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REVIEW

Mass spectrometry based metabolomics for small molecule metabolites mining and confirmation as potential biomarkers for schistosomiasis – case of the Okavango Delta communities in Botswana

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ABSTRACT

Introduction: Metabolomics for identifying schistosomiasis biomarkers in noninvasive samples at various infection stages is being actively explored. The literature on the traditional detection of schistosomiasis in human specimens is well documented. However, state-of-the-art technologies based on mass spectrometry have simplified the use of biomarkers for diagnostics. This review examines methods currently in use for the metabolomics of small molecules using separation science and mass spectrometry.

Area Covered: This article highlights the evolution of traditional diagnostic methods for schistosomiasis based on inter alia microscopy, immunology, and polymerase chain reaction. An exhaustive literature search of metabolite mining, focusing on separation science and mass spectrometry, is presented. A comparative analysis of mass spectrometry methods was undertaken, including a projection for the future.

Expert Commentary: Mass spectrometry metabolomics for schistosomiasis will lead to biomarker discovery for noninvasive human samples. These biomarkers, together with those from other neglected tropical diseases, such as malaria and sleeping sickness, could be incorporated as arrays on a single biosensor chip and inserted into smartphones, in order to improve surveillance, monitoring, and management.

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1. Introduction

Schistosomiasis is a parasitic helminth infection and is one of the most prevalent neglected tropical diseases (NTDs) in the world. It is estimated that more than 250 million people are infected globally [1]. In the past decade, schistosomiasis accounted for 1.7 to 4.5 million disability-adjusted life years [1,2]. Schistosomiasis is endemic in low- to middle-income countries where universal health coverage does not prevail. In these countries, schistosomiasis is contracted during childhood in tropical environments through bathing, swimming, or fishing [3]. Infection is associated with months or years of chronic illness, including hepato-intestinal and urogenital infections.

1.1. Schistosomiasis infection of humans

Schistosoma parasites emerge during daylight from a snail's sporocysts which produce cercaria, i.e. the larval form [4], and drive themselves into water where they use their bifurcated tail to seek out a final host. When they encounter human skin, infection is initiated. The skin reaction that evolves into a rash within a week is an indication of cercaria secretion seeping into the host epidermis down to the superficial dermis [5]. The cercaria creep along the skin in search of a suitable penetration site, which is usually a hair follicle. The skin is penetrated through the epidermis, via cytolytic secretions from the cercarial post-acetabulum and the preacetabular glands. The schistosomulum's energy stores for traversing the bloodstream lasts for approximately 48 h [6]. This affords sufficient energy to penetrate the host dermis and venule wall to reach the circulatory system [7,8]. The escape gland secretions, which contain the first immunogenic material, invade the dermal vessels, which then stimulate the host immune response [9,10]. In the thin pulmonary capillaries, the schistosomulum embolizes and thus migrates through the pulmonary vein. The schistosomulum then metamorphoses into a lung-stage schistosomulum [11,12]. Embolization allows the small schistosomula to stretch its body, so that it is long and thin enough to move across the capillary web of the lungs to reach the left ventricle, within six days. At this phase, the lung-schistosomulum migrates further, while blood-feeding its way into the portal vessels. The specific venule location varies depending on the species. However, studies present conflicting evidence for the ultimate location of *Schistosoma mansoni*: it is either the superior or inferior mesenteric and hemorrhoidal plexus, which drains into the large intestine [13,14].

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Article highlights

- Reviews traditional or 'gold standard' methods for diagnosis of schistosomiasis from a layperson's perspective
- Highlights metabolomics with respect to schistosomiasis for metabolite mining and catabolic perturbation studies
- Assesses state-of-the-art methods for small molecule metabolite mining for schistosomiasis with respect to mass spectrometry and separation science
- Comparatively analyzes data acquisition, filtration and analysis strategies for schistosomiasis
- Relates the findings to the situation in the Okavango Delta of Botswana
- Predicts the trajectory of the research in this area in the near future with respect to both the Okavango Delta and globally

