LABORATORY DETECTION OF TRICHURIS TRICHIURA, AND ITS IMPACT ON HUMAN PATIENT

Maher Futael Obead Alqethami[1]*, Mohammed Saleh Alghamdi[2], Muath Abdullah Ahmed Alghamdi[3], Saleh Eidhah Ali Almalki[4], Faisal Abdullah Alibalghamdi[5], Abdullah Hameed Mohammed Almalki[6], Abdulrahman Mohammed Bamalik[7], Moneira Affar Masoud Alsobei[8], Munirah Ali Mohammed Hadadi[9], Faisal Ayban Alanazi[10]

Abstract:
Trichuris trichiura is a parasitic worm that is transferred through soil and infects people worldwide. There have also been recorded cases of humans being infected by Trichuris suis and Trichuris vulpis. Identification of Trichuris species infecting human populations at the molecular level. In this study, we examined the laboratory identification of Trichuris trichiura and its effects on human patients. Traditional techniques encompass microscopy, culture, and egg quantification. Serology plays a significant role, particularly in cases involving S. stercoralis, as standard approaches have low sensitivities. The use of quick and extremely sensitive molecular techniques, such as quantitative polymerase-chain reaction, is well-suited for diagnosing STH compared to insensitive and labor-intensive traditional approaches. Previously, the detection of STH (Soil-Transmitted Helminths) at the molecular level was primarily limited to research environments. However, there is currently a suggestion to incorporate molecular testing into the STH eradication programs endorsed by the World Health Organization.

[1]*Laboratory technician, Laboratory, health affairs in Mecca
[2]Laboratory technician, Laboratory, health affairs in Mecca
[3]Laboratory specialist, Laboratory, King Faisal hospital - Makkah
[4]Laboratory technician, Laboratory, King Faisal Hospital in Mecca
[5]laboratory technician, laboratory, King FAISAL HOSPITAL
[6]Laboratory, laboratory technician, King Faisal Hospital
[7]laboratory technician, laboratory King Faisal Hospital
[8]Medical laboratory specialist, Laboratory, Al-Kharma General Hospital
[9]Laboratory specialist, Laboratory, Maternity and Children Hospital
[10]laboratory technician, Dammam Medical Complex, Laboratory-Dammam Medical Complex

***Corresponding Author: -** Maher futael obead AlQethami
*Laboratory technician, Laboratory, health affairs in Mecca

DOI: 10.53555/ecb/2022.11.8.64

Introduction:

Trichuris trichiura, commonly referred to as the human whipworm, is a kind of roundworm that induces trichuriasis in people. The whipworm is named such due to its resemblance to a whip, having large grips at its posterior end. The whipworm possesses a slender anterior esophagus and a large posterior anus. The worms often exhibit a pink coloration and fasten themselves to the host by means of their short anterior end. The dimensions of these worms range from 3 to 5 cm. Females often exhibit a greater size than males [1].

The female worm has the capacity to deposit a range of 2,000 to 10,000 eggs on a daily basis. The eggs are deposited in soil by the excretion of human feces. Following a period of 14 to 21 days, the eggs reach maturity and transition into an infectious phase. When people consume the fertilized eggs, the eggs begin to hatch in the small intestine and use the microorganisms and nutrients in the gut to reproduce and develop. Most larvae go to the cecum, infiltrate the mucosa, and undergo maturation into adulthood. Infections with a significant number of worms usually affect the lower sections of the large intestine [1].

Trichuriasis is one of the three well-documented illnesses caused by soil-transmitted helminths (STH). The other two infections are ascariasis and hookworm infection. The World Health Organization (WHO) and the Centers for illness Control and Prevention (CDC) classify it as a neglected tropical illness.

Trichuriasis is mostly caused by the consumption of contaminated eggs present in soil. This is frequently attributed to unsatisfactory sanitary conditions, such as the practice of open defecation and the utilization of human excrement as fertilizer. Recent research indicate that individuals with specific chromosomal characteristics may have a higher likelihood or greater vulnerability to developing trichuriasis[2].

