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LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP): SENSITIVE AND RAPID DETECTION OF *SCHISTOSOMA HAEMATOBIUM* DNA IN URINE SAMPLES OF EGYPTIAN SUSPECTED CASES.

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Abstract

Schistosomiasis *haematobium* is a major endemic parasitic disease in many tropical regions including Egypt. Typical infection results in haematuria, dysuria, anaemia, genital as well as urinary tract lesions, with prospect of kidney damage in complicated cases. In addition, deposited eggs in the tissue, eventually leads to squamous cell carcinoma of urinary bladder in chronically infected individuals. Microscopic detection of excreted ova in urine samples remains the gold standard diagnostic method, in spite of its inherited low sensitivity, inconsistent egg excretion and unreliable results in chronic phase of the disease. Moreover due to pre-requisite for skilled personals and pricey equipment, PCR-based technologies are of limited use especially in low-income endemic countries. So emergence of loop-mediated isothermal DNA amplification (LAMP) seemed a promising technique. Our study evaluated application of LAMP technique in detection of *S. haematobium* DNA in 69 urine samples of suspected patients for urogenital schistosomiasis, versus conventional urine filtration followed by microscopy ova detection method. Specificity of LAMP was tested using other parasites DNA samples that showed no cross reactivity. Furthermore our results of the calculated diagnostic parameters for sensitivity and specificity for LAMP assay were 100%, with 95% CI (88.78%-100%), and 63.16%, with 95% CI (45.99%-78.19%) respectively, moreover Positive likelihood ratio (LR+) 2.7, and Negative likelihood ratio (LR-) 0.0, which display that LAMP technique is an up-to-date simple, sensitive, diagnostic important tool that could be employed in clinical diagnosis in poorly equipped facilities, as well as in surveillance of infectious diseases. As authors knowledge, this is the first national report evaluation of LAMP technique as a promising diagnostic tool for urogenital schistosomiasis.

Key words: Urogenital schistosomiasis, *S.haematobium,* loop-mediated isothermal DNA amplification (LAMP).

Introduction

Schistosomiasis is a parasitic disease caused by the trematode of the genus *Schistosoma*, commonly known as blood flukes. It is a major endemic parasitic disease in many tropical regions of Asia, Africa and South America, which affects more than 240 million people worldwide. *Schistosoma haematobium*, accounts for about half of this number (Fu *et al*, 2012). The morbidity and mortality is mainly associated with the long lasting chronic stage of infection, when egg deposition followed by the granuloma formation in the different affected tissues, especially urogenital organs. With continuous irritation due to strong inflammatory response around deposited trapped eggs, and over time cellular metaplasia sets in, and eventually the development of cancer is anticipated (Shiff *et al*, 2006).

Most epidemiological assessments of the schistosomiasis burden had relied on the microscopy, providing a comparatively easy and cheap tool to detect the intensity of *Schistosoma* eggs in urine samples. How-

ever, unsatisfactory sensitivity due to great fluctuations of egg output in urine and the difficulties in meeting the multiple sampling requirements lead to unreliable results (Aryeetey *et al*, 2013). A study on the sensitivity and specificity of various diagnostic tools (haematuria, egg detection, antigen detection and antibody detection) showed equivocal results and ensured the need of a more sensitive and specific test to improve the schistosomiasis diagnosis (Shiff, 2012).

Bearing in mind no available vaccine to protect against schistosomal infection, and with mass praziquantel treatment campaigns of population at risk of urogenital schistosiomiasis are being conducted especially in endemic areas, still reinfections can occur simply because of recurrent direct contact with parasites contaminated water, which is part of routine daily activity in farmers' life (Rollinson *et al*, 2013). The existing parasitological, serological and molecular methods limitations in schistosomal infections (Gomes *et al*, 2014) necessitate new, simple, precise and economical tools to accurately diagnose the acute as well as chronic stages. Subsequent effective management will be optimistically reflected on the success of control campaigns of urogenital schistosomiasis in endemic areas; besides it will help monitoring drug efficacy.