S. haematobium lodges specifically in the venous plexus of the bladder and rarely in the rectal venule [9,11].

The acute stage is commonly a manifestation of schistosomiasis eggs deposited in host tissues. Hematuria is a common symptom of *S. haematobium* in the acute stage of infection, and is usually a result of granulomatous inflammation caused by the deposition of eggs into the host tissues. At the acute stage, *S. mansoni* deposits eggs into the gut wall, inducing inflammation, ulceration, and polyposis. Symptoms usually include blood in the feces or diarrhea, and colicky, hypogastric pain in children [15]. Colonic polyposis and an inflammatory mass in the colon are associated with protein leaching [15,16]. When eggs are voided to the outside of the host in urine or feces, they need fresh water to hatch and continue the life cycle [8,14]. Eggs not voided are swept into the blood circulation, and the periportal tract of the liver filters them. Some eggs are trapped in the spinal cord, brain, cerebellum, leptomeninges, and choroid plexus [8,14,17]. These manifest in central nervous system (CNS) infections and cause symptoms such as dizziness, vomiting, headache, and even paralysis [18,19]. Schistosomiasis disrupts the host's metabolic regulation host, such as the tricarboxylic acid (TCA) cycle and glycolysis, as the schistosomula consumes glucose [20,21]. Although there is limited information on the disruption of energy production systems in humans as a result of parasitic infection, there is evidence that schistosomiasis in birds interferes with the molecular pathways critical for protein production and lipid biogenesis in tissues that are along the schistosomiasis migration pathway [22].

1.2. Surveillance and monitoring

Surveillance is the systematic collection, analysis, and dissemination of health data that drives planning, intervention, and evaluation of public health programs. In many developing countries, the prohibitive costs of surveillance lead to a suboptimal standard of sensitivity, specificity, and representativeness [23]. Despite national efforts to reduce the prevalence of schistosomiasis, accurate diagnosis still remains poor in low- and middle-income countries (LMICs) [24,25]. In Botswana, despite the low sensitivity reported for the Kato Katz method and urine microscopy, schistosomiasis surveillance and monitoring in the Okavango Delta have been performed exclusively by microscopy [26].

This review explores the global state of schistosomiasis in relation to the current status of the Okavango Delta communities in Botswana, where the infection is still endemic, albeit on a small scale. Future trends are also explored with an accompanying expert situational analysis.

2. Methodology

The literature in this review was retrieved from a variety of sources, some of which are housed at the University of Botswana Library as database subscriptions. These databases include the Web of Science, Science Direct, Scopus, Springer Nature, Ebscohost, Scifinder, Taylor and Francis, and Google Scholar. A systematic search was adopted using the parent keyword 'schistosomiasis,' followed by search refinements using sub-keywords like 'Metabolites' and 'Liquid Chromatography – Mass Spectrometry' and then the publication year range i.e. '2010–2020' in order to retrieve literature pertaining to the metabolomics of schistosomiasis using liquid chromatography-mass spectrometry. The LC-MS biomarker mining section returned 28 hits in Scifinder after removing replicates. These searches were repeated for each subsection in all databases. Unless otherwise reported, the articles were selected from high-impact sources to ensure the accuracy of the data. Unpublished local data were verified through interviews with the owners of the repositories.

3. Body

3.1. Analytical strategies for the diagnosis of schistosomiasis

Global efforts to prevent and treat diseases are a function of the proper detection of the causative agent for accurate and proper diagnosis. For proper patient management, evaluation of treatment, and monitoring of programs meant to control schistosomiasis in endemic areas, early diagnosis is recommended. Communities where the disease is prevalent require accurate diagnosis to contain or limit its spread [27,28]. Biomarkers offer improved diagnostic tools for various infections that cannot be diagnosed with traditional analytical techniques. While other diagnostic techniques focus on organ dysfunction, tools derived from biomarkers enhance the sensitivity and specificity often lacking in traditional methods [29]. Biomarker-based techniques are important for enhancing the sensitivity and specificity of microscopy as well as overcoming limitations in nucleic acid-based methods (i.e. PCR), including interference of primers during amplification and the risk of false positives [30]. The diagnosis of schistosomiasis has evolved over time, from counting of excreted eggs following copulation [31], to improved techniques such as PCR [32] and biomarkers, which are more sensitive and specific [33–35]. There is also a need for sensitive and specific diagnostic strategies that can discriminate between treated and active cases [36]. Field-applied parasitological techniques, which although they are increasingly insensitive, continue to be considered as the 'gold standard' for diagnosis [36]. This is despite evidence that the increase in infection

rates is proportional to the decrease in sensitivity of the diagnostic technique. As such, there might have been possible misdiagnoses that resulted in reinfection and increased morbidity [37]. A discussion of some of the diagnostic techniques used today is presented below.

