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Recent Development and Evaluation for Effective Diagnosis and Treatment of Human Onchocerciasis, Using an Antigen Detection Dipstick Techniques: A Review

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Abstract*: Onchocerciasis still remains a major hindrance to sustainable public health, farming and livestock development. However, the type of diagnostic test used for the detection of infections caused by onchocerciasis varies according to the epidemiological characteristics of the disease and the strategy for control. The clinical signs are not pathognomonic and the standard techniques for the detection of onchocerca species are not sufficiently sensitive. Despite several improvements in the techniques for onchocerca species detection, a high proportion of infections still go undetected as the majority of infections are chronic. This diagnostic challenge has led to the development of alternative methods, for instance molecular techniques, because they are extremely sensitive and better diagnostic tools. This write-up therefore, underscores the relevance of various diagnostic techniques in the epidemiological study of onchocerciasis. It concluded that, these current techniques can allow the investigation of the phylogeny and diversity of onchocerciasis species that would help in devising appropriate control measures to limit the public health and the economic losses arising from the disease.*

Key words: *Onchocerciasis, Onchocerca volvulus, Diagnosis.*

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I. Introduction

Background to the Study

Onchocerciasis is a chronic and disabling disease caused by the parasitic nematode *Onchecerca volvulus* (WHO, 2016). The parasite is transmitted from one person to another through the bite of a blood sucking blackfly of the genus Simulium (Lavebratt *et al*., 2016). An estimated 17.7 million people currently suffer from the infection worldwide;the vast majority of affected people live in Sub-SaharanAfrica (Burnham, 2017). The disease, if not treated causes pruritus, populareruption of the dermis, skin dyspigmentation, serious blindness, kidney disease, epilepsy and hypo sexual dwarfism (WHO, 2015). There is neither vaccine nor a suitable microfilaricidal drug against the infection (Chandrashekar *et al*. 2014). Onchocerciasis control programme require a non-invasive, highly sensitive and specific diagnostic test for use in low transmission in disease free areas (Ngu *et al*, 2013).

Definitive proof of active infection due to *Onchocerca volvulus* is by microscopic demonstration of worms in skin snips or in nodules surgically excised from patients (Weil *et al*, 2000). Unfortunately, the commonly used skin snip method is insensitive in low transmission areas and in areas where long-term use of the microfilaricidal ivermectin has resulted in significant reduction of both individual and community skin microfilariae loads, with a consequential reduction in the prevalence of most signs and symptoms of the disease (Mbacham *et al*, 2016). Though, the skinsnip procedure is painful and involves a high risk of other bloodborne infections such as HIV, which may result in refusal of the test by population under investigation (Boatin *et al*, 2016). To improve upon the diagnosis and treatment of onchocerciasis, alternative methods based on the detection of antibodies or antigens in body fluids have been developed (Mbacham *et al*, 2017). Although, antibody detection analysis are sufficiently sensitive but unable to distinguish between active and past infections (Weil et al, 2015; Chandrashekar *et al*, 2009). On the other part, developed antigen detection assay are either not sufficiently sensitive or specific or are laborious and time consuming (Vincent *et al*, 2016, Ngu *et al*, 2008; Mbacham *et al*, 2017). Currently, the development of polymerase chain reaction (PCR) based methods for the

detection of parasite DNA in skin snips has greatly improved the diagnosis of onchocerciasis given its highsensitivity and specificity (Meredith *et al*. 2014; Zimmerman *et al*, 2015). This extensive research has been invested to look into alternative techniques that provided indirect evidence of infection. This will also go along line towards effective epidemiological studies of onchocerciasis that will help in designing appropriate control strategies to limit if not eliminate life and economic losses arising from the onchocerciasis disease.

II. Options For The Diagnosis Of Onchocerciasis

Generally, the diagnosis of onchocerciasis is classified into: Clinical, microscopy, immunological and molecular diagnosis (Zimmerman *et al*., 2016).