For urogenital schistosomiasis, detection of parasite-specific DNA in urine is feasible, highly sensitive, and specific. However, application of this technique in endemic areas is problematic where it is hard to operate thermocyclers and electrophoretic equipment. For this reason, isothermal amplification technique emerged as an alternative method (Shiff, 2012). Isothermal amplification initially described in 2000 and applied for detection of a variety of infectious agents (Boehme *et al*, 2007).

Piepenburg *et al*. (2006) reported the isothermal nucleic acid amplification as a relatively new, single tube reaction which might replace traditional PCR. The process of amplification is accomplished by the aid of certain enzymes without the need of thermal changes. So, isothermal nucleic acid amplification needs much more simpler equipment than PCR. The technique is operating best at distinct temperatures that should be calculated and standardized due to the primers used to detect different infectious agents in an acceptable time, reported to be faster than conventional PCR, which made it an excellent candidate for introduceing low-cost, rapid, point-of-care molecular tests (Abbasi *et al*, 2010).

The current study aimed to evaluate reliability of isothermal nucleic acid amplification assay in detection of urogenital schistosomiasis in our laboratory, especially in highly suspected cases, and to overcome conventional diagnostic methods limitations.

Material and Methods

Patients´ urine samples: A total of 69 urine samples were collected from suspected *S. haematobium* patients attending outpatient clinic from April 2015 to April 2016 at Tudor Bilharz Institute, Imbaba, Egypt. Suspicious criteria for *S. haematobium* infection were as follow: bilharziasis history (history of previous bilharzial treatment and/ or canal water contact), urinary symptoms especially haematuria, dysuria or frequency, presence of bilharzial complications as the urinary bladder mass or kidney back pressure changes with all relevant data.

Urine samples were subjected to two different concentration methods before microscopic egg detection. Firstly direct microscopic examination for egg detection after centrifugation (Strasinger and Di-Lorenzo, 2008), and to microscopic egg detection after urine filtration (Nuclepore®), briefly filtration of 10ml of urine through a polycarbonate filter of 13mm diameter and a pore size of 12-20µm was performed, followed by microscopic egg counting if proved positive (WHO, 2003).

All patients were subjected to several microbiological & parasitic diagnostic tests to exclude other infectious agents according to standard routine laboratory procedures,

and samples proved positive to other parasitological or infectious diseases.

DNA extraction form urine samples: Before DNA extraction; certain additional steps were performed to eliminate effect of inhibitors in urine sample, firstly centrifugation pellet was boiled for 10 minutes, with 200µL of PBS, followed by adding 20 µL of proteinase K enzyme (PK) (934U/ml, 18.5mg/ml) (Promega, USA), mixed well and incubated for 30 minutes at 50° C. For urine samples DNA extraction; we used the commercially available Wizard® genomic DNA Purification kit REF A1125 (Promega corporation, USA) following manufacturers´ instructions. Extracted DNA samples were stored at -20°C until use in LAMP reactions.

Positive control: DNA was first extracted from *S. haematobium* eggs, Egyptian strain that was obtained from Tudor Bilharz Institute, Schistosome Biological Supply Center using above mentioned kit. DNA concentration (100ng/µL) was confirmed by measuring with Nanodrop ND-100 (Nanodrop Technologies), and then was diluted with ultrapure water to a final concentration of 50ng/µL. Subsequently, serial 10-fold dilutions were also prepared with ultrapur water (nucleases free water) ranging from 5ng/µ L to 0.5atg/µ L and stored at -20°C until used. Thus, prepared DNA was used as positive control in LAMP reactions and also to assess sensitivity of the assays.

S haematobium LAMP primers: Sets of 4 oligonucleotide primers were used, targeting specified region of *S. haematobium* ribosomal intergenic spacer (IGS) DNA retrieved from GenBank (Accession No. AJ223838). The outer forward primer (F3), outer backward primer (B3), forward inner primer (FIP), backward inner primer (BIP) primers were ordered according to the general criteria described by Notomi *et al*.(2000) and finally selected based on the criteria described in "A Guide to LAMP primer designing" (http://primerexplorer.jp /e/v4_manual/ index.html). The oligonucleotide sequences of the 4 primers are shown (Tab. 1). All the primers were purified high performance liquid chromatography (HPLC grade) (Thermo Fisher Scientific Inc., Madrid, Spain).

