Serological and parasitological assessment of onchocerciasis transmission and associated factors at Abeshige district, Guragae zone, Southwest Ethiopia: Cross-sectional study

Sisay Dagnew Arega (sisydagnew074@gmail.com)
Department of Medical Laboratory, Yekatit 12 Hospital Medical College, Addis Ababa, Ethiopia

Sindew Mekasha Feleke
Department of Parasitology, Ethiopian Public Health Institute, Addis Ababa, Ethiopia

Tadesse Kebede Zeleke
Department of Microbiology, Immunology and Parasitology, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia

Wuletaw Tadesse Mekonnin
Department of Medical Parasitology, College of Health Science, Salale University, Addis Ababa, Ethiopia

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Abstract

Background: One of the crippling neglected tropical diseases, onchocerciasis (river blindness), is caused on by the skin-dwelling filarial nematode *Onchocerca volvulus*, an important vector-borne neglected tropical disease transmitted by the bite of infected black fly. The adult worm lives in the subcutaneous tissues producing thousands of microfilariae that cause skin and eye disease. In Ethiopia, onchocerciasis elimination program has been under way for more than two decades and has been mainly targeting hyper-endemic districts. However, the transmission status in hypoendemic areas wasn't assessed and targeted for intervention. This study was therefore carried out to assess onchocerciasis transmission status and associated factors in the hypoendemic Abeshige district and generate evidence for program decision making.

Objective: This study assessed onchocerciasis transmission status and associated factors in first line villages of Abeshige districts.

Method: A community-based cross-sectional study was conducted in three purposively selected first line villages of the Abeshige district from April to May 2022. The study participants (n=300, 100 for each village) were randomly selected and enrolled using single proportion formula. Demographic information and participant's knowledge, attitude and practices data were collected using a standard questionnaire. Finger prick blood sample for serologic test and skin snip samples for microfilariae examination were collected and examined. Data was entered into Epi-info software version 7.1, and imported to SPSS version 26 software for analysis. The association between dependent and independent variables was done using bivariate logistic regression and the P-value <0.05% was considered statistically significant.

Result: out of the 300 examined study participants, 31 (10.1%), 17 (5.7%), 1 (0.33%) were positive by Ov-16 ELISA, Ov-16 RDT and skin snip microscopy, respectively. The participants have poor knowledge, attitude and practice about disease causation, transmission and prevention. None of the study participants have demonstrated palpable nodules, skin depigmentation, blindness, eye inflammation and itching. Age, sex and distance from the river were the independent variables those had significant associations with positivity for Ov-16 ELISA results (P<0.05).

Conclusion: the serological prevalence of onchocerciasis was above the WHO recommended threshold (5%) for the implementation of elimination programs. Age, sex and distance from the fast-flowing rivers had significantly associated with positivity of Ov-16 ELISA test. The majority of the population had poor knowledge, attitude and practice about onchocerciasis. Therefore, ivermectin treatment intervention is recommended to be implemented in the district to eliminate the disease. Inclusion of health education in the intervention program is also very important.

Author Summary

The fight against onchocerciasis has been applied over the past decades aimed to achieve elimination by 2025. However, not all areas are included in the elimination programs as is the case in Abeshige district,
south west Ethiopia. In the study area we checked high abundance of the black fly vector. Besides this the area is located close to the endemic areas of onchocerciasis where elimination programs are being practiced by bi-annual administration of ivermectin. Therefore, the chance of cross border transmission is very high. This study was able to show ongoing transmission onchocerciasis using serological and parasitological assessments. Therefore, we suggest the need for the implementation of elimination programs using biannual application of ivermectin. In the areas close to fast-flowing rivers, health education should be given with other elimination program packages.

Introduction

Background

Onchocerciasis (river blindness) is one of the debilitating neglected tropical diseases that are caused by a filarial nematode, *Onchocerca volvulus*, which resides in the skin. The parasite transmits from person to person by the bite of female black fly vector (1). *Simulium* black flies are the vectors that transmit the causative agent *Onchocerca volvulus*. *Simulium damnosum* is the major vector that spreads the causative agent which causes blindness and skin pathologies in humans. These black flies mainly breed in fast-flowing rivers and streams and the females may fly away from the breeding sites to search for people to suck blood for the maturation of eggs. These flies are active during the daytime and outdoors, so they make contact with their hosts in these circumstances (2).