Clinical diagnosis

Onchocerciasis is characterized by severe itching, skin changes, nodules and alteration in vision which could lead topermanent blindness(Mackenzie *et al*., 2017). Also among the characteristic are the loss of skin elasticitycausing hanging groin. Sometimes the pigmentation layer of the skin is also affected; particularly in the lower legs known as leopard skin (APOC, 2015). However, the clinical manifestation of onchocerciasis may develop between 3 months to 3 years after, depending on the intensity of the parasite burden (Kain, 1999 and Nozais *et al.,* 1997). Thus, owing to these varied clinical manifestations, diagnosis of onchocerciasis cannot be based on clinical signs alone; therefore making
Laboratory confirmation of onchocerciasis becomes an absolute necessity.

Microscopy

Microscopy entails demonstration of parasite based on standard *Onchocerca volvulus*detection methods as developed by Manson (1893) and their modifications. The methods include skin snips, Micro-haematocrit Centrifuge Techniques (MCT), thick blood films, Quantitative Buffy Coat Method (QBCM) and chromatography (Dickerson *et al*., 2017). Skin snips is used for detection of the microfilariae of *Onchocerca volvulus* that reside in the skin (Dickerson *et al.,* 2017). Thick blood film is used for speciation based on microfilariae motility.*Onchocerca volvulus* moves or swims very fast across the microscope field (Dickerson *et al*., 2017). The sensitivity of direct microscopic examination was improved through concentration of the microfilariae by centrifugation (WHO, 2016). Micro-haematocrit is used for the diagnosis of onchocerciasis infections, especially when the numbers of microfilariae present are too small for efficient detection by the thick blood films (WHO, 2016). Also quantitative buffy coat has been reported to be an acceptable rapid diagnostic test for the detection of microfilariae, with sensitivity equivalent to that of the thick blood film (WHO, 2016). Microfilariae counts are used for accurate counting of microfilariae from stained thick blood films of measured volume. Counting requires careful systematic scanning of the blood film with the low-power objective of the microscope. The stained slides can be kept as a permanent record (Fleck *et al.*, 2014). Equally reliable counts can be made from membrane filters which, if mounted with a coverslip, can be retained as a permanent record. Some researchers prefer using a counting chamber technique, which is very reliable butdoes not lend itself to species identification or permanence (Fleck *et al*., 2014)

Immunodiagnostic Techniques

An Immunodiagnostic technique has recently been made available by the development of various recombinant filarial antigens (Ramachandram, 2016). This test, however do not play an integral part in the control programme of the onchocerciasis, this is because it cannot reliably distinguish between past and present infection (Nutman *et al*., 2017).

Antibody Detection Test

Antibody detection test does not distinguish between active and past infections. Various antibodies have been tested asshown below.

- Onchocerca volvulus 16 card test(Ov16): Antibodies against this antigen have been shown to yield high sensitivity (approximately 80%) and specificity (approximately 85%) and may yield positive results in early infections when skin – snip results are negative (Maso *et al.,* 2015). Capillary blood samples are collected by finger prick. The immunochromatographic card test is also used to detect the presence of immunoglobulin G4 (IgG4) antibodies to recombinant Ov16 antigen (Buttner *et al*., 2016).
- Recombinant hybrid proteins (OvH2 and OvH3). This test is based on hybrid proteins (Ov20 and Ov33). High sensitivity and specificity has been described in this enzyme – linked immunoassay (ELISA) based antibody detection test (Weil *et al*., 2000).

