

DNA Extraction and PCR Detection of *G.Lamblia* Cyst from Human Fecal Samples in Some Sudanese Suspected Patients

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Abstract: The study aim to evaluate guanidine hydrochloride (HCL) as DNA extraction method from Giardia Cyst in human fecal samples and PCR detection using triose phosphate isomerase gene (tpi). A total of 30 positive fecal samples of Giardia were collected from Hospitals and health centers from three states of Sudan (Khartoum, Gazeira and Sennar) during the periods from June to September 2017 by Convenient sampling method the samples were purified in Phosphate buffer saline, treated with Liquid Nitrogen and boiling, then guanidine hydrochloride method was used for DNA extraction 1ml of sample added to 1ml lysis buffer, 1ml guanidine hydrochloride, 300µl ammonium acetate, and 10µl proteinase K and incubated in 37°C overnight, DNA was harvested, confirmed and evaluated by Nanodrops can, and spectrophotometer for concentration, then followed by PCR detection, using ready prepared commercial product (Maxime PCR PriMex (i-Taq 20µl) Macro gene Company, Korea). In the reaction 1.5µl forwards and 1.5µl reverse primer were added to 5µl DNA template and complete the volume to 20µl with sterile D. W. the reaction was conducted in 94°C for 5min as initial temperature 94°C for 30 sec, 55°C for 45 sec, 72°C for 2min, 72°C for The result found that DNA concentration was 34.5000±2.1213ng/ml 62.4000±0.56569ng/ml 4.6800±1.46803ng/ml for Khartoum, Sennar, and Gazeira states while PCR only 5 samples were +ve 2 (20%) samples Khartoum and 3 (30%) samples Sennar states but no +ve PCR result in Gazeira state. which confirmed by agarose gel electrophoresis, the study conclude that chemical methods of DNA extraction for examples guanidine HCL is not less effective than other, and PCR it is high sensitive to inhibitors. Also it is possible to obtain DNA from G.cyst after treated with heat and liquid nitrogen.

Keywords: DNA Extraction, *G. Lamblia*, G. Cyst, Guanidine HCL, PCR Detection, Sennar

1. Introduction

Giardia duodenalis (syn. *Giardia intestinalis* and *Giardia lamblia*) it is a protozoan flagellate which is the only species with in the genus *Giardia* that infects humans, [1]. The infection has world wide distribution, Globally, there are

greater than 700,000 death per year associated with diarrheal throughout the world [2] An estimated 2.8-108 cases per year, represents the most common gastrointestinal parasitic infection in developed countries [3]. In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis, with some 500,000 new cases reported each year

[4]. The epidemiology of *G. duodenalis* influence by several characteristics: (i) in humans, the infective dose is about 10 to 100 cysts; (ii) cysts are immediately infectious when excreted in feces and can be transmitted by person-to-person or animal to-animal contact; (iii) cysts are remarkably stable and can survive for weeks to months in the environment; and (iv) environmental contamination can lead to the contamination of drinking water and food [5]. Traditionally, the laboratory diagnosis of intestinal protozoan infections relies on the detection of trophozoites and cysts by microscopic stool examination. However, the majority of diagnostic methods used in the clinical practice have a limited application regarding the parasite detection [6]. These methods do not allow on the identification of *Giardia* species, as well as the origin of species, or the pathogenic potential of cysts, so molecular techniques provide powerful analytical tools for the epidemiology of human and animal giardiasis. Polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) analysis showed that *G. intestinalis* is a complex organism, made up of morphologically indistinguishable isolates that are genetically and phenotypically distinct [7]. Using phylogenetic analysis of nucleotide sequence and all ozymedata, [8] divided isolates of *G. intestinalis* into seven different lineages, designating assemblages A-G [9]. also Molecular tools have been used recently to characterize the giardiasis, and isolates of *G. duodenalis* from humans and various animals which are morphologically similar, distinct host-adapted genotypes have been demonstrated with in *G. duodenalis* [8, 10]. Two major groups of *G. duodenalis* have been recognized as infecting humans world wide, but there are some differences in naming of these groups, as evidenced by the following categorizations; Polish and Belgian genotypes groups 1, 2, and 3 [11, 12]. and assemblages A and B [13]. So far, no general consensus has been reached concerning the nomenclature of these genotypes, but the term assemblages has been more widely used. All characterized isolates from humans fall with in two major genetic assemblages, A or B which also include many isolates from animals. Such data can be obtained with the use of one of the molecular methods based on the nucleic acids isolated from the resistant forms of these parasites, Because samples of *Giardia* cysts in the presence of many other microorganisms, the recovery of *Giardia* DNA during DNA extraction is very important [14, 15]. *Giardia* cyst wall varies from 0.3 to 0.5 μm in thickness that is formed by an outer filamentous layer and an inner membranous layer including two membranes that enclose the periplasmic space [1]. The biochemical composition of the cyst wall is Composed of carbohydrates, in the form of N-acetyl galactos amine polymers [16], and cyst wall proteins (CWPs) [16, 17]. The presented studies have shown that the extraction of DNA from *Giardia* cysts not preceded with any actions that could destroy the cyst wall is not effective [18]. *Crypto sporidium* oocysts have similar features, because before the DNA extraction they are subjected to different actions in order to destroy their wall [19, 20].