3.2. Kato Katz

The Kato Katz (KK) microscopy technique relies on counting helminth eggs in small fecal samples. It has been shown to be specific for schistosomiasis in endemic regions [38]. However, studies [39,40] have reported low sensitivity of the technique due to variation in the day-to-day excreted eggs per worm in fecal samples. The varying results with the time of sample collection, deterioration of eggs, low throughput, and poor test performance are some of the limitations that need to be carefully managed to improve the specificity and sensitivity of this technique [41]. The Kato Katz technique compares well with urine microscopy, as both are highly effective standalone diagnostic techniques that have been used successfully in endemic areas [42]. KK is affordable, even in resource-poor countries [43]. However, this technique cannot assess true prevalence when the infection has been brought under control using schistosomiasis therapeutics such as praziquantel [40]. Low sensitivity and specificity at low infection levels for this technique have been demonstrated [44,45]. As such, even though the Kato Katz technique has been considered as the 'gold standard' for the diagnosis of schistosomiasis, these shortcomings lead to speculation about its association with the ever-escalating infections in endemic regions where it is used. The false negatives that may result from this technique make it inappropriate for monitoring the effect of mass drug administration on prevalence and infection intensity. This is further complicated by the fact that little is known regarding the dynamics of infection following chemotherapy.

3.3. Helmintex

The limitations of the KK technique noted above make it imperative that an increase in sensitivity and specificity for schistosomiasis diagnostic tests be achieved. Strong binding between eggs and paramagnetic particles enables the direct isolation of *Schistosoma* eggs from clinical samples for subsequent microscopic examination, using the Helmintex [HTX] technique [46]. While HTX has shown superior performance in sensitivity and specificity, KK remains the preferred diagnostic technique in endemic areas and, to some extent, in low-intensity transmission regions [47]. Calderia *et al*. [47] emphasized the increasing appreciation of HTX as the final step in KK analysis, mainly because of its low cost and simplicity. Some limitations of HTX, such as its complexity, continue to overshadow the exceptionally high sensitivity that has been reported [46]. The affinity of the schistosomiasis eggshell to paramagnetic beads remains an outstanding issue. The unpaired electrons in paramagnetic particles underlie the affinity principle; however, the affinity is typically weak and transient. Candido *et al*. [48] proposed that electrostatic forces are a function of the zeta potential difference of the eggs. This uncertainty in the binding of microspheres to different *Schistosoma* eggs further emphasizes the need to elucidate the affinity dynamics.

3.4. Nuclear magnetic spectroscopy (^{1}H NMR)

Wang et al. (2024) [49] pioneered the first metabolomics application by designing a study in which *S. mansoni* was analyzed using ^{1}H NMR. This study aimed to identify the metabolic signatures of schistosomiasis infection that could be used to fabricate diagnostic tools for improved disease detection and surveillance. ^{1}H NMR was used to characterize the metabolic profile of *S. mansoni* infection in mice. In this study, several metabolites associated with infected mice were identified, in comparison to uninfected mice. Subsequently, a number of NMR-based metabolomics studies have emerged, with several compounds being postulated as potential biomarkers of schistosomiasis infection [49–53]. In one study [48], *S. mansoni* was observed to reduce the levels of tricarboxylic acid (TCA) cycle intermediates, including citrate, succinate, and 2-oxoglutarate, and increase levels of pyruvate, suggesting the stimulation of glycolysis. There is also a range of microbial-related metabolites, including trimethylamine, phenylacetylglycine, acetate, p-cresol glucuronide, butyrate, propionate, and hippurate. Urinary 3-ureidorpopionate has also been observed to be elevated as a result of *S. japonicum* infection [50], with others identified in related studies [51]. Another ^{1}H NMR study of *S. mansoni* infection identified 2-C-methylerythritol as an indicator of heavily infected subjects [53]. ^{1}H NMR affords simplified sample handling procedures and short acquisition times [54] but suffers from low sensitivity, which is a disadvantage for the holistic identification of metabolite features when compared to mass spectrometry (MS).

3.5. Immunological diagnosis

Immunological diagnosis analyzes molecules released by the host's immune response. It monitors antibodies released following schistosomiasis infection [55]. Although there are many immunological techniques such as agglutination, immunodiffusion/precipitation reaction, and immunohistochemistry, few of these have seen significant clinical application in the detection of schistosomiasis. Immunoassays and immunoblotting have mainly been used as complementary techniques in cases of light infection [56,57]. Enzyme-linked immunosorbent assay (ELISA) has been applied in the fecal diagnosis of *S. mansoni* and has proven to be more sensitive than the KK technique [58]. ELISA detects hormones, peptides, nucleic acids, and metabolites, among others, as medical analytes commonly assayed in blood and excretions. These hormone or metabolite molecules are attached to a substrate, followed by migration on a hydrophilic substrate [56] and detection using antibody staining. Immunological diagnosis is considered a 'gold standard' technique in the case of low parasite burden, or in the absence of tissues necessary for parasitological diagnosis [59]. It is not labor-intensive, and point-of-care testing (POCT) kits are available. However, the differences among epitopes of different antigens remain a major

drawback. Incidents of low specificity and cross-reactions with other parasites known to result in false positives have been reported [56,58]. Further, misdiagnosis has been reported in 40% of cases that are egg-positive [60] due to species-specific antibodies or insensitive kits. This is mainly due to a seronegative window that lasts only between 6 and 12 weeks. The inability to discriminate active from resolved infections has also been associated with immunological diagnostic techniques. Surprisingly, one study on parasitology and immunological tests [61] revealed 100% specificity. The study suspected bias; however, this could not be established.