When infected black flies bite people, they pass the third stage larvae to the skin. Then male and female adult filarial worms of the parasite coupled each other and form nodules in subcutaneous tissues. The fertilized females produce millions of microfilariae which live and freely migrate in the intercellular spaces of the skin tissues. The death of these microfilariae (mf) causes a wide range of signs and symptoms of skin (onchodermatitis) and eye disease (3). The immune response to the dead larvae can result in dermatitis, skin atrophy and inflammation in the eye (4). The disease is usually characterized by severe itching and pruritus which may progress to chronic skin changes leading to loss of elasticity, skin thickening and premature ageing. In some localities of Ethiopia, this acute itching and pruritus are known as “foket” (severe itching). Chronic scratching may lead to pigment changes like “leopard skin” or depigmentation. Other manifestations associated with onchocerciasis are hanging groins, hernia and elephantiasis. When the microfilariae invade the eye tissues, they cause damage to different parts of the eye including the cornea, conjunctiva, anterior and posterior eye segments. The chronicity and morbidity of the disease is associated with microfilariae (first stage larvae) that migrate through the skin and the eye. The parasite induces an immune response when the mf dies (3).

Onchocerciasis has also been associated with a variety of psychosocial and economic impacts. It results in social stigma of infected persons and their families, disturbed sleep and reduced earnings among infected adults, poor school performance and higher dropout rates of children and high health costs (5).

The rate of transmission is commonly assessed using standardized procedures with a standard fly collector catching all *Simuliid* female flies which come to feed on the fully exposed body of the fly
catcher. Dissecting the head part of these flies will give us the number of infective L3 larvae, which also tells the daily transmission potential. The characteristics vector, such as suitable ecology and population dynamics of the vector, as well as vectorial capacity and human socio-economic activities that take place around vector breeding sites, determine the intensity of transmission and level of disease endemicity (6).

The world distribution of onchocerciasis showed that 37 million people in 34 endemic countries are currently at risk of infection and about 99% of the global burden currently occurs in Africa (7). The African Savanna and Tropical Forest Zones South of the Sahara Desert are the endemic areas where the disease is widely distributed (8). This parasitic filarial disease is endemic to many Sub-Saharan countries, parts of the Arabian Peninsula and Latin America (9).

The control of the disease through applications of insecticides against larvae of *Simulium damnosum* in the endemic areas of the Volta River Basin, West Africa, has been in progress since 1973 by the Onchocerciasis Control Programme (OCP) supported by the World Health Organization, World Bank, etc. (10). Onchocerciasis in Ethiopia was first reported from Bonga by Italian investigators in 1939 (11, 12). Since then, several epidemiological studies have been conducted in Ethiopia and revealed the presence of the disease in different localities at varying levels of endemicity. According to these studies, the prevalence of the disease ranges from 0% in Eastern Ethiopia to as high as 84% in Southwest Ethiopia (12).

In Ethiopia, more than 25 million people live in endemic areas and are affected by the disease, or are at risk of infection. According to the studies done so far in Ethiopia, the disease is mostly found in South-Western, Western and North-Western parts of the country, including the whole of Benishangul-Gumuz, a portion of Oromia, Amhara, Gambella, and Southern Nations Nationalities Peoples (SNNP) Regions (1, 12). The Ethiopian onchocerciasis control program was launched in 2001 and run until 2012 through annual ivermectin mass drug administration in all meso and hyper endemic areas of the country. In 2012, the program goal changed from control to elimination with a biannual MDA approach. Ivermectin has some activity against adult worms, but predominantly reduces skin microfilarial loads through its microfilaricidal and embryostatic effects, thereby reducing symptoms and transmission. Therefore, female flies that come to bite infected people will take blood only (13). The dynamics of transmission of *O. volvulus* indicate that transmission intensity is strongly affected by the rate of host-vector contact, and reducing vector densities can be an effective method of suppressing transmission (14). Since the program goal is elimination, assessing the transmission status in all suspected area that are nearby to known endemic districts and have fly breeding sites is becoming important. Even though, elimination programs are being implemented in different endemic areas of the country, there were poor knowledge, attitude and practice on disease causation, transmission, control and elimination in the study area. Therefore, this study aimed to determine current status of onchocerciasis transmission in Abeshigae district of the Gurage zone, Southern Ethiopia.