Table 1: Lamp primer set targeting the selected sequence (GenBank Accession No. AJ223838) for ribosomal intergenic spacer *S.haematobium* DNA region amplification: Sequence of LAMP primers: F3, forward outer primer; B3, reverse outer primer; FIP, forward inner primer (comprising F1c and F2 sequences); BIP, reverse inner primer (comprising B1c and B2 sequences).

Primer	Length	Primer sequence (5' to 3')
F3	18 bp	CTTTCTAAGCCCGCGATA
B3	18 bp	GCGCATTACACTTGGTCT
FIP (FIc-F2)	41 bp	TACCCCTAACTTCGTGGTCTCCCCCCCTTATTTTAGGGTGC
BIP (BIc-B2)	48 bp	CTCCCTATATAACATGGCGAGTAAGACTATGAAATCAGTGTTTTTCGG

LAMP reaction: LAMP reactions mixtures with total volume 25µL, contained 1.6µM of each FIP and BIP primers, 0.2µM of each F3 and B3 primers, 0.4µM of each LB and LF primers, 1.4mM of each dNTP (Promega, USA.), 1x Isothermal Amplification Buffer - 20 mM Tris-HCl (pH 8.8), 50mMKCl, 10mM $(NH4)_2SO_4$, 2mM $MgSO_4$, 0.1% Tween20- (New England Biolabs, UK), betaine 1M, supplementary $MgSO_4$ 6mM, and 8U of 0.2ml Bst Warm-Start DNA Polymerase, with 2µL of template DNA. All amplification reactions included positive (*S. haematobium* DNA) and negative (ultrapure water or no DNA template) controls were always included in each LAMP assay.

Analysis of LAMP products: Initially, when possible, turbidity caused by the accumulation of magnesium pyrophosphate (a by-product of the reaction) was visually inspected by the naked eyes. Positive results were also visually detected by adding 2µL of 1:10 diluted 10,000x concentration fluorescent dye SYBR Green I (Invitrogen) to the reaction tubes. Green fluorescence was clearly observed in a successful LAMP

reaction, whereas it remained original orange in the negative one.

Only for initial establishment steps and standardization of used LAMP techniques, 3-5µL of only one positive sample and both positive and negative controls LAMP products were used for 2% agarose gel electrophoresis stained with the ethidium bromide. A GelDoc imaging system (UVItec, UK) was used to observe the band patterns.

Specificity and sensitivity of the LAMP assay: To determine specificity of LAMP assay used, a panel of 6 DNA samples from other helminthes and protozoa were used as heterogeneous control samples including, *S. mansoni*, *Fasciola hepatica*, *Taenia* spp., *Cryptosporidium parvum*, *Giardia intestinalis*, *Entamoeba histolytica.* Concentration of these DNA samples was also measured by using Nanodrop ND-100, and then diluted with ultrapur water to a final concentration of 5.0ng/µL. All these DNA samples were kept at -20°C until use in molecular assays. Sensitivity of LAMP assay to amplify only *S. haematobium* DNA was tested by determining lower detection limit of used LAMP assay, genomic DNA from *S. haematobium* 10-fold serially diluted-ranging from 0.5ng /µL to 0.5fg/µL subjected to amplification.

Statistical analysis: Data were coded and entered using the statistical package SPSS (Statistical Package for the Social Science) version 23. Data was summarized using frequency (count) and relative frequency (Percentage) for categorical data. Standard diagnostic indices including sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), positive likelihood ratio (LR+), and negative likelihood ratio (LR-) were all calculated as described by**.** For comparing categorical data, Chi square ($\chi 2$) test was performed (Galen, 1980). *P-value* less than 0.05 were considered as statistically significant.

Ethics statement: Human urine samples used in this study were obtained as part of public health diagnostic activities at the out-patient clinic from at Tudor Bilharz Research Institute, Imbaba, Egypt. Human urine samples were not collected specifically and all were obtained under written informed consent and coded and tested as anonymous samples. All participants were given detailed explanations about the aims, procedures and possible benefit of the study. The study protocol was approved by the departmental research committee and Ethical approval was obtained from the Research and Ethics Committee of Faculty of Medicine, Cairo University.

