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Molecular–Physiological Detection as a More Accurate Diagnostic Method for Giardia lamblia Infections in Al-Muthanna Province, Iraq

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Abstract

Giardiasisa common diarrheal illness that has major health consequences worldwide, particularly in developing nations, is caused by the flagellated intestinal protozoan Giardia lamblia. The primary diagnostic technique is still conventional microscopy, although it frequently has low sensitivity and operator dependence. This study aimed to evaluate the diagnostic accuracy of polymerase chain reaction (PCR) relative to light microscopy for detecting G. lamblia in diarrheic patients in Al-Muthanna Province, Iraq, and to analyse the distribution of infection concerning demographic and environmental factors. During a six-months period, 100 stool samples were collected from patients with diarrhoea receiving treatment at Al-Rumaitha General Hospital and the Maternity and Childrens Hospital. Samples underwent microscopic examination, subsequently followed by reexamination using PCR to target the small subunit ribosomal RNA gene of G. lamblia. Microscopic analysis revealed intestinal parasites in 34.4% of samples, with G. lamblia as the predominant species at 53.8%. PCR analysis detected G. lamblia DNA in 96% of samples, demonstrating enhanced sensitivity. Men (54.2%) exhibited a higher propensity for infections, with children under age of ten being the most susceptible. Sequencing analysis revealed minimal genetic variation (0.01-0.002%) among isolates, which are stored in GenBank (OL719308). In conclusion, PCR is a dependable and highly sensitive technique for diagnosing giardiasis that can supplant microscopy. The elevated infection rates among rural children underscore the necessity of improving sanitation, hygiene education, and access to potable water in Al-Muthanna Province.

Keywords: Giardia lamblia; Giardiasis; Polymerase Chain Reaction (PCR); Molecular diagnosis; Intestinal protozoa; Epidemiology; Microscopy; Al-Muthanna Province; Iraq.

Introduction

Giardiasis, caused by Giardia lamblia (also known as G. intestinalis or G. duodenalis), is amon the most prevalent parasitic infections of the intestines globally. The World Health Organization (WHO) identifies G. lamblia as a significant contributor to global morbidity, particularly in low-income regions where children are predominantly impacted [1]. The primary mode of transmission for illness is the consumption of cysts contaminated by water, food, or direct contact with faeces [2]. Symptoms can vary from none to prolonged diarrhoea, malabsorption, childrens slow growth, and weight loss [3]. Chronic infections can lead to issues with thinking and eating [4]. Approximately 200 million people worldwide are afflicted with giardiasis each year. Africa, Asia, and Latin America have the highest rates, and the disease is still evolving in these regions [5].

Intestinal parasitic infections remain a considerable health risk in Iraq. Previous studies have shown that the prevalence rates among children vary from 20% to 40%, mainly because of poor sanitation, bad water quality, and limited access to healthcare [6–8].

For a long time, the standard way to find G. lamblia was to look for cysts and trophozoites under a microscope. It takes a long time, needs skilled workers, and can give false negatives if cysts are released at random times or if faecal debris gets in the way [9Molecular diagnostic techniques, such as PCR, have become superior alternatives, offering high sensitivity and specificity by identifying parasite DNA even at low concentrations [10–12].

This study compares traditional microscopy and PCR-based detection of G. lamblia among diarrhoeic patients in Al-Muthanna Province, Iraq, and examines correlations between infection rates and demographic variables, such as age, gender, and residence. The study also examines the molecular composition of local isolates to identify genetic variations and confirm their authenticity.

Materials and Methods

Study Area and Sample Collection

The study was conducted in southern Iraq, Al-Muthanna Province, which is notorious for its inadequate sanitation and reliance on river water for daily necessities. From January to June 2024, 100 stool samples were taken from patients aged 1 to 40 who had diarrhoea and were being treated at Al-Rumaitha General Hospital and the Maternity and Children's Hospital.

The institutional ethical committee of Al-Muthanna University (Ref. No. 2024/237) approved the study, and each participant gave their informed consent. Samples were collected in sterile containers and transported to the parasitology laboratory under cold conditions within two hours of collection.

Microscope Examination

The samples were first looked at with the naked eye to check for color, consistency, and visible blood or mucus. We made direct wet mounts with physiological saline and Lugol's iodine solution and looked at them under a light microscope with ×10 and ×40 objectives. We used WHO guidelines [13] to identify

the cysts and trophozoites of G. lamblia, Entamoeba histolytica, and other intestinal protozoa based on their shapes.