**Materials And Methods**
**Study area**

The study was conducted in Abeshige district, Gurage zone, Southern Ethiopia. The district contains many kebeles with fast-flowing rivers, among those Gibe, Wabe, Walga, Darge and Kulit are the major ones. The Gibe River is the biggest of all these rivers. It flows between Abeshige district and Jimma zone. It is located 1710m above sea level, at a distance of 185 km along with the Addis Ababa-Jimma main road in the Southwestern part of Ethiopia. This river is extensively used by the inhabitants of both Abeshige and Jimma zone for different activities.

Walga village is another kebele in the district where there are many cash crop production lands and many daily laborers come to the area for farm activities. The Walga River is the biggest river in the kebele which used as to take shower, wash their clothes, irrigation activities, etc. for the inhabitants. The other first line village in the district is Tachgeraba, which is the nearest village to Wolkite city. The biggest fast-flowing river in the kebele is Wabe. It is surrounded by densely packed bushes. This river is also used for many activities of the community.

The weather condition of the district is considered to be convenient with some hot villages. The latitude and longitude of the area are 81,48N and 37 044"E respectively. The area around the fast-flowing rivers where the study had undertaken is a flat plain with sparse vegetation that is dominated by different acacia species. Hence, it is the ideal site for black fly breeding. The societies of the district are highly dependent on the river for different activities. Frequent river contacts expose people to black fly bite.

**Study design**

A community based-cross-sectional study was conducted to investigate the transmission of onchocerciasis in selected first-line village residents. Parasitological investigations were done by microscopic examinations of skin snip samples and serological investigations were done using blood samples. The knowledge attitude and practices of the societies living in the first line villages have been assessed using structured questionnaires.

**Study period**

The study was conducted from April to May, 2022.

**Population**

**Source population**

The residents of Abeshige district including those who are above five years old and live in the area permanently are the source population.
Permanent inhabitants of the selected first-line villages (Gibe, Walga and Wabe) of the Abeshige district who had willingness to participate.

Measurement and Data collection

Sample size calculation and sampling method

The skin snip and blood samples were collected from each study participants from first-line villages for parasitological and serological analysis. Samples were taken from three first-line villages located around fast flowing rivers. All individuals above 5 years and live in the three first-line villages were included. A total of 300 participants (considering the 10% non-respondents) were taken from using single proportion formula first line villages for serological and parasitological analysis.

\[
\text{Therefore: } n = \frac{(z_{\alpha/2})^2 p(1-p)}{d^2}
\]

Where, \( n \) = Sample size

\( z_{\alpha/2} \) = Critical value =1.96

\( p \) = Percentage of onchocerciasis prevalence in Jimma zone = 0.23

\( d \) = Precision (margin of error) =0.05

\[
\begin{align*}
\text{n} &= (1.96)^2 \times 0.23(1-0.23) / (0.05)^2 \\
\text{n} &= 272
\end{align*}
\]

Substituting the values for each of these variables in the above formula, the sample size was estimated to be 272. Adding non response rate of 10%, the final sample size became 300.

Sampling technique

A purposive sampling method was conducted. Three first line villages were purposively selected based on their proximity to fast flowing rivers. The selection of first line villages was based on proximity to fast-flowing rivers; complain of black fly bite and proximity to endemic areas. From those purposively selected first line villages, 300 participants whose age was \( \geq 5 \) years were randomly selected.

Measurement and Data collection

Each step of the sample collection procedure was done in separate room with adequate illumination to maintain the privacy of study participants. DBS samples were collected using sterile lancet by finger pricking. The blood was dropped into the circles of filter paper. The drying procedure was done with care to avoid dust and direct sunlight. For RDT tests, a gentle finger prick was made and put a drop of blood into sample well of the RDT kit. A drop of buffer was added to move the sample to test and control zones. Finally, the result was recorded after the recommended time carefully.
Skin snip samples were collected from both sides of iliac crust of participants by sterile bio-puncher. The sites were cleaned by 70% prior to punching. Each skin snip sample was put in coded 96 well microtiter plate wells containing normal saline and incubated at room temperature for 12 to 24 hours. Each skin snip sample was examined under binocular compound microscope and the number of microfilariae was counted.