Numerous direct DNA extraction methods has been available in the preparation of DNA from *G. intestinalis* cysts, Several genes including the β -giardin (bg), the glutamate dehydrogenase (gdh) and the triose phosphate isomerase (tpi) among others are used to amplify *Giardia* DNA by PCR. [14, 21, 22] In recent study we aim to evaluate guanidine HCL DNA extraction from *Giardia* Cyst in human fecal sample and PCR detection using tpi gene.

2. Material and Methods

2.1. Samples and Samples Collection

A total of 30 positive fecal samples were collected from Hospitals and health centers from threes tates of Sudan (Khartoum, Gazeira and Sennar) during the periods from June to September 2017. Convenient sampling method was used, purified and washed in phosphate buffer saline PBS confirmed microscopically, counted before used and preserved in-20 for further experiments.

2.2. DNA Extraction and PCR

2.2.1. DNA Extraction

DNA was extracted according to the protocol that developed by Adams ka *et. al.* 2010 [18] with slight modification, Cyst were treated by heat, boiling in 100°C for 2 min and Deeping in liquid nitrogen (DAL Group Company of liquid air Khartoum, Sudan) for 2 min these steps were repeated 3 times before DNA extracting. A guanidine hydrochloride method was used as the following.

1ml of sample added to 1ml lyses buffer, 1ml guanidine hydrochloride, 300 μl ammonium acetate, and 10 μl proteinase K and incubated in 37 Cover night, DNA was harvested, confirmed and evaluated by Nanodrops can, and spectrophotometr for concentration, protein purification and kept in-20 as stock for further PCR experiments.

2.2.2. PCR Amplification

PCR reaction was conducted according to Soliman *et. al* 2003 [23] with slightly modification TPI fragment 605 bp was amplified by using primers AL3543 [5-AAATIATGCCTGCTCGTCG-3] forward and AL3546 [5-CAAACCTTITCCGCAAACC-3] reverse as target gene sequence. The reactions were performed by using ready prepared commercial product (Maxime PCRPrIMex (i-Taq 20 μl) Macro gene Company, Korea). In the reaction 1.5 μl forwards and 1.5 μl reverse primer were add to 5 μl DNA template and complete the volume to 20 μl with sterile D. W. the reaction was conducted in 94°C for 5min as initial temperature 94°C for 30 sec, 55°C for 45 sec, 72°C for 2min, 72°C for 10min to complete 30cycles. a PCR product was confirmed by run in 1.5g agarose with ethidium bromide and the result was read by Gel Documentation System for target visible bands. Control of PCR inhibitor test was used by adding 10 μl of the positive PCR samples to the negative samples in order to control inhibitor substances and run it in thermo cycler with same PCR program then the results was

records.

3. Results and Discussion

3.1. The Results

DNA concentration was calculated by ng/ml. Mean \pm STD in (Nano dropscansystem) Table 1 and PCR results in Table 2 same result after PCR control test was obtain while the result of PCR positive observed in Figure 1 that show a clear bands.

