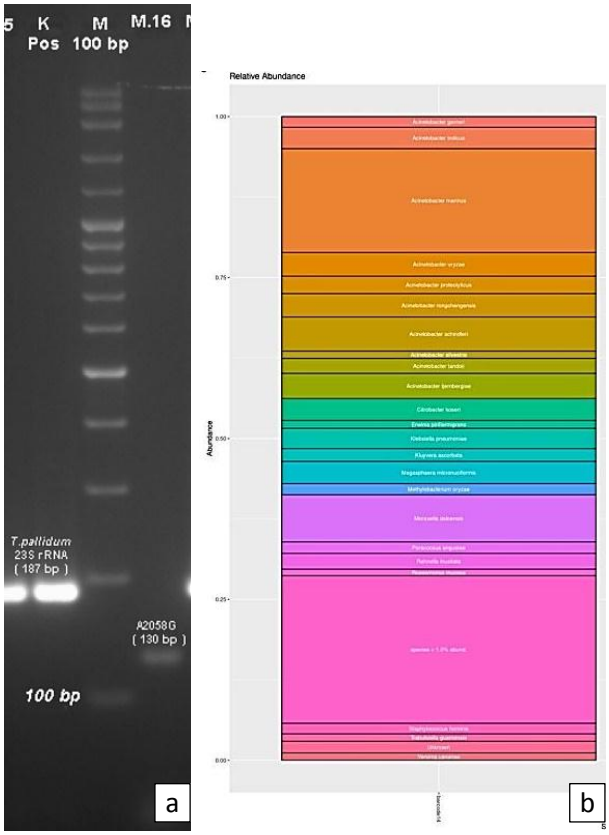

Figure 3 PCR (a) and Whole Genome Sequencing (b) results.

Additionally, the patient received topical therapy using a cream containing 3% salicylic acid, 6% benzoic acid, and 30 grams of miconazole applied in the morning and evening on the black patches in the groin and buttocks. Education was provided for sexual abstinence during treatment and condom use if abstinence was not possible. For the ocular condition, the patient received methylprednisolone 4mg every 8 hours orally and citicoline 500mg orally, daily. The patient also received Tenofovir 300mg/ Lamivudine 150mg/ Dolutegravir 50mg orally daily, and was advised a follow-up after 6 months post-treatment.

During the second-month follow-up, the skin condition improved, although the black patches persisted. Blurred vision in the eye was still present but showed signs of improvement. Dermatological examination revealed

hyperpigmented macules in the gluteal and inguinal regions. Ocular examination showed visual acuity for the left eye (OS) at 20/80 without improvement with a pinhole and for the right eye (OD) at 20/20 without improvement with a pinhole. Fundoscopy results for the right eye showed a positive fundus reflex; optic nerve (N.II) with clear boundaries; cup-disc ratio (CDR) of 0.3; A:V ratio of 2/3; positive macular reflex; and normal peripheral retina. Fundoscopy results for the left eye showed a positive fundus reflex; optic nerve (N.II) with unclear boundaries; CDR of 0.3 suggestive of hyperemia; A:V ratio of 2/3; positive foveal reflex in the macula; and normal peripheral retina. At present, there have been no new complaints reported by the patient.

Discussion

Latent syphilis is a diagnosis of exclusion after primary, secondary, and tertiary syphilis (including neurosyphilis) have been ruled out. Latent syphilis diagnosis can also be established in individuals with a history or serological evidence of syphilis but who have never received treatment for the disease and do not display clinical symptoms.[4] Treatment for late latent syphilis requires an extended therapeutic program.[5] In the patient in this case, no skin or genital complaints were found, but there was a history of self-resolved genital ulcers and a past occurrence of red spots on the palms and soles one year ago, categorizing the patient as having late latent syphilis.

Neurosyphilis with complaints of blurred vision or ringing in the ears can occur at any stage of syphilis. Acute eye inflammation can happen during the early stages, i.e., primary, secondary, and early latent syphilis. More chronic inflammation occurs during the later stages of syphilis.[6] In patients diagnosed with optic neuritis, ocular syphilis typically affects both eyes and is more common in men. Furtado *et al*. reported that optic neuritis is a frequently encountered eye disorder in syphilis.[7] Failure to establish a diagnosis and delays in treatment increase the risk of infection transmission, carry a poor visual prognosis (blindness), and lead to neurological deficits that may progress to neurosyphilis.[8] Syphilis can affect all eye structures, including the conjunctiva, sclera, cornea, lens, uvea, retina, and optic nerve. Ocular syphilis can mimic various other eye diseases such as interstitial keratitis, anterior and posterior uveitis, chorioretinitis, retinitis, retinal vasculitis, and optic neuropathy. Therefore, ocular syphilis is often referred to as "the great masquerader".[9]