The results of serology and microscopic examinations were recorded in a structured check list. The clinical examination for signs and symptoms of onchocerciasis such as palpable nodules on their bodies, pruritis, leopard skin and hanging groin were collected by trained and experienced health professionals.

**Demographic and clinical data**

Socio-demographic data was collected using a structured questionnaire by trained data collectors. The level of exposure to the black fly bite was collected by the principal investigator using interviews. Each individual was physically examined for skin lesions as well as palpated for onchocerca subcutaneous nodules.

**Sample collection for laboratory analysis**

Blood samples were taken from participants based on standard finger pricking procedures for serological examinations. Skin snip samples were taken from three first line villagers of Abeshige district to see microfilariae of *Onchocerca volvulus*. Left and right iliac crust area is the site for skin snip sample collection. These sites were cleaned using 70% alcohol before applying biopuncture to avoid contamination. Blood free skin samples were taken using sterile biopuncture and put the sample in the wells of microtiter plate that contains normal saline. Finally, the microtiter plate were labeled, incubated overnight and examined at 40X magnification power of light microscopy.

**Data Quality Assurance**

Pretest was on 5% of the total sample size to assess the understandability of the questionnaire. The questionnaire was prepared in English and translated to Amharic then translated back to English to ensure consistency of translation. A comprehensive training was given to data collectors. Daily quality controls were run to ensure the quality of each lot of the RDT kit prior to screening participant's blood. The serological tests were done by trained and experienced laboratory professionals with strict supervision of principal investigator. Blood samples were immediately tested using RDT based on standard operating procedures. DBS samples were also prepared by well-trained data collectors and analyzed by experienced laboratory professionals by ELISA machine in parasitology laboratory located at EPHI. Both the quality controls and samples were diluted and run in duplicates. The wells of the microtiter plates were checked for clarity to avoid detergents and other dirt's which could affect the quality of our test. Quality controls and samples were in run duplicates according to manufacturer's instructions and the results were recorded properly. skin snip samples were collected by well trained professional from each participant. After incubation microscopic examinations were done at the right time and with the
right quality of binocular microscope to increase the chance of getting microfilariae and the results were collected recorded immediately. All fields of microscope were examined to increase the chance of finding microfilariae.

**Inclusion and Exclusion Criteria**

**Inclusion criteria**

All individuals above 5 years of age and who live in the first-line villages.

**Exclusion criteria**

Anyone who is too sick or not willing to participate and under 5 children were excluded.

**Study variables**

**Dependent variables**

Ov-16 antibody prevalence

Skin microfilariae prevalence

**Independent variables**

Sex of participants

Age of participants

Occupation

Distance from the river

Educational status

**Laboratory testing and analysis**

Blood samples were tested using serological testing methods. The RDT was done using drops of blood taken from each participant by finger pricking. The blood sample was dropped into Ov-16 RDT kit-based on standard operating procedures. The result was recorded immediately for each of the participants. The dried blood sample was brought to parasitology laboratory located in EPHI and analyzed using ELISA using standard operating procedures. The results were properly read using standards and recorded immediately.

Collected skin snip specimens were immersed into 3 drops of normal saline. The microliter plates that bear the specimen was covered by adhesive plaster and labeled properly. Incubated samples were examined under a microscope carefully. The results of the microscopic examination were recorded...
immediately using structured check-lists prepared for each individual. Positive skin snip samples were transferred to screw capped tubes and preserved in absolute alcohol for further investigations.

**Data analysis and interpretation**

All the data were checked for completeness, then entered into Epi-info version 7.1, and analyzed using SPSS version 26 statistical software for windows. The data were checked for normality of its distribution Therefore, the nonparametric methods for the determination of serological prevalence were used as recommended by clinical laboratory sciences (CLSI) 5 Median, central 95 percentiles, and 95% confidence interval (CI) were calculated. Binary logistic regression was the statistical model used to determine the serological and parasitological prevalence of onchocerciasis. P-value < 0.05 is considered as statistically significant