Molecular Diagnostic Methods

Recent developments in molecular biology technique have produced more sensitive and specific methods for detecting *Onchocerca volvulus*(Egbert *et al*., 2005)

Therefore, DNA – based methods have a wider range of applicability and efficacy in epidemiological studies than morphological examination alone and are now indispensable tools for the study of onchocerciasis because of the improved sensitivity and specificity (Duke *et al*., 2017). Molecular detection techniques have been developed for the diagnosis of infection with *Onchocerca spp* in humans,animals and blackflies (Fuglsang *et al*., 2016). For instance, polymerase chain reaction (PCR) first performed in 1983 now has various primer sets available that can amplify different *Onchocerca spp* and type (Gasser, 2006). Additionally, species – specific probes are now available to identify Onchocerca to sub species level (Maia *et al.*, 2015). PCR can detect infection as early as 5 days following an infective blackfly bite (Ramzy, 2002). Moreover, using the quantitative PCR confers an additional advantage of identification as well as establishing the parasite burden (Medeiros *et al*., 2016). The PCR techniques apply the uses of machine to amply parasite DNA sequences in the skin snip specimens (Molyneux, 2009). This method is highly sensitive and can be used to diagnose patients with low-level infections (Shelley *et al*., 2001). Therefore, the specimen could be collected with a skin scratch in which the superficial layer of the epidermis is carefully removed with a lancet (Morales, 2009). The major disadvantage of the PCR techniques is its high cost and complication (Vincent *et al*., 2000).

Other techniques for the diagnosis of onchocerciasis

These are old techniques used in diagnoses of onchocerciasis disease.

- Palpating techniques: this is a very simple technique in which the patient's skin is patted for nodules. These nodules are very common in patients with onchocerciasis because the microfilariae congregate near the skin surface and swelling (kirch *et al*., 2003). Palpating only indicates swelling and does not provide concrete proof of onchocerciasis; therefore, other techniques are often used to provide an exact diagnosis (WHO, 2011).
- Skin snips: one of the most common diagnostic techniques is the skin snip (Thylefors, 2004). It involves the removal of some skin from an inflamed area, placing the skin snip into saline to encourage microfilariae to leave the skin and microscopic examination to determine microfilarial load (Little *et al*., 2004). This diagnostic technique is not sensitive enough to detect an early stage infection (little *et al*., 2004).
- Mazzotti Test: This test is rarely used anymore because it can cause a severe allergic reaction which is possibly leading to death (Egbert *et al*., 2005). This technique has been replaced by the DEC (Diethycarbamazine) patch test (Egbert *et al*.,2005). The techniquerequires the oral administration of 6mg of DEC and positive result isindicated when pruritus and intense inflammation occur due to the death of microfilariae (WHO, 1997).
- DEC patch techniques: the DEC technique was created as an alternative to the mazzotti test because of the potential for serious side effects associated with the large dose of DEC (Blank *et al*., 1998). In the patch test, gauze pad is soaked in a 20% solution of DEC and placed on the hip; the application site is later examined for skin inflammation due to DEC induced microfilariae death (Boatin *et al*., 1998). All these olden diagnostic techniques are not been quantified in terms of sensitivity and predictive values (Stingl *et al.*, 1984).

Prospects of High Throughput Molecular Techniques

Newer molecular techniques are being developed for the effective diagnosis of onchocerciasis. This technique is known as oncho-dipstick test (Ngu *et al.*, 2017). In this test, each test strip was designed to have a positive control band (+), as well as negative control band (-) and a test band (T) (Taylor *et al.*, 2016). Following a series of assays with nitrocellulose strips that sensitized with various amount of recombinant antigen (0.1-5µg/ml) or specific antibodies (50-200µg/ml),the positive control band were sensitized by incubation with 2µg/ml of oncho-C27 antigen prepared in PBS,pH7.2 (Vincent *et al.*, 2017). While, both the negative control and test bands are sensitized with 150µg/ml of pre-immunization. Rabbit IgG and anti-oncho-C27 IgG antibodies respectively (Vincent *et al.*, 2017). Sensitization of the NC bands was by overnight incubation of the strips in respective solutions at 4^0C (Burnham, 2017). This test can be carrying out using urine and tears (Chandrashekar *et al.*, 2017). Each patient provided 20ml urine in a screw-capped vial prior to clinical examination (Vincent *et al.*, 2017). For tears, a single strip of absorbent paper was gently placed inside each lower eyelid and left to absorb tears secretions for 5min (Vincent *et al.*, 2017). The wetted strip was then immersed in 500µl of PBS, pH7.2 in a small plastic vial and gently shaken to facilitate diffusion of proteins from the strip into the solution (Toe *et al.*, 2015). The oncho-dipstick test uses non-invasive diagnostic sampling which avoids the risk of blood-borne contamination (WHO, 2015). Given the high concordance value (87%) between the results obtained on urine and tears. Urine would be the preferred sample for the dipstick test (Vincent *et al.*, 2017), because the test was marginally more sensitive on urine than on tears (Tume *et al.*, 2016).