The whipworm's egg is the stage at which it becomes infectious, and it requires a warm and humid atmosphere to mature under ideal conditions. Hence, the majority of the illness load is prevalent in tropical regions, particularly in Asia, and to a lesser extent, in Africa and South America. It is also seen in rural areas of the southeastern United States. Global estimates suggest that the number of active cases ranges from 450 million to 1 billion, with the majority of diagnoses occurring in youngsters. Evidence suggests that there is a degree of partial protective immunity that develops with increasing age [3].

Globally, about 50% of the 5 billion individuals residing in underdeveloped nations are affected by at least one type of soil-transmitted helminth, whereas 10% are infected with two or more helminth species. Adolescent males are more susceptible to the negative impacts of this phenomenon due to their higher likelihood of engaging in outdoor activities and displaying pica behavior [4].

Review:

Soil-transmitted helminths (STHs) are a group of intestinal nematodes, including hookworms (Ancylostoma duodenale, Necator americanus), roundworm (Ascaris lumbricoides), whipworm (Trichuris trichiura), and Strongyloides stercoralis. However, the World Health Organization (WHO) does not officially include S. stercoralis in the STH list. STH has been placed in the World Health Organization's list of 17 neglected tropical diseases (NTDs) because to its association with poverty, substantial morbidity, and loss of disability-adjusted life years (DALYs) [5]. The majority of illnesses occur in nations located in the tropical and subtropical regions, such as India. Approximately 1.5 billion individuals worldwide are afflicted with soil-transmitted helminths (STHs) [5]. The prevalence of ascariasis is estimated to be between 771.7 and 891.6 million individuals, whereas trichuriasis affects around 429.6 to 508 million people. Hookworm infection is recorded in roughly 406.3 to 480.2 million individuals [6]. STHs, or somatotropic hormones, lead to substantial hindrance of both mental and physical development as well as anemia, particularly in youngsters. STH infections frequently exhibit no symptoms, however they may lead to severe gastrointestinal (GI) symptoms. The World Health Organization (WHO) has established a target of eradicating soil-transmitted helminths (STHs) as a public health issue in kids by the year 2020 [7].

The laboratory diagnosis of trichuriasis relies on the microscopic analysis of stool samples to identify the presence and, if feasible, quantify the quantity of eggs [5]. In cases of severe infestation, the eggs may be seen on a stool saline smear, however this method has limited sensitivity. The World Health Organization (WHO) advocates for the utilization of the Kato-Katz technique to quantify the number of eggs per unit weight of excrement. Additionally, it is advised to utilize 2 slides for each sample [5]. A drawback of stool sample evaluation is the presence of a three-month time lag between the ingestion of eggs and the growth of the mature worm. During this duration, there can be an absence of indications of an infestation, and the feces may not exhibit any evidence of eggs or shedding.

Stool samples can also show the presence of red blood cells (RBCs) and white blood cells (WBCs), particularly eosinophils. Anemia may be indicated by a comprehensive blood count. There have been instances where individuals in well-equipped regions have reported symptoms, and their diagnosis has been confirmed with sigmoidoscopy or colonoscopy. An observed phenomenon is the presence of mature worms in white masses, like coconut cake, hanging from irritated mucosa in the rectum. Recent research have revealed the presence of a phenomenon called "whipworm dance" on ultrasonography, characterized by the constant wriggling movement of the lumen of the appendix. This method is readily applicable in situations with limited resources [7].

PCR tests, which utilize the polymerase chain reaction technique, are presently under development and implementation in research environments. As a result, the ability to identify the whipworm has been enhanced in terms of both specificity and sensitivity [5,8].