Results

Among the study group (n=69); 28 urine samples (40.6%) were positive for *S. haematobium* eggs by the direct microscopic examination of urine sediment after centrifugation. The number increased to 31/69 (44.9%), when urine samples were subjected to microscopic egg detection (Tab. 2).

Table 2: Microscopic examination of urine samples by microscopic egg detection after two concentration methods

		Microscopic egg detection after filtration	
		Positive Count	Negative Count
Microscopic examination of urine sediment.	Positive	28 (40.6%)	0 (0.0%)
	Negative	3 (4.4%)	38 (55.0%)

Results of the LAMP amplification was detected by simple visual observation of turbidity due to accumulation of white precipitate of magnesium pyrophosphate, which goes in proportion to accumulated amplified DNA products as observed in positive control tube (Fig. 1A), or by visual detection of color change to green, when incorporated with a fluorescent intercalating dye (SYBR Green I) and easily distinguished from reddish orange color of negative control tube (Fig.1 B). Fluorescent intercalating dye (SYBR Green I) fluoresce brightly when illuminated with UV lamp (Fig. 1C).

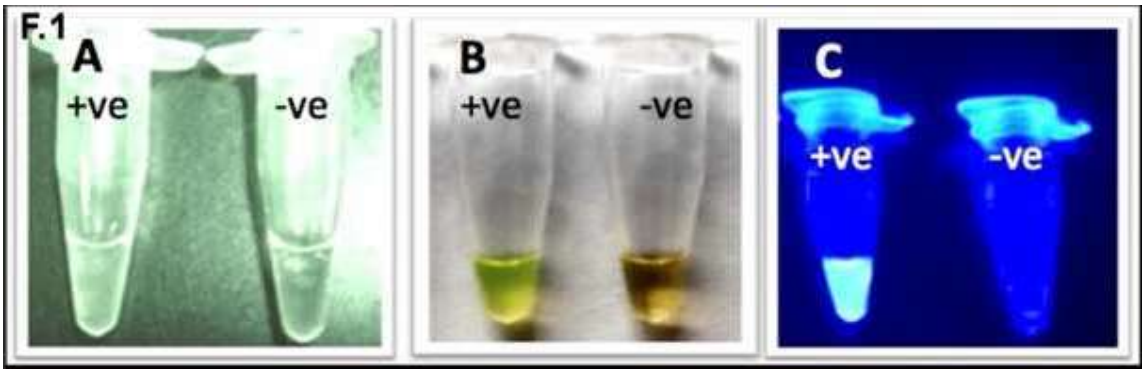

Fig. 1: Detection of LAMP products. (A) Turbidity observation; turbidity increases in positive reaction due to accumulation of magnesium pyrophosphate. (B) Detection of visible green color change in positive reaction after addition of SYBR green I dye, which was be easily distinguished from reddish orange color indicating a negative reaction. (C) showed fluorescent LAMP product with SYBR green I dye under UV light in positive reaction tub

Only for initial establishment and consistency of the used LAMP techniques in this study, LAMP products of one positive sample and positive & negative controls were electrophoresed on 2% agarose gel stained with ethidium bromide. The results were respected positive if they showed a characteristic ladder-like band pattern of the targeted DNA fragments (Fig. 2).

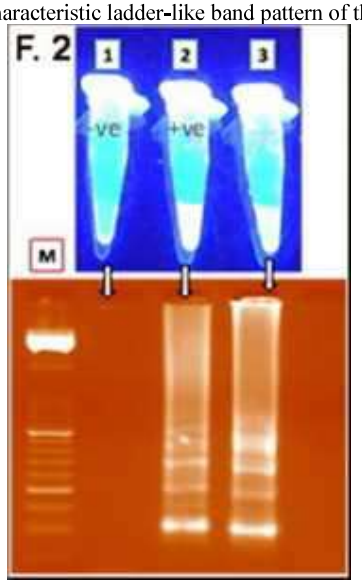

Fig. 2: LAMP products visualized by bright fluorescence in positive control tube (tube 2) and one of microscopically confirmed positive sample (tube 3) (up), and 2% agarose gel electrophoresis of same tubes reaction products, showing characteristic ladder-like band pattern of targeted DNA fragments *(down):* M a DNA ladder (50bp) (Molecular weight marker XIII, Roche), tube 1 a negative control, tube 2 is a positive control and 3 is a confirmed positive *S. haematobium* sample.

