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Recapitulation on the Recent Advancements in the Diagnosis andTreatment of Neglected Tropical Disease - Onchocerciasis

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Abstract: ***Onchocerciasis is a parasitic tropical disease under the category of neglected tropical diseases(NTD) of WHO. This disease is caused by the bite of Onchocerca volvulus infected black flies.Commonly affects the residents of the African region and even the tourists who visit the region.Sometimes this infection is associated with microfilariae. It is estimated that about 5% deathsare caused by this infection. The nematode provokes the person's inflammatory response causing lesions and even impacts the eyes causing blindness which is termed as river blindness.The diagnosis of the disease is available even for low intensity of infections. The treatment ofthe disease is limited to Ivermectin and few antibiotics which is given every 6 to 12 months. But these drugs do not cure the disease rather just control the infection.***

Keywords: Onchocerciasis, Neglected tropical diseases, Microfilariae, Caseate, Skin snipbiopsy

1. Introduction

Onchocera volvulus is a spirurid nematode belongs to the Superfamily Filarioi-p dea, Family Onchocerciadae. The disease caused by Onchocerca tends tomainly affect the rural people of central and South Africa, Sub-Saharan Africa and Yemen. Like malarial parasites the lifecycle of the worm involves two hosts:

1) A definitive host - Humans
2) An intermediate host- The black fly of genus *Simulium*.

In humans life cycle begins when the infected female *Simulum damnosum* transmits the third stage larvae which is an infective stage into the human body. Larvae undergo molting twice before they develop into adult worms; they cause characteristic subcutaneous Collagenous nodules in humans. Unsheathed microfilariae (usually found in the dermis) produced by the female vector, migrate to different parts of the body including the eyes. In the eye, they provoke an immune response leading to inflammatory lesions and progressive eye damage, sometimescausing blindness, also called “river blindness”.

River blindness is one of the most common cause of infectious blindness in the world, estimated to have caused one million cases of visual impairment and340,000 cases of blindness.

In hyper-endemic communities more than 60% of the population is infected with microfilariae and about half of these develop symptoms; about 15% suffer from serious eye disease and up to 5% become blind [(1)].

Figure 1: Blackfly

River blindness, continues to impact the lives of millions of people, particularly in sub- Saharan Africa. Through effective vector control and mass drug administration, significant strides have been made in controlling and eliminating the disease.

2. History

The first observation of the symptoms of onchocerciasis was already described in the 17th century, mainly in Africa and Latin America. Indigenous populations living in endemic areas recognized the disease and referred to it as “river blindness” due to the close association of the disease with rivers where black fly vectors usually breed.

Onchocerciasis was first described in 1874 by Irish surgeon John O'Neill when it was discovered as a skin infection found in West Africa where it was at that time known as‘craw-craw’. It is a skin disease characterizedby severe itching and a red rash and was also observed to be transmitted from person toanother.

In 1893, the small worm Onchocercavolvulus was discovered by the Scottish physician Sir Patrick Manson. He had found that microfilariae (larva nematode) werepresent in the skin nodules of patients who had the disease.

In the year 1915 a physician, Dr. Rodolfo Robles observed that in an 8-year-old patient having a subcutaneous nodule present, in which it was found that the adult worms of Onchocerca volvulus were present and in subsequent research principally retrieved for the first time that onchocerciasis caused visual loss. In honor of his important contribution to research, onchocerciasis is also called as 'Robles Disease'.

Originally found mainly in Africa, onchocerciasis was brought to the Americas by the slave trade as part of the Colombian trade and spread by immigration [(2)].

3. Epidemiology

According to an article "Recent Updates onOnchocerciasis: Diagnosis and Treatment" by Don N. Udall, published in 2007, river blindness which is caused by Onchocerca volvulus affected about 34 countries around the world mainly in Africa, the Middle East, South America, and Central America. About 17.7 million people are affected, among which 500,000 people experienced secondary visual impairment and 270,000 people suffered from blindness[(3a)].

It was found that the maximum prevalence in history of onchocerciasis was observed mainly in 11 sub-Saharan West African nations which included Ghana, Nigeria, Liberia, and parts of Mali [(3b)].