**Ethical considerations**

All study participants were given full and detailed information regarding the study procedures and objectives based on their local language. The procedures of skin snip and blood sample collection was explained in detail to participants. The degree of pain while taking the samples was explained frankly. Participants were guaranteed that their confidentiality would be maintained while exposing the skin. Separate rooms were prepared for sample collection. For children, the same agreement was reached between parents/guardians and the principal investigator. Assent was obtained from children who are 12-18 years old. Participants were convinced that their results would be kept confidential. Since; properly collected skin snip samples were bloodless there is no fear of bleeding. The site where the sample is taken was cleaned to avoid contamination. There was tolerable slight pain while pinching the sin using a sterile bio-puncher and puncturing finger using sterile lancet. Each participant agreed verbally and signed informed consent to participate in the study. All above 5 years old members of the Abeshige society would be given ivermectin, if an appropriate government decision would be made based on the recommendations of this study. It is a greater advantage to be part of the national onchocerciasis elimination programs. Therefore, the participants should be responsible for the reliability of the information they provide. Their active participation was vital to show the real picture of onchoceriasis transmission in the district and this could have an impact on society. The study was approved by the community leaders, zone and district health bureau of Gurage zone and the South region bureau. Ethical approval was obtained from Addis Ababa University College of Health Science Department of Microbiology Immunology and Parasitology Research Ethics Review Committee.

**Results**

**Socio-demographic Characteristics of the study participants**

A total of 300 participants were enrolled with 100% response rate from three first line villages of the Abeshige district. More than half of the participants 165 (55%) were females. The mean age of the respondents was 32.28 ± 20.83 years. The majority of the study participants 211 (70.33%) were below 41
years, while 89 (29.67%) were 41 and above years. The majority 224 (74.67%) were Orthodox religion followers while 73 (24.33%) were Muslims and protestants were around one percent. More than half of the participants 175 (58.33%) were married and 125 (47.67%) were not married. The educational status of the study participants was summarized as, 130 (43.33) were illiterate, 151 (50.33) attended primary education and those who attended secondary education and above were 19 (6.33%). More than half of the participants 185 (61.67%) were farmers, 110 (36.67%) of them were students, 5 (1.67%) of them were government employee.

Table 1 Socio demographic characteristic of study participants for parasitological and serological indicators of onchocerciasis in the Abeshige district, Southern Ethiopia, 2022
<table>
<thead>
<tr>
<th>Variable</th>
<th>No of Participant</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>135</td>
<td>45</td>
</tr>
<tr>
<td>Female</td>
<td>165</td>
<td>55</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-10</td>
<td>50</td>
<td>16.7</td>
</tr>
<tr>
<td>11-20</td>
<td>64</td>
<td>21.3</td>
</tr>
<tr>
<td>21-40</td>
<td>97</td>
<td>32.3</td>
</tr>
<tr>
<td>≥41</td>
<td>89</td>
<td>29.7</td>
</tr>
<tr>
<td>Religion</td>
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<td></td>
</tr>
<tr>
<td>Orthodox</td>
<td>224</td>
<td>74.67</td>
</tr>
<tr>
<td>Muslim</td>
<td>73</td>
<td>24.33</td>
</tr>
<tr>
<td>Protestant</td>
<td>3</td>
<td>1.0</td>
</tr>
<tr>
<td>Marital Status</td>
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<td></td>
</tr>
<tr>
<td>Married</td>
<td>175</td>
<td>58.33</td>
</tr>
<tr>
<td>Unmarried</td>
<td>125</td>
<td>47.67</td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No formal education</td>
<td>130</td>
<td>43.33</td>
</tr>
<tr>
<td>Primary education</td>
<td>151</td>
<td>50.33</td>
</tr>
<tr>
<td>Secondary and above</td>
<td>19</td>
<td>6.33</td>
</tr>
<tr>
<td>Occupation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Farmer</td>
<td>185</td>
<td>61.67</td>
</tr>
<tr>
<td>Student</td>
<td>110</td>
<td>36.67</td>
</tr>
<tr>
<td>Employee</td>
<td>5</td>
<td>1.67</td>
</tr>
</tbody>
</table>