Table 3: Loop mediated iso-thermal amplification (LAMP) in relation to microscopic egg detection after filtration.

		Microscopic egg detection after filtration	
		Positive Count	Negative Count
Loop-mediated iso-thermal amplification (LAMP) technique	Positive	31 (44.9%)	14 (20.3%)
	Negative	0 (0.0%)	24 (34.8%)

The assay sensitivity was laboratory evaluated using *S.haematobium* DNA 10-fold serially diluted, and the lower limit of detection of the used LAMP assay was 1 fg/ml, furthermore calculated sensitivity of used LAMP assay was 100%, in relation to conventional microscopic egg detection after filtration "standard detection assay", as the used LAMP assay didn't miss any positive sample detected by the microscopic detection of eggs after filtration see Table (3). Considering specificity, when the 6 DNA samples obtained from other parasites included

in current study were subjected to this LAMP assay, no cross-reactions were found, when using DNA as a target from all tested other parasites, as the target amplicons were never amplified versus positive (*S. haematobium* DNA) & negative controls (Fig. 3).

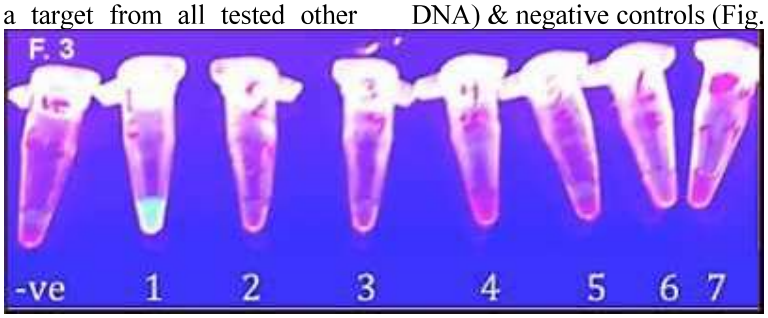

Fig. 3: LAMP amplification products of a panel of 6 DNA samples from several other helminthes and protozoa tested as heterogeneous control samples including in order to test specificity, tube 1: *S. haematobium* DNA, tube 2: *S.mansoni* DNA, tube 3: *Fasciola hepatica* DNA, tube 4: *Taenia spp.* DNA, tube 5: *Cryptosporidium parvum* DNA, tube 6: *Giardia intestinalis* DNA, tube 7: *Entamoeba histolytica* DNA.

Fig (4): showed example of products of LAMP amplification, visualized by fluorescence after incorporating a fluorescent intercalating dye SYBR Green I, and illuminated with a UV lamp. First 2 tubes are negative & positive controls, samples 1, 2, 3, 5, 6, 8, 9 & 10, were positive for *S. haematobium* while samples 4, 7, 11 & 12 were negative samples .

All 69 patients´ urine samples by LAMP technique for *S. haematobium* DNA detection compared to conventional microscopic detection of eggs after filtration (Tab. 3). The LAMP positive results in 45/69 (65.22%), 14 (20.4%) were negative by microscopy examination after filtration, with calculated sensitivity 100%, with 95%CI (88.78%-100%), while the specificity 63.16%, with 95% CI (45.99%-78.19%). The calculated positive predictive value (PPV) 68.89%, with 95%CI (53.35%-81.83%), and negative predictive value (NPV) was 100%, with 95%CI (85.75%-100%), furthermore; positive likelihood ratio (LR+) was 2.7, and Negative likelihood ratio (LR-) was 0.0. Figure (4) showed patients example of positive and negative samples for *S. haematobium* by LAMP assay, when illuminated by UV lamp.