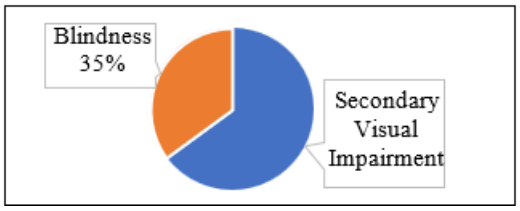

Figure 2: Percentage of people affected with eye related issues in the year 2007

In the temporal and regional boundaries an estimate of 5% of the deaths was recorded in a study which attributed to an interrelation between *O. volvulus* microfilarial load which caused mortality and severe morbidities. A recent update showed that a higher prevalenceof onchocerciasis mainly in Ghana was seen in patients suffering from glaucoma[(3c)] .

According to the latest article "Onchocerciasis" published by World HealthOrganization (WHO) in 2022, it was reported that more than 99% of people affected with the disease were living in 31 countries of sub-Saharan Africa mainly from Angola Democratic Republic of the United Republic of Tanzania. It was also reported that Yanomami area of Brazil and Venezuela (Bolivarian Republic of Venezuela) as well as Yemen were among the countries where onchocerciasis was transmitted [(1)].

According to WHO the first ever country inthe world to be declared free fromonchocerciasis infection was COLOMBIA inthe year 2013. Nigeria had the greatest prevalence of this infection globally, which imputed upto 30.2% of the cases associated with blindness in the year 2021.

4. Pathogenesis

Simulus blackflies are the obligate intermediate hosts of O. volvulus. The flies breed in rivers mainly in the fast-flowing rivers. The microfilariae reach the thoracic muscles from the infected blackfly's midgut via haemocoel [(4b)].

That is the location where microfilariae multiply in to first stage larvae and subsequently infective third stage larvae (L3). During blood meal, an infected blackfly (genus Simulium) introduces third-stage filarial larvae onto the skin of the human host,where they penetrate into the bite wound [(4c)].

L3 larvae undergoes two molting process to become adult worms, which can be detected in subcutaneous fibrous nodules of primary host.

Females release on an average of 1,000microfilariae (L1) per day over a 9- to 14-year period and the cycle is continued after uptakeby blackflies during a blood meal. Disease will not be developed unless there are bites from many infected blackflies.

The bites from the infected blackflies resultsin inoculation of infective larvae (L3), whichdevelops into its adult form of worm within 12 to 18 months. Adult female worms have alife cycle of 15 years in the definitive hosts subcutaneous nodule[(4a)].

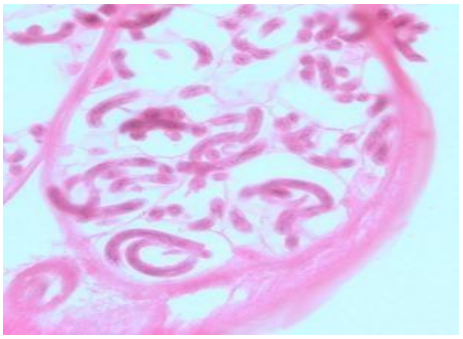

Figure 3: Larval development

In some filarial nematodes causing Onchocerciasis also contain intracellular bacteria symbiont like Wolbachia species. The initial report of such mutualistic endosymbiosis was reported by Kozek and Marroquin in the year 1977.

This symbiosis helps in exacerbating proinflammatory pathogenesis and enhances the chances of survival of the filariae. In somecases, they even lead to onchocercal keratitis and dermatitis [(4d)].

Onchocerca volvulus posses their owninnate immunity to protect against other pathogens and have evolved mechanisms to protect themselves from the insect and human host's immune system. *Onchocerca volvulus* has C-type lectins, galectins, jacalins and scavenger receptors which are responsible fortheir immunity[(4e)].