Typically, the diagnosis of STHs involves examining fecal or other gastrointestinal samples to detect the presence of helminthic eggs, larvae, or occasionally adult worms or their segments. In order to detect parasites, it is necessary to collect numerous specimens over a 10-day period, as eggs and/or larvae are shed intermittently [5]. A fresh fecal specimen, consisting of roughly one big teaspoon or about 10 ml of liquid stool sample, must be sent to the laboratory within 1 hour of collection.

Direct microscopy technique:

Create a homogeneous and thin solution (using saline and iodine) and then place a cover glass on top. To handle dysenteric and unformed specimens, extract a sample that includes both blood and mucus. Create a smear by placing the sample on a slide, without adding any saline or dye. Finally, cover the sample with a cover glass [9]. Inspect the specimen under a microscope to detect any larvae or eggs of parasitic worms. They should be meticulously monitored for their morphology, dimensions, presence of bile pigmentation, and other relevant characteristics. The eggs of A. lumbricoides exhibit bile staining, whereas the eggs of other soil-transmitted helminths do not. The fertilized eggs of A. lumbricoides are spherical, measuring between 45 and 75 μm in length, and possess a robust shell with an outer layer that is covered with little projections. Occasionally, the outer layer is missing, resulting in decorticated eggs. Unfertilized eggs are elongated and bigger than viable eggs, measuring around 90 μm. Their shell is thinner and the mammillated layer is more varied, exhibiting either prominent protuberances or virtually none [10]. Microscopic differentiation between the eggs of Ancylostoma and Necator is not possible. The eggs have a thin shell, lack color, and have dimensions of 60–75 × 35–40 μm. The eggs of T. trichiura measure 50–55 × 20–25 μm, have a thick barrel-shaped shell, and include a pair of polar "plugs" at each end. When examining S. stercoralis, one may discover larvae that are 200–250 μm long and 16 μm wide, without a protective covering. The specimen has a characteristic rhabditiform esophagus with a prominent bulbous structure. If a stool sample is left in a warm and humid setting for more than 24 hours, the hookworm eggs may hatch and release larvae. It is important to distinguish them from the larvae of S. stercoralis. Eosinophils and Charcot-Leyden crystals may be seen in the feces during T. trichiura infection [9].

Fecal concentration methods:

If the fecal population is less, it is advisable to concentrate the stool whenever feasible. The most highly suggested process is the formal-ether concentration (FEC) method. The formalin-ether solution has a lower specific gravity compared to the parasitic organisms, resulting in the concentration of the organisms in the sediment [8,9]. The limited usage of floating techniques (such as zinc sulfate floatation and saturated sodium chloride floatation) is due to their inability to effectively collect sterile eggs of Ascaris and larvae of Strongyloides, as they do not float.

Kato-Katz method & McMaster technique:

This approach is widely used for quantifying helminth eggs. The World Health Organization (WHO) advises the inspection of replicated slides in the Kato-Katz (K-K) technique [10]. It is necessary to inspect the slides after a glycerol-clearing period of 40-60 minutes, otherwise hookworm eggs have a tendency to dissolve and vanish.

The World Health Organization (WHO) has established that infection levels of 5000 to 50,000 EPG for A. lumbricoides, 2000 to 4000 EPG for hookworm, and 1000 to 10,000 EPG for T. trichiura are considered moderate and heavy infections, respectively. The user's text is "[10]". K-K is effective in detecting infections caused by A. lumbricoides and T. trichiura, but it has limited sensitivity in detecting hookworm eggs. This is because hookworm eggs have a tendency to dissolve if there is a delay in examining the samples [11]. Presently, this approach is extensively utilized

in epidemiological field surveys because of its economical nature and very little skill requirements [12].

This approach offers a simpler standardization process compared to K-K, making it easier to establish consistent measurements. Additionally, its performance is similar to that of K-K. In this procedure, a specified amount of fecal suspension is analyzed under a microscope using a counting chamber. The EPG is determined by multiplying the total number of eggs or oocysts by 50. In this case, the eggs are separated from any dirt or waste, which allows for a reasonably fast and accurate counting process. The sole drawback in this case is the requirement for a specialized counting chamber [13].