Table 4: Summarizes demographic & clinical findings of 14 patients proved to be positive by loop mediated iso-thermal amplification (LAMP), and negative by microscopic egg detection. Comparing categorical data, Chi square ($\chi 2$) *P-values*< 0.05 were considered as statistically significant*.

Variables	Patients diagnosed positive only by loop mediated isothermal amplification (LAMP) (n=14) Number (%)	*P value*
Sex (male)	14/14 (100%)	0.00*
Age: 50-59 years old 60-69 years old >69 years old	6/14 (42.86%) 6/14 (42.86%) 2/14 (14.28%)	0.00*
Occupation (farmers)	8/14 (57.14%)	0.01*
Residence (rural areas)	14/14 (100%)	0.00*
History of canal water exposure	10/14 (71.43%)	0.01*
History of anti bilharzial treatment	4/14 (28.57%)	0.11
Haematuria	10/14 (71.43%)	0.01*
Dysuria	4/14 (28.57%)	0.11
Cancer Urinary bladder	8/14 (57.14%)	0.00*
WBCS (>50/HPF)	14/14 (100%)	0.00*
Proteinuria	4/14 (28.57%)	0.11

By LAMP assay (14/69) 20.4% were positive but negative by conventional microscopic egg detection after filtration. Briefly, it was found that all were males, living in rural areas, mostly farmers (57.14%), with routine contact of water canal (71.43%). Clinically, haematuria was detected (71.43%), WBCs in >50/HPF indicating urinary tract infection detected in all and cancer urinary bladder was found in 8 patients (57.14%).

Discussion

Urogenital schistosomiasis due to *S. haematobium* continues as a serious undervalued public health problem and worsened by its devastating complications, particularly in developing African countries including Egypt, hence, acquired the title of the neglected Schistosome (Rinaldi *et al*, 2011).

Microscopic detection of *S. haematobium* eggs in urine especially with concentration method remains the gold standard for diagnosis, in spite of its inherited low sensitivity, but may be due to low operational costs. Many of the immuno-based assays provided higher sensitivity than conventional techniques, but show cross-reaction and false positive results especially related to patients who have already eliminated the parasite after the efficient chemotherapy (Doenhoff *et al*, 2004; Sorgho *et al*, 2004).

In general all still showed problems in diagnosis, particularly early in infection course before excretion of eggs, and in chronic stage of the disease, in which patients pass only small numbers of eggs due to bladder wall fibrosis, or even in individuals with very low worm burden in low disease transmission areas (Enk *et al*, 2008). This impels clinicians towards using invasive techniques, in order to confirm diagnosis of *S. haematobium*, which is unfavorable approach (Ibironke *et al*, 2011). Thus an efficient, rapid, simply applicable& low cost diagnostic tool is a prerequisite to accomplish proper early management before chronicity & decompensating sequelae of the urogenital schistomiasis.

LAMP assays are comparatively new DNA amplification technique, though the LAMP assays were generally used for diagnosing infectious diseases such as tuberculosis (Geojith *et al*, 2011), malaria (Poon *et al*, 2006), and sleeping sickness (Njiru *et al,* 2008) in developing regions, thus far it was comprehensively validated for the other pathogens (Sen and Ashbolt, 2011).

In current study; the sensitivity and specificity of the used LAMP assay through application of the assay on urine samples of highly suspected human patients, in statistical comparison relative to the gold standard method, in addition to laboratory testing. Sensitivity was evaluated in lab through determination of the lower limit of detection of the used LAMP assay, which was 1 fg/ml of parasite DNA. By means of reference, Schistosome genome contains approximately 580fg of DNA (Gomes *et al*, 2006), therefore, theoretically LAMP assay would detect *S. haematobium* diluted DNA in urine samples corresponding to a great extent less than the equivalent to a single parasite cell. Furthermore sensitivity was calculated statistically in comparison to conventional microscopic egg detection after filtration, which reported 100% as agreed with others (Fernandez-Soto *et al*, 2014; Gandasegui *et al*, 2015).