DNA Extraction and PCR Amplification

Following the manufacturer's instructions, the QIAamp DNA Stool Mini Kit (Qiagen, Germany) was used to extract genomic DNA. We used a spectrophotometer to measure the DNA's purity and concentration at 260/280 nm.

We used primers that targeted the 18S rRNA gene of G. lamblia to do PCR:

5′-GACGGCTCAGGACAACGGTT-3′ is the forward primer.

5′-TTGCCAGCGGTGTCCG-3′ is the reverse primer.

The PCR reaction mixture (25 µL) was made up of 12.5 µL of Master Mix (Promega, USA), 2 µL of template DNA, 1 µL of each primer (10 µM), and water that didn't have any nucleases in it. The thermal cycling conditions were as follows: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 30 s), annealing (58°C for 45 s), and extension (72°C for 60 s), with a final extension at 72°C for 7 min. Amplified products were seen on 1.5% agarose gels that had been stained with ethidium bromide and looked at under UV light.

Sequence and Phylogenetic Analysis

Macrogen Inc. (Seoul, South Korea) sequenced the positive PCR amplicons after they were cleaned up with a DNA purification kit. We used the NCBI BLAST tool to check the identity of the sequences we got and see how similar they were genetically to other G. lamblia isolates. MEGA X software (version 10.2) created a phylogenetic tree using 1000 bootstrap replicates and the neighbor-joining method.

Statistical Analysis

SPSS version 25 (IBM Corp., USA) was used to enter the data. To determine whether infection rates varied by age group, gender, and place of residence, chi-square tests are employed. Statistical significance was defined as a p-value of less than 0.05.

Results

Microscopic Detection

Intestinal protozoa were detected in 34 (34.4 %) of the 100 samples. G. lamblia was the most prevalent species (53.8 %), followed by Cryptosporidium parvum (11.8 %) and E. histolytica (29.4 %). Microscopic identification showed higher infection rates in males (55.1 %) than females (44.9 %) and higher prevalence in rural females (44.9 %) than in urban areas (39.8 %).

PCR Detection

It is found that 96 out of 100 samples (96%) contained G. lamblia DNA, according tp PCR testing. Compared to microscopy, PCR found more positive cases and revealed multiple infections that were

missed by microscopic examination. According to the PCR test, men (56.2 %) and those who resided in rural areas (54.1 %) had the highest infection rates.

Age Distribution

Children ages 10 and under had the highest infection rate (32.2 %), followed by those ages 11 to 20 (14.8 %). Infection rates decreased with age. This because people hygiene habits changed and they became partially immune.

Molecular Characterization

All PCR-positive samples were confirmed to be connected to G. lamblia assemblage A by sequencing. Nucleotide changes were negligible (0.01-0.002 %) in comparison to reference sequences (DQ157272.1). the local isolate was assigned the accession number OL719308 and entered into GenBank.

Discussion

The study demonstrated how much simpler it is to correctly diagnose G. lamblia using molecular techniques, particularly PCR. Because microscopy relied on the examiner morphological knowledge, it understated prevalence. These findings are in line with those of McHardy et al. [14] and Caccio & Ryan [15], who reported that molecular methods can detect low-intensity infections that microscopy often misses.

Because of their lifestyle and the places they live, men and those who live in rural areas are more likely to become ill. Males, especially kids, are more likely to engage in outdoor activities, which increases their exposure to contaminated soil and water [16]. The higher prevalence in rural areas comparable to findings in Ethiopia and Nigeria, where poor sanitation and contaminated water sources were significant risk factors [17,18]. Children under the age of ten were most impacted, which is consistent reports from Iraq, Ethiopia, and India [19–21]. Young children's are more vulnerable to parasitic infections due to their behavioural patterns, including eating street food and playing in contaminated areas, as well as their immature immune systems. In line with earlier regional studies, sequencing analysis showed little genetic variation among local isolates [22]. Given that assemblage A is known to infect both humans and animals, its prevalence suggests zoonotic potential [23].

The importance of molecular surveillance in tracking genetic variants that may impact virulence or treatment response is highlighted by these findings. Additionally, the associations found with sociodemographic variables highlight the need for community-level initiatives focusing on personal hygiene, water safety, and parasite screening in medical facilities.

Conclusions

Compared to traditional microscopy, PCR-based molecular techniques provide significantly higher sensitivity and specificity for Giardia lamblia detection. Particular public health measures, like improved water, sanitation, and hygiene education programs, are desperately needed, as evidenced by the high rate

of infections among children in rural areas. Controlling giardiasis in Iraq may be considerably simpler if molecular diagnostics are added to routine parasitological testing.

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