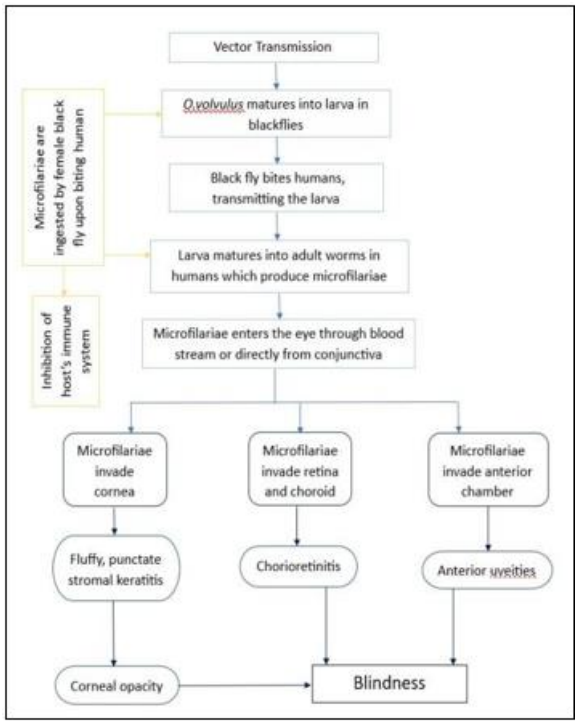

Figure 4: Flowchart of Pathogenesis ofOnchocerciasis

5. Symptoms

Primary symptoms are:
- Corneal Opacification
- Neovascularisation
- Systemic Symptoms

1) Eye
- *Vision impairment*: Onchocerciasisprimarily affects the eyes, and vision problems are common. They canrange from mild visual disturbances tocomplete blindness.
- *Eye inflammation*: Inflammation of the conjunctiva (the thin membranecovering the white part of the eye) andother eye structures can occur, leading to redness, itching, and sensitivity to light.
- *Eye lesions:* Onchocerciasis can cause eye lesions, including small white nodules in the iris, corneal clouding, and in severe cases, optic atrophy maylead to sclerosing keratitis.
- *Other symptoms:* Some symptoms include pupil deformation, opticneuritis and chorioretinitis are also observed.

2) Skin
- *Intense itching*: This is a hallmark symptom of onchocerciasis. Itching can be severe and persistent, particularly in the affectedareas(pruritis).
- *Skin rash*: The skin may develop a rash or small bumps, which can become thickened and rough over time.
- *Skin hypopigmentation*: Areas of the skin may lose their normal pigmentation, resulting in light patches also known as 'Leopard Skin'.
- *Other skin symptoms:* Onchocercal dermatitis, Intense pruritis, There is loss of elasticity of skin including thinning of skin occurs, In the lower abdomen and upper medial thighs atrophic skin develops in severe conditions.

3) Nodules
Nodules are either palpable orasymptomatic.

Nodules may calcify and caseste [4a]. (Cassation is where nodule tissue forms a firm, dry mass like cheese in appearance which slowly tend to undergo necrosis while Calcification is where there is calcium deposition in the nodules after infection).

4) Systemic Symptoms
- *Lymphadenopathy*: ln the early stages of infection Enlarged lymph nodes areobserved.
- *Musculoskeletal pain*: Some individuals may experience joint and muscle pain, typically associated with the migration of the parasite (female microfilariae).

The severity of symptoms can also fluctuate over time, depending on factors such as reinfection, immune response, and the effectiveness of treatment.

6. Diagnosis

6.1. Skin snip biopsy

The pores and skin snip biopsy is the standard golden test for the analysis of onchocerciasis.

Procedure-
The biopsy test is done using a needle or needle to take a small cup (3 mm in diameter)from the skin and shave it with a spoon. Thisremoves approximately 2 mg of tissue.The tissue is then incubated in saline for 24 hoursat room temperature to allow microfilariae (larvae) to emerge. Microfilariae can be seenunder the microscope.

Skin incisions are usually made on the iliac crest, scapula, and lower extremities. Six fragments show the highest sensitivity in diagnosis. Sensitivity to skin incisions may be restricted in low-intensity infections andin the pre-patent phase of the infection, which may last for 12 to 18 months.

Pros-
1) Ease of performance
2) Simple equipment
3) Accuracy: Skin biopsy has high sensitivity and specificity for *Onchocerca volvulus* microfilariae detection in the skin. It involves removing a small piece of skin (usually from the iliac crest or shoulder) and examining it in order to check the presence of microfilariaeunder the microscope
4) Quantification of microfilariae burden: Biopsy of a skin section allows the quantification of microfilariae burden in the skin. The number of microfilariae per skinscraping can provide information about the intensity of the infectionand aid in treatment decisions.
5) Assessment of disease activity: Microfilariae viability can be assessed by examining a biopsy of a skin section. This information can be useful in determining disease activity and monitoring the effectiveness of treatment over time.