For specificity; it was tested in current study through laboratory testing by performing amplification reaction using 6 different measured DNA samples from several other helminthes and protozoa as heterogeneous control samples including, *S. mansoni*, *F. hepatica*, *Taenia spp.*, *C. parvum*, *G. intestinalis*, and *E. histolytica.* All tested samples showed negative results, against positive (*S. haematobium* DNA) & negative control tubes, which assured high specificity of the used LAMP assay, and were in agreement with Gandasegui *et al.* (2015). Additionally, specificity was calculated by statistical analysis, which irrationally was biased to the gold standard method, seeing the results of positive samples only by LAMP assay & not

by microscopic egg detection as non-specific reaction. In actual fact; considering that primers used in LAMP assay in present study were highly purified by high performance liquid chromatography (HPLC grade), which was an efficient method for analysis and purification of synthetic primers oligonucleotides. The primers' synthesis involves a large number of individual reactions, which leads inevitably to the accumulation of impurities such as truncated oligonucleotides and sequences containing chemically modified bases. So, the HPLC technique guarantee highly pure synthesized oligonucleotides primers that subsequently assured the highest specificity (Gerber *et al*, 2004). The present results agreed with Gandasegui *et al*. (2015) who tested urine samples of murine experiment-tally infected by *S. haematobium* parasite. In this study, high sensitivity was shown by laboratory detection of lower limit of detection of parasite DNA, explains the detected positive samples only by the LAMP assay 14/69 (20.3%), and not by conventional egg detection after filtration. Besides, detected non-cross reactivity, when testing the heterogeneous other parasites DNA denies false positive results of these samples.

In current study, statistical analysis of evidence-based medicine; Assay likelyhood ratio was calculated used for assessing the value of performing any diagnostic test. An assay likelihood ratio of > 1 indicates the test result is associated with the disease. While an assay likelihood ratio < 1 indicated that the result was associated with absence of the disease. Positive likelihood ratio (LR+) of used LAMP assay=2.7 (if this LAMP assay showed positive result, this increased probability of patient infection) and negative likelihood ratio (LR-) of used LAMP assay=0 (meaning that if this LAMP assays showed negative result, this decreases the probability of patient infection).

Considering the present results and with thoroughly analysis about patients those diagnosed only positive by loop mediated iso-thermal amplification (LAMP) (n=14). All cases were male older than 50 years old, live in rural areas, majority were farmers or with history verified contact of canal water, and presenting with haematuria & leucocytes in urine samples. These data agreed with the risk factors for *S. haematobium* infection shown by (El-Khoby *et al*, 2000), with only one exception related to age. These authors in an epidemiological cross sectional survey in nine governorates in Egypt; concluded that the risk factors associated with *S. haematobium* infection were; males of an age <21 years old, living in smaller communities, with exposures to canal water, history of burning micturition or blood in urine, and detected haematuria.

The difference in the age found in the present results seems to be due to different patients study group, as the present study selected highly suspected cases presenting to hospital clinic with disease urogenital complications, these chronic sequelae and associated morbidity of *S. haematobium* that occur later on in older age.

Among this 14 patients 8 (57.14%) suffered cancer urinary bladder, which was suggestive that chronic, long lasting, and repeated *S.haematobium* infection could be the precipitating factor of urinary bladder cancer. This might be explained of many years of exposure to infection and a steady accumulation of parasite eggs in the urinary tract tissue, resulting in long-term chronic inflammation (Vennervald *et al*, 2015).

Actually none of them revealed *S. haematobium* ova in his urine using the direct microscopy parasitological egg detection method.

Conclusion

As far to the present authors' knowledge this is the first national report evaluating application of the loop mediated isothermal DNA amplification technique as a promising diagnostic tool for urogenital schistosomiasis. The LAMP is isothermal assay offers high specificity & sensitivity that primarily eliminates the need for expensive thermo-

cyclers used in PCR. Furthermore, owing to its simplicity, low cost and well-defined product interpretation; it may be a valuable method for infectious disease diagnosis in low and middle-income countries.

Also, additional field & clinical evaluation of LAMP assays is still required, especially for testing other parasitic diseases. LAMP assay has the potential to be used as a simple screening assay in the field or at the primary health care delivery by clinicians, permitting early management that could avoid the pathology associated with chronic infections

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