Conclusion:

Presented is the molecular evidence of the presence of T. trichiura and T. suis in people. It is evident that there is a need to increase knowledge regarding the zoonotic capacity of T. suis. Molecular data is crucial for conducting systematic, taxonomic, and diagnostic studies on human populations affected by T. trichiura and T. suis. It plays a vital role in ongoing epidemiological investigations, population genetics research on T. trichiura and T. suis, and the development of prevention and control programs aimed at reducing animal-to-human transmission in this area. An effective surveillance program should be implemented, considering the involvement of reservoir hosts (namely pigs) in the natural transmission of human trichuriasis caused by T. suis. Molecular approaches are becoming more often employed as auxiliary tools for species-level identification. Various molecular markers, including nucleotide sequences of internal transcribed spacers 1 and 2 (ITS1 and ITS2) and nuclear small subunit rRNA, can be employed to identify Trichuris spp.

References:

1. Bansal R, Huang T, Chun S. Trichuriasis. Am J Med Sci. 2018 Feb;355(2):e3.
2. Williams-Blangero S, Vandeberg JL, Subedi J, Jha B, Dyer TD, Blangero J. Two quantitative trait loci influence whipworm (Trichuris trichiura) infection in a Nepalese population. J Infect Dis. 2008 Apr 15;197(8):1198-203.
3. Truscott JE, Turner HC, Anderson RM. What impact will the achievement of the current World Health Organisation targets for anthelmintic treatment coverage in children have on the intensity of soil transmitted helminth infections? Parasit Vectors. 2015 Oct 22;8:551.
4. Ranjan S, Passi SJ, Singh SN. Prevalence and risk factors associated with the presence of Soil-Transmitted Helminths in children studying in Municipal Corporation of Delhi Schools of Delhi, India. J Parasit Dis. 2015 Sep;39(3):377-84.
5. World Health Organization. *Investing to Overcome the Global Impact of Neglected Tropical Diseases: Third WHO Report on Neglected Diseases.* Geneva, Switzerland: WHO Press; 2015. pp. 1–191.
6. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors.* 2014;7:37.
7. World Health Organization. *Eliminating Soil-Transmitted Helminthiases as a Public Health Problem in Children, Progress Report 2001-2010 and Strategic Plan 2011-2020.* Geneva, Switzerland: WHO Press; 2012. pp. 1–78.
8. Tarafder MR, Carabin H, Joseph L, Balolong E, Jr, Olveda R, McGarvey ST. Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* infections in humans in the absence of a 'gold standard'. *Int J Parasitol.* 2010;40:399–404.
9. Cheesbrough M. Direct Laboratory Practice in Tropical Countries (Part-1) New York: Cambridge University Press; 2009. pp. 29–35.
10. WHO Expert Committee. Prevention and control of schistosomiasis and soil-transmitted helminthiasis. *World Health Organ Tech Rep Ser.* 2002;912:i.
11. Knopp S, Mgeni AF, Khamis IS, Steinmann P, Stothard JR, Rollinson D, et al. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: Effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Negl Trop Dis.* 2008;2:e331.
12. Tarafder MR, Carabin H, Joseph L, Balolong E, Jr, Olveda R, McGarvey ST. Estimating the sensitivity and specificity of Kato-Katz stool examination technique for detection of hookworms, *Ascaris lumbricoides* and *Trichuris trichiura* infections in humans in the absence of a 'gold standard'. *Int J Parasitol.* 2010;40:399–404.
13. Nikolay B, Brooker SJ, Pullan RL. Sensitivity of diagnostic tests for human soil-transmitted helminth infections: A meta-analysis in the absence of a true gold standard. *Int J Parasitol.* 2014;44:765–74.