Identification of other skin conditions: A skin biopsy also allows the identification of other skin conditions that may present withsimilar symptoms to onchocerciasis. This can

help distinguish onchocerciasis from other diseases and ensure an accurate diagnosis.

Cons-

1) Used only in low intensity infections.
2) Invasive procedure: A skin biopsy involves removing a small piece of skin tissue, usually with a punch or scraper, scalpel or knife. The complex nature of the procedure can lead to discomfort, pain, and complications such as infection or scarring.
3) Technical experience: Performing askin biopsy requires an experienced professional to perform the procedure. Proper preparation is required to ensure accurate acquisition and interpretation of results. Lack of experience can leadto insufficient samples or incorrect diagnosis.
4) Variable sensitivity: The sensitivityof a skin biopsy can vary depending on the site of infection and convalescence. Early-stage infectionscan be difficult to detect, especially when adult worms have not yet produced microfilaria (microscopic larvae) that can be seen in broken skin. As a result, false negativeresults may occur.
5) Labor process: The process ofpreparing and testing skin prick samples is time-consuming and labor-intensive. After taking a skin swab, it should be incubated and checked for the presence ofmicrofilariae by examining under the microscope. This can be time- consuming, especially in areas where onchocerciasis is endemic and where large-scale trials are needed.
6) It is less sensitive than newer biochemical methods including ELISA, skin patch PCR, EIAs and antigen assays[3a].

6.2. Antibody tests

The diagnosis of onchocerciasis can be assisted with antibody tests but it's not feasible to perform it outside the researchor laboratory setting.

Pros-

1) Mainly useful in determining asymptomatic victim.
2) Specificity: Antibody tests foronchocerciasis can be very specific, meaning they are less likely to produce false-positive results. This helps to correctly identify people whoare infected with parasites.
3) Sensitivity: Antibody tests can detect the presence of antibodies produced by the immune system against infection. It may be sensitive enough to detect early or low-gradeinfections, increasing the chance ofan accurate diagnosis.
4) Convenient: Most antibody tests are non-invasive and involve a simple blood sample, making them easier to perform and tolerated by patients.
5) Diagnostic tools in non-endemic areas: Antibody tests in non-endemic areas of onchocerciasis can be useful to identify people who have contracted the infection throughtravel to endemic areas or by other routes. This can lead to appropriate treatment and prevent the spread of the disease.

Cons-

1) Availability: As previously mentioned, onchocerciasis antibody tests may not be widely available outside the research setting. Thislimits their availability in many regions where the disease is endemic,preventing early diagnosis and treatment.
2) Cost: Antibody tests can beexpensive, especially if they are not part of routine diagnostic procedures. This may present a barrier to their implementation in resource-limitedsettings where onchocerciasis isprevalent.
3) Technical expertise: Some antibody tests require specialized equipment and trained personnel to accuratelyperform and interpret results. The lack of such resources in certain settings may further limit the use of these tests.
4) False negatives in early infections:

Antibody tests may not always detect

the presence of antibodies during the early stages of infection when antibody levels are low. This can result in false negative results and delayed diagnosis if you rely on antibodytesting alone.

It is worth noting that other diagnostic methods such as skin incisions and polymerase chain reaction (PCR) testing are used to diagnose onchocerciasis, especially in endemic areas. These methods have their advantages and limitations. The choice of diagnostic method depends on factors such as availability of resources, expertise and thespecific context in which the diagnosis is made [5a].

6.3. Antigen detection dipstick assay:

The antigen detection dipstick assay is a newly advanced technique which is a promising diagnostic method and has an antigen detection threshold of 25 nanograms per ml only.

Pros-

1) It is having 100% detection rate in patients having positive results of skin snip microscopy.
2) In the study area where the disease is highly endemic, this method gives positive results for urinary worm antigen in subjects with predominantly having negative results from microscopic view of skin incision.
3) 100% specificity among a low- prevalence population.
4) This method does not show cross- reaction with any of the co-endemicparasites.