**Knowledge, attitude and practice of the community about onchocerciasis**

The KAP analysis about onchocerciasis and its mode of transmission showed gaps as presented below (Table 2). 88 (29.33%) of the participants have heard about onchocerciasis, 111 (37%) of the participants knew that onchocerciasis is a transmittable disease, 75 (25%) thought that onchocerciasis is transmitted
by the bite of black fly, 95 (31.7%) of them were those who think contact to infected person could transmit the disease, 48 (16%) thought that bite of mosquito transmits the disease, 56 (18.7%) of them think by house fly bite and 40 (13.3) of them think that it is aerosol transmitted. In addition, 84 (28%) of the participants believe that spending time near river for washing clothes, body washing and day time irrigation practice expose for black fly bite. The majority of the participants did not know the sign and symptoms of onchocerciasis. Accordingly, 60 (20%) of them know the sign and symptoms of the disease, 54 (18) skin rash, 59 (19.7%) itching, 25 (8.3%) blindness, 52 (17. 33%) skin depigmentation 41 (13.7%) inflammation of the eye and 47 (15.7%) skin lesion were the signs and the symptoms which are believed to be occur due to onchocerciasis infection. The study participants also have little knowledge about the prevention of black fly bite and transmission of the disease. The majority of them do not know the drug used to treat the disease. Only 17 (5.7%) of the participants know the drug used to treat onchocerciasis. 155 (51.7%) of the participants thought that there is higher risk of contracting onchocerciasis and it is dangerous to the community. More than half of the participants 179 (59.1%) believed that onchocerciasis curable disease, 180 (60%) of them believed that communities become free after MDA is given and 171 (57%) of them believed that onchocerciasis can be eliminated by proper use of MDA. 82 (27.3%) of the participants know the ways of controlling onchocerciasis. 68 (22.7%) of the participants believed that wearing cloths prevents black fly bit, 81 (27%) of them believed that MDA prevents onchocerciasis and 66 (22%) of them believed that vector control measures help to prevent onchocerciasis. Washing with soap, keeping personal hygiene, avoid contact with infected person, avoid river contact, health education and vaccination are mentioned by 20 (6.6%) of the participants as additional ways of preventing onchocerciasis.

Table 2 Knowledge attitude and practices study participants in the parasitological and serological indicators of onchocerciasis in the Abeshige district, Gurage zone southern Ethiopia, 2022.
Variables | Response (Yes %)
---|---
Have you ever heard about onchocerciasis | 88 (29.33)
Do you think onchocerciasis is a transmittable disease | 111 (37.0)
Do you know that onchocerciasis caused by a filarial worm | 16 (5.3)
Onchocerciasis is transmitted by
- mosquito biting | 48 (16.0)
- house fly biting | 56 (18.7)
- contact with infected person | 95 (31.7)
- Aerosol | 40 (13.3)
- black fly biting | 75 (25.0)
Do you know how to prevent onchocerciasis | 82 (27.3)
MDA | 81 (27.0)
Wearing clothes | 68 (22.67)
Vector control | 66 (22.0)
Do you know what exposes for biting of onchocerciasis | 24 (8.0)
Do you know the sign and symptoms of the disease | 60 (20.0)
Do you think the person with the disease should be isolated | 160 (53.3)
Do you think it can be eliminated by the MDA | 171 (7.0)

Clinical characteristics and laboratory findings of study participants

In the clinical examination, each participant was visually examined for skin lesions as well as palpated for onchocerca subcutaneous nodules. They were asked whether they faced itching, inflammation of eyes, skin discoloration and other signs and symptoms of onchocerciasis. The clinical examinations revealed that, there were no skin lesions and palpable nodules in the participants. Although some of the participants complained itching and skin discoloration, these were checked to be non onchocercal itching and discoloration. Blood sample was taken from each participant and examined immediately using Ov-16 kits. DBS samples were taken for further serological examinations. Skin snip samples were taken from the participants to be examined for microfilariae. From the participants 17 (5.7%) were positive by Ov-16 RDT tests and 31 (10.33%) of them were positive by ELIZA tests. The skin snip examination showed that 1 (0.33%) was positive under microscopic examinations.