Table 1. DNA concentration mean \pm Std. Deviation.

| | | |
|----------------------|-----------------------|-------|
| DNAconcKhartoumstate | 34.5000 \pm 2.12132 | ng/ml |
| DNAconcsennarstate | 62.4000 \pm 0.56569 | ng/ml |
| DNAaoncGazeirastate | 4.6800 \pm 1.46803 | ng/ml |

Table 2. PCR Results.

| locality | PCR+VE | PCR-VE | TOTAL |
|----------|---------|-----------|-------|
| Khartoum | 2 (20%) | 8 (80%) | 10 |
| sennar | 3 (30%) | 7 (70%) | 10 |
| Gazeira | 0 (0%) | 10 (100%) | 10 |
| Total | 5 (17%) | 25 (83%) | 30 |

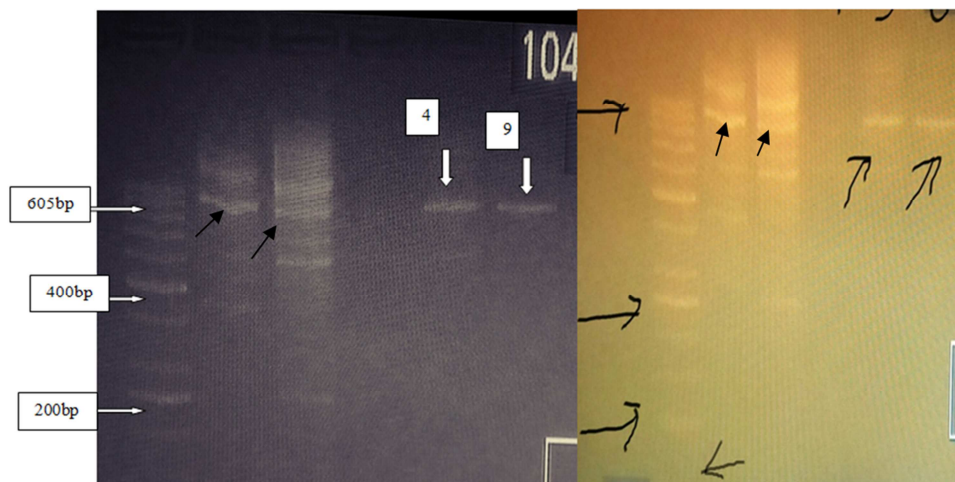


Figure 1. Specific band for positive sample on Gel electrophoresis (1.5 % agarose and 50bp DNA marker) (4, 9).

3.2. Discussions

At present, Light microscopy or immune fluorescence assay has been used to identify *G.intestinalis* in most laboratories. However, these techniques might not be sensitive enough to detect giardia cysts [24]. Recently, Molecular detection methods based on PCR have been developed to detect *G.intestinalis* cysts in feces. These techniques have numerous advantages interms of sensitivity, speed, and specificity in comparison to conventional methodologies [8]. So the recovery of this pathogen DNA during DNA extraction becomes important and there is no much data in the available literature relevant to initial treatment of cysts before the DNA extraction [13] despite the *Giardia* cysts are characterized with high resistance on externals influence [15]. Study found that DNA extraction from G.cyst when treated previously with heat and liquid nitrogen to obtain DNA is very effective to enhanced DNA extraction, the concentration was measured and calculated in Table [1] show that how ever no DNA was obtained from cyst with out treatment by heat and liquid nitrogen this was agree with Adams ka M *et, all* (2010) [18]. PCR detection by using tpi gene result was obtain in Table [2] show that 2 (20%) for +ve 8 (80%) for -ve in Khartoum state and 3 (30%) for +ve, 7 (70%)-ve in Sennar state while there are no result was obtained from Gazeira State, because of PCR inhibitor factors in human feces, which was controlled by adding 10 μ l from +ve sample to -ve one but there was no result was obtained. This findings similar to Lane, S *et, all*

(2002) [3] and no similar result in Sudan.

3.3. Conclusion

study conclude that chemical methods of DNA extraction for examples guanidine HCL is not less effective than other, and PCR it is high sensitive to inhibitors. also it is possible to obtain DNA from G.cyst after treated with heat and liquid nitrogen.

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