3.6. The polymerase chain reaction (PCR) technique

PCR is based on selective DNA amplification. The high sensitivity and specificity possible with PCR diagnosis is vital for effectively assessing interventions in the control and elimination of schistosomiasis infection [39]. Although it is a costly assay, especially for resource-poor countries, the high sensitivity of PCR can be used effectively as a diagnostic tool in a subset of endemic populations to monitor intervention and implementation strategies [33]. A limitation of this technique is contamination during sample collection or DNA extraction and downstream handling [62,63]. A recent study [64] reported on direct diagnosis using PCR that discriminates DNA sequencing, thus minimizing costs and time. Although PCR is commonly used in a variety of diagnostics, its versatility for cellular level analysis is compromised by false negatives, especially in the early stages of infection, and by reported interferences with primers [30,65]. However, the early detection of parasites has been reported using conventional PCR [66]. Despite the low sensitivity of detection of parasites at a late stage, real-time PCR offers the advantage of being more useful in routine laboratory settings than conventional PCR [67]. Isolating high-molecular weight DNA of quantities above ≥ 12 µg, freeze-drying, and grinding in liquid N_2, or bead beating are recommended strategies prior to the application of chemical or kit-based extraction methods. These strategies require the absence of RNA, protein, and carbohydrate impurities as well as intense mechanical disruption that can cause heat-induced DNA shearing, resulting in lower-molecular weight DNA [68]. As such, these remain significant challenges in DNA purification. Owing to their high sensitivity and versatility [65], metabolomics-based tools have emerged as excellent analytical platforms that simplify the analysis of lower-molecular weight species, and can be used with low quantities of sample [68]. This underscores the NMR and MS detection of small molecules that are similar to the phenotype and change rapidly in pathophysiological mechanisms using metabolomics [69].

3.7. Metabolomics

Metabolomics employing separation techniques, conjugated to mass spectrometry, is now of growing interest. To unravel a holistic schistosomiasis-host interrelationship, systems biology experts advocate for 'omics' technology [70]. 'Omics' has the potential to unveil the mystery between biological information and functionality, as influenced by the *Schistosoma*'s co-existence with the host organism [71]. One of the 'omics' is the totality of all low-molecular weight cell products. The launch of the *Schistosoma* genome project in 1994, by the World Health Organization (WHO), led to substantial progress dealing with *Schistosoma*-containing aquifers, applying genomics, transcriptomics, proteomics, and metabolomics [72]. Metabolomics is the end product of the cellular genome. The promise in using the overall changes that occur with metabolism inside host tissues and biofluids, before and after *Schistosoma* infection, has attracted attention. The capability for proper surveillance is directly dependent on robust schistosomiasis diagnostic techniques capable of informing accurate infection assessments [73]. The potential of the metabolomics technique was first pioneered in 2004 by Wang, who visualized metabolite changes using ^{1}H NMR and multivariate statistics [49]. This study paved the way for innovative sensitive techniques for detection of biomarkers induced as a result of infection with schistosomiasis, such as liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis-mass spectrometry (CE-MS) [74]. Metabolomics advances have not been limited to experimental research, but have also revolutionized data standards and bioinformatics [75]. Despite the high cost of instrumentation involved, the aim is to use the instruments to mine biomarkers that can be used to fabricate inexpensive POCT devices which can be afforded by communities in low-resource endemic regions.

MS is usually interfaced with a variety of separation techniques to provide high selectivity and specificity for the qualitative identification of appropriate metabolites. On the other hand, NMR is nondestructive, noninvasive, and fast. However, while MS can be coupled to LC, GC, or CE to enhance its robustness, NMR is limited by its lower sensitivity and unaccounted loss of low concentration metabolites, or overlay of signals in some regions that interfere with metabolite identification [76]. The key focus of both platforms is the identification of metabolites in infected versus non-infected control samples for possible diagnostic biomarkers using data processing and multivariate techniques. These techniques, though not labor-intensive, require stringent and laborious data manipulation procedures that often employ a combination of software packages for data filtration. Data filtration enables the removal of false