Clinical characteristics and laboratory findings of study participants in the parasitological and serological indicators of onchocerciasis in the Abeshige district, Gurage zone southern Ethiopia, 2022.
<table>
<thead>
<tr>
<th>Variable</th>
<th>No of Participant</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ov-16 RDT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>283</td>
</tr>
<tr>
<td>Ov-16 ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>269</td>
</tr>
<tr>
<td>Skin Snip</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Neg</td>
<td>299</td>
</tr>
</tbody>
</table>

Factors that influence the results of onchocerciasis test

Binary logistic regression analysis was done to see the association of independent variables to the positive findings of onchocerciasis. The independent variables, age, sex and distance from fast-flowing rivers were analyzed by binary logistic regression to check the association of the variables to positive findings of onchocerciasis test. These variables were found to be significantly associated to the positive results of onchocerciasis test with P-value of <0.05.

Table 4 Binary logistic regression analysis result showing factors associated with positivity for OV16 ELISA for onchocerciasis in Abeshige district Gurage zone, southwest Ethiopia; 2022
### Performance of the test Ov-16 ELISA as a reference standard for Ov-16 RDT

In this study only one of the participants was positive for microfilariae of *Onchocerca volvulus*. The Ov16 ELISA served as gold standard for Ov16 RDT and skin snip tests. The sensitivity, specificity, positive predictive value, and negative predictive value were respectively 46.88% (29.09-65.26%), 98.88% (96.76-99.77%), 83.33% (60.47-94.23%) and 93.97% (91.84-95.57%).

### Discussion

Many countries have passed through several years of onchocerciasis elimination program implementation, but onchocerciasis still remains a disease of public health importance in many African
countries. As different studies showed, there are numerous challenges that affect the existing efforts to eliminate onchocerciasis from Africa. The major challenges include an incomplete mapping of all transmission zones, possible emergence of ivermectin resistance, weak cross-border elimination efforts, poor health care coverage, uncoordinated program implementation and technical and financial challenges (6). The laboratory findings showed that sex, age and distance from the river had significant association with positivity of Ov-16 ELISA tests. Communities living near to the river were more likely to be positive than those who live far, males were likely to be more positive than females and active age groups (11-20 years) were more victimized than the rest of the age groups.

It was found that 10.33% of the participants were positive for Ov-16 ELISA test, 5.7% for Ov-16 RDT test and one (0.33%) for skin microfilariae examination. This finding is consistent to a study done in northern and central Togo for Ov-16 ELISA (15). In contrast the prevalence rate of onchocerciasis in this study is lower compared to the results of the studies conducted in Jimma 22.5% and Gurage zone 17.1%, southwest Ethiopia. The lower prevalence might be due to the implementation of CDTi in the adjacent kebeles of the study site that could hinder further transmission. The Ov-16 ELISA result is in agreement with the finding of the researches done in Gurage and Jimma zones southwest Ethiopia (6, 16). The value 10.33% Ov-16 ELISA test is higher than the findings done in Ethiopia Metema Galabat focus (16). This discrepancy may be due to the implementation of elimination program in the Metema Galabat focus for the last two decades.

This study revealed that the study participants have poor knowledge of causation, exposure, transmission, treatability and prevention of onchocerciasis. Only 88 (29.33%) of them have heard about onchocerciasis and 111 (37%) of them thought that it is a contagious disease but only 16 (5.33%) of them believed that onchocerciasis is a filarial disease. 75 (25%) of the participants mentioned that onchocerciasis is transmitted by the bite of black fly, while the majority of the them 225 (75%) believed that the disease is transmitted by other means such as 95 (31.67%) contact with infected person, 48 (16%) mosquito bite, 56 (18.7%) housefly bite and 40 (13.33%) aerosol. Only 24 (8%) of the participants mentioned the exposing factors to black fly bite and 82 (27.33%) of them knows the ways of avoiding disease transmission. Similarly, poor knowledge and practice towards onchocerciasis was also reported from other places in Ethiopia (17). In contrast a research done in Nigeria showed good knowledge, attitude and practices to the cause, transmission, morbidity and prevention of onchocerciasis (18, 19).

The present study showed that there is significant association between participant sex to positivity of Ov-16 ELISA test. Males were more likely to be positive to Ov-16 ELISA test than females. The rationale behind this finding might be, the potential outdoor activities make males more exposed to black fly bite. This study is in agreement with the study done in Yeki and Asosa districts in Ethiopia. This finding is not in agreement with the researches done in Cameroon (20, 21). Distance of residential areas from fast-flowing rivers showed significant association with the positivity of Ov-16 ELISA test. Participants who lived near to fast flowing rivers are four times more positive than those who lived at far distances from the river. This might be due to the more frequent river contact of the societies who live nearest to the river.
than those who live farther. The same findings were reported in the researches done in Equatorial Guinea and South Sudan (16, 22).