Cons-

More substandard at ruling out infection in symptomatic people [5b]

6.4. Mazzotti test:

Using the drug diethylcarbamazine (DEC) a skin patch test is performed named as Mazzotti test. Severe symptoms are observed because of the death of microfilariae which is caused by DEC.

1) In two ways diagnosticians may use DEC to test for onchocerciasis. One way is by administering the medication by oral route. If a subject is infected, this would within two hours cause severe itching.
2) Putting DEC on a skin patch is the other method involved. That will cause a rashand localized itching in people with river blindness.[5c]

The Mazzotti test is a diagnostic test that wasformerly used to aid in the diagnosis of oncology. However, it is no longer recommended or commonly used due to safety concerns and the availability of more reliable diagnostic methods.

Mazzotti's experiment involved the administration of small doses of diethylcarbamazine (DEC) to people suspected of having oncosis. DEC is an effective antiparasitic agent against Onchocerca volvulus, the parasite that causes onchoceriasis. The rationale behindMazzotti's test is that if a person hasonchoceriasis, the dead microfilariae (a form of parasitic worm) will release antigens that cause inflammation.

The standard procedure for the Mazzotti test includes the following steps:

Individuals will be given a single oral dosage of diethylcarbamazine. For several hours after administration, a person may experience symptoms which includes rash, rash, fever, joint pain, or swelling. These symptoms are considered a positive result forthe Mazzotti test, indicating that the person is infected with Onchocerca vulva.

However, the Mazzotti test has several important limitations and risks which include Safety Precautions: Testicular Onchocerca volvulus can cause severe and potentially fatal systemic reactions in individuals

6.5. PCR:

To increase the sensitivity and specificity,polymerase chain reaction (PCR) can be performed for skin snip, but this is not commercially available.

Pros-
1) More Sensitive.
2) More Specific.
3) Only requires a small skin sample

Cons-
1) Concede for diagnosis if the larvae are not seen microscopically.
2) Expensive [5d]

6.6. Slit Lamp eye exam:

A slit-lamp eye examination may be performed to visualize microfilaria or lesions in the eye for patients whose eyesare affected due to the disease. [3c]

7. Treatment

7.1. Ivermectin

The most common treatment for onchocerciasis is said to be Ivermectin whichcan also be given as prophylaxis. A dosage ofabout 150 µg/kg is administered every 6 to 12months as a single dose by oral route. Ivermectin being highly lipophilic [6a] due to the presence of a 16-membered macrocyclic lactone and it is obtained from the source *Streptomyces avermitilis*. Ivermectin does notcure the disease but helps in controlling the disease.

Single-dose of ivermectin was proven to kill microfilariae effectively as it works by blocking the glutamate-gated chloride ion channels which are present postsynaptically and thereby inhibit the transmission of the disease and causes paralyzation of the nematode which is responsible for causingriver blindness. [6b]The immune system response in ivermectin treated hosts showed enhancedimmunity against O. volvulus. It was proven from a study which was performed for 15-months that ivermectin treatment is highly efficacious at preventing further reactiveonchocercal skin lesions rather than eliminating existing lesions [6b]

7.2. Moxidectin

A propitious new drug has been found called Moxidectin. In animal studies it had shown some significant macrofilaricidal activity and was found safe for human use. This drug has undergone phase II trials already[6c]. In the year 2018, U.S. FDA had approved this drug for use in ages 12 and older. The oral dosage of the drug in adults and children from age of 12 is upto 8 mg in asingle dose.

7.3. Doxycyline

It is found that doxycycline of dose 100 mg per day for a period of 6 weeks, followed bya single dosage of about 150 µg/kg of ivermectin given in a nonrandomized, placebo-controlled trial involving humans, showed results in patents wherein up to 19 months had no microfilaridermia, as well as there was 100% elimination of Wolbachia species (Intracellular bacterial symbiont harboured along with *Onchocerca volvulus* infection containing nematodes) from the isolated and immunohistologically tested worms [6d] .

8. Adverse effects of the treatment

Skin reactions like rashes and inflammatoryreactions occurred after receiving treatment with ivermectin, which was frequently reported in patients having high densities of microfilaria.