Ocular syphilis in patients with HIV tends to progress more rapidly.[10] Ocular syphilis does not correlate with CD4+ cell count and typically occurs in patients with normal CD4+ counts.[11] In this patient, the CD4+ count was 236 cells/ml, indicating an HIV infection. According to CDC recommendations, patients with uveitis or other ocular manifestations of syphilis (neuroretinitis and optic neuritis) may be associated with neurosyphilis.[12,13]

The immunoserological tests in our patient showed a reactive result for VDRL at 1:128 and TPHA at 1:5.120, thus establishing the diagnosis of late latent syphilis. The treatment plan involved intramuscular injections of Benzathine Penicillin G at a dose of 2.4 million units intramuscularly once a week for three weeks. Since 2006, the CDC has recommended the use of intravenous Penicillin G at a dosage of 18-24 million units daily for 10-14 days or Procaine Penicillin G at a dose of 2.4 million units intramuscularly plus Probenecid 500 mg orally four times a day for 10-14 days.[12]

In this case, PCR examination results showed the presence of the A2059G gene mutation.

Mutations A2058G and A2059G on the 23S rRNA gene of Treponema pallidum are associated with macrolide resistance. T. pallidum resistance to macrolides has been found in many countries with varying prevalence rates. The prevalence of T. pallidum resistance to macrolides can reach 100% in China, 93.1% in Ireland, 84.4% in Australia, and 64.3% in the United States.[14,15]

In several studies, the A2059G mutation has been less frequently detected compared to A2058G, with proportions of A2059G mutations reported at 4% and 2% in America and Europe, respectively. The A2059G mutation in T. pallidum strains was first reported in the United States in 2011, in which 10% of T. pallidum strains originating from men who have sex with men.[16,17]

Sensitive diagnostic examinations detecting T. pallidum are crucial for establishing an early syphilis diagnosis. Nested PCR is a relatively sensitive method for detecting T. pallidum DNA in the blood, exhibiting better sensitivity than single-step PCR. A study indicated that nested PCR can be used to detect T. pallidum DNA across various stages and complements other examination results to confirm a syphilis diagnosis.[18] Nested PCR can also aid in diagnosing syphilis in patients with negative Treponema serology results. Another study stated that blood-based Nested PCR has 3.25 times better sensitivity than serum-based testing.[2,19]

In this case, the whole genomic sequencing (WGS) did not show T. pallidum. Whole-genome sequencing is an alternative promising procedure for clinical diagnosis and surveillance of several pathogenic species. However, contamination can affect WGS outcomes. A study showed that contamination in WGS samples can introduce bias in bacterial variant analysis, leading to false-positive or false-negative results. In that study, contamination affected the WGS's ability to detect only 40% of T. pallidum-positive specimens.[3]

WGS is considered a better genomic variant monitoring platform compared to whole-exome sequencing (WES), due to its perceived broader coverage, higher accuracy level, and potential for identifying structural variants. Continuous comprehensive evaluation of next-generation sequencing (NGS) pipelines is necessary to minimize false-negative results.[20]

The examination of T. pallidum using WGS is also challenging due to low bacterial loads or difficulties in obtaining adequate samples for testing. Additionally, molecular studies of T. pallidum strains pose challenges due to the limited amount of whole genome obtained directly from clinical specimens. Molecular epidemiological studies indicate that the considerable diversity of T. pallidum strains based solely on a few genetic loci may not fully represent the entire T. pallidum genome. This could be a factor contributing to false negatives in WGS. One study also suggests that most false-negative results in WGS occur due to excessive filtration processes.[21]

In assessing the response to syphilis treatment, the CDC recommends clinical and serological evaluations at 3, 6, 12, and 24 months post-treatment in HIV patients.[13] In this case, satisfactory results were obtained with an improvement in the left eye's visual acuity from previously 20/200 PH(-) to 20/80 PH(-) after the third injection. Most cases have a good prognosis if diagnosed early and treated with appropriate antibiotic therapy.[7]

Conclusion

Ocular syphilis can occur at any stage, including

late latent syphilis. Ocular syphilis can mimic various other eye diseases, earning it the moniker "the great masquerader". The presence of HIV infection causes a faster progression of ocular syphilis.

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