The oncho-dipstick test would be useful for early diagnosis of onchocerciasis as it detectedinfection in patients who had lived in the endemic areas for less than 1 year (Tume *et al.,* 2016).

Treatment of Onchocerciasis

The treatment of onchocerciasis was revolutionized with the introduction of ivermectin in 1987. Ivermectin is now the drug of choice in the treatment of onchocerciasis (Hoerauf, 2003). Suramin may be indicated for use only if ivermectin cannot adequately control the disease (Hoerauf, 2003). Amocarzine has not been shown to be effective in treating onchocerciasis, combination of suramin and amocarzine are capable of destroying adult worms (Awadzi *et al.,* 2016). DEC (DiethylCarbamazine) therapy is no longer recommended (Stingl *et al*., 2015). Ivermectin therapy does not have the adverse reactions of DEC and it eliminates the need for 6weekly injections of suramin (Ogbuagu *et al*., 2015). The treatment of onchocerciasis with ivermectin is suitable for both clinical use and mass distribution in endemic areas (Ogbuagu *et al.,* 2015). Ivermectin is a compound derived from the bacterium *Streptomyes avermitilis* (Michael *et al.,* 2014). The drug causes nematode paralysis by impairing neuromuscular function (Michael *et al.,* 2014). Ivermectin not only prevents ocular disease but also improves and eliminates the skin disease (Awadzi *et al.,* 2016). A single dose of 150µg/kg clears the microfilariae from the skin for several months (Pacque *et al.,* 2017). It temporarily decreases the release of microfilariae, but it does not kill adult worms (Pacque *et al.,* 2017). Adverse reactions are similar to the responses of the body to dying microfilariae, but the intensity and rate of development are increases (Lazarov *et al.,* 2014). Some of adverse reactions include fever, body pains, nausea, pruritus, edema, lymphadenitis and headache (Lazarov *et al.,* 2014). Researchers are still debating on the frequency and duration of ivermectin therapy (Hoerauf, 2003). As many as 33% of onchocerciasis patients mostly in nonendemic areas are cured with only 1 dose of ivermectin, but most patients require additional therapy (Pacque *et al.,* 2017). In endemic areas, the ivermectin drug is given from every 3 months to every year depending on the degree of symptoms, cost constraints and patient compliance (Mackenzie *et al*., 2003). In non-endemic areas, a reasonable approach is the administration of a single dose of ivermectin (Pacque *et al.,* 2017). Depending on the patient's skin symptoms, the dose can be repeated every 3-6 months as needed (Pacque *et al.,* 2017). Recently (Keiser *et al.,* 2014) a treatment with doxycycline for 4 weeks, accompanied by two doses of ivermectin has been found effective (based on bacterial endosymbionts of *Onchocerca volvulus*) (Keiser *et al*., 2014).

III. Conclusion

Onchocerciasis is still a major obstacle to sustainable public health, farming and livestock development due to it negative contribution to food security in Nigeria. Disease diagnosis may be based on the clinical signs and symptoms, by demonstration of the causative organism or by reactions to diagnostic tests. However, the type of diagnostic techniques used for the detection of infections caused by onchocerciasis will vary according to the epidemiological characteristics of the disease and the strategy for control. This diagnostic challenge has had significant improvements although a high proportion of infections still remain undetected. Consequently, alternative techniques of diagnosis have been developed which can give a measure of relatedness and subsequently allow the study of the phylogeny of onchocerca species that would culminate in divisive appropriate control measures to limit the public health and economic losses arising from the disease.

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