Participant's age is another variable that is found to be associated to the positive results of IgG4 recombinant Ov-16 ELISA test. The age group 11-20 was found to be significantly associated to the positive Ov16 ELISA test result. This finding agrees to the findings of the research done in Equatorial Guinea. The reason behind this high prevalence in this age group might be due to the increased of economic activity they play around fast-flowing rivers in their locality. They are active age groups who are responsible for keeping cattle near rivers, irrigation farms, collecting fire wood in the bushes located around rivers and fetching water from the rivers. In addition to these activities these age groups may take showers and swim on rivers. These activities expose these people to intense black bite. Three (1%) children from 5-10 years’ age group were positive for Ov-16 ELISA test, which indicates that there is an ongoing transmission in the district. This finding is lower than study done in Nigeria. The rationale behind this discrepancy might be the study done Nigeria was undertaken after ten rounds of ivermectin administration. But the value 1% suggests the ongoing transmission of the disease in the district that needs intervention (23, 24).

The present study was undertaken in villages where there are numerous fast-flowing rivers which provide ideal breeding sites for the vector. 201 (67%) of the participants live within 1-2 kilometers range from the river. This makes them highly exposed for black bite, thus there will be high potential of onchocerciasis transmission.

**Strength and limitation of the study**

**Strength of the study**

Before starting the actual study, river prospection was done to search for the presence of the right vectors around fast flowing rivers. Community members were involved in the processes to check community complaints of black fly bite. This helps to identify the right target areas of study. All the necessary variables were used to dig out the right indicators of ongoing transmission of onchocerciasis. Microscopic examinations of skin snips and serological were carefully done by well trained and experienced professionals.

**Limitation of the study**

The sample size was not enough for hypoendemic areas of Abeshige district to see the actual picture of skin microfilariae. Collagenase enzyme was not added to enhance digestion of the skin snip to increase sensitivity of the microscopic examination. Entomological transmission indicators such as, vector infectivity, transmission potential was not covered due to resource limitations.

**Conclusion And Recommendation**

**Conclusion**
The serological prevalence of onchocerciasis was above the WHO recommended threshold (5%) for the implementation of elimination programs. Age, sex and distance from the fast-flowing rivers had significantly associated with positivity of Ov-16 ELISA test. Therefore, intervention should be implemented using ivermectin treatment in the district to eliminate the disease. The majority of the population had poor knowledge, attitude and practice about onchocerciasis. Therefore, inclusion of health education in the intervention program is important.

**Recommendation**

Health extension workers and other health professionals of the district should give health education to the inhabitants of the district on the transmission, etiology, sign and symptoms, exposure, the nature of the vector and preventive measures in an organized way. Officials of the health bureau of the district should support the community mobilization process.

Researchers who are interested to do the same research are recommended to achieve the following:

- Increase sample size to maximize the probability of finding microfilariae in the skin.
- Allocate enough resource to study all the necessary aspects of onchocerciasis transmission such as vector infectivity, parity, transmission potential and serological prevalence.
- Use sophisticated diagnostics like molecular diagnosis (PCR) to show the real picture of onchocerciasis transmission in the districts.

Apply collagenase enzyme to incubated skin snip samples so that the skin can be digested to release the microfilariae in to the solution.

**Declarations**

**Ethical considerations**

Ethical approval for the study was provided by Addis Ababa University College of Health Science Review Committee. The study was approved by the community leaders, zone and district health bureau of Gurage zone and the South region bureau. All study participants were given full and detailed information regarding the study procedures and objectives based on their local language. The procedures of skin snip and blood sample collection was explained in detail to the participants.

**Consent for publication**

Not applicable

**Availability of data and materials**

All materials and data are available in the hands of corresponding author if requested.

**Competing interests**
We declare that we do not have competing interests.

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Author contribution

SD engaged in the conception of design of study, performed data collection, analysis, interpretation and drafted the manuscript. SM carried out supervision field data collection and laboratory analysis. TK, SM and WT involved in the study design, reviewed data analysis and manuscript writing.

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References


**Figures**
Figure 1

map of the study site