Rarely seizures associated with ivermectin treatment is reported. It was also found that there was decrease in counts oof circulating eosinophil and increase in IL-5 and eosinophil-derived neurotoxin levels in patients undergoing ivermectin treatment [7a].

Resistance to ivermectin treatment has been observed in four species of nematode parasites but these usually do not affect human beings. The four species of nematodesare: *H.contortus* (Blackhall et al., 1998; Xu etal.,1998; Sangster et al., 1999), *Ostertagia circumcincta* (Prichard, 2001); *Tricho- strongylus colubriformis* (Prichard, 2001); and *Cooperia oncophora* (Prichard, 2001) [7b].

9. The current scenario and challenges in control of onchocerciasis

According to an article *"Reaching the last mile: main challenges relating to and recommendations to accelerate onchocerciasis elimination in Africa"*published in the year 2019 by Chinese Medical Association Publishing House, there were rigorous control interventions to eliminate

Onchocerciasis. The control programme includes:

1) Onchocerciasis Control Programme in West Africa (OCP): From the year 1974 to 2002
2) African Programme for Onchocerciasis Control (APOC): From the year 1995 to 2015
3) Expanded Special Project for Elimination of NTDs (ESPEN): Their ultimate goal unlike the previous programmes shifted from control to elimination of onchocerciasis by inhibiting the transmission of the parasite from its territory.

In the year 2016, WHO had published certain guidelines to stop mass drug administration and verifying transmission and elimination of the disease. This was done by creating three phases for elimination of onchocerciasis in the guidelines.

Phase 1 included active transmission and mass drug intervention, Phase 2 was related to post treatment surveillance for newer infections and finally Phase 3 dealt with post elimination surveillance to detect relapse of onchocerciasis infection.

In spite of these programmes and guidelines there are some challenges which majorly affect the elimination of onchocerciasis mainly in Africa. The challenges include: Co-endemicity of onchocerciasis, resistance to ivermectin treatment, incomplete elimination of the disease in the transmission zones, various financial and technical challenges [(8)].

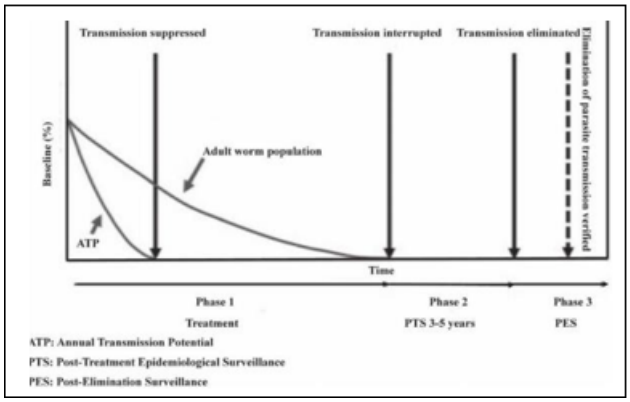

Figure 5: Phases in the elimination ofOnchocerciasis

10. Developments in treatment strategies for control of onchocerciasis

The possible treatment strategies for control of Onchocerciasis will not be restricted only to curing the patients but also prevention of the disease by vaccines. In-silico studies have been done and some researchers are developing multi-epitope vaccine candidates against Onchocerciasis.

Possible commercial development of anti- onchocerca metabolites from Cyperus articulates, a plant. The secondary metabolites obtained from the hexane extractof this plant species can be commerciallyproduced in large amounts to treat against Onchocerciasis.

Costunolide, a naturally occurring sesquiterpene lactone under went in-silico screening by molecular docking for its inhibitory capacity against the protease ofOnchocerciasis.

11. Conclusion

The complete elimination of onchocerciasisfrom the endemic and non-endemic regions isa major concern but many control programmes and newer discovery of drugs will soon eradicate the disease from many regions including Africa. Extensive research is being carried out all over the world to develop specific drug candidates acting against *Onchocerca volvulus.*

With advancements in research and technology there is possibility of development of proper treatment for Onchocerciasis in the future.

Ethics Approval

The article was reviewed and approved by the Department of Pharmacology, M.S. Ramaiah university Of Applied Sciences

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All authors have read and approved the finalmanuscript.

Declaration of Competing Interest

The authors have no competing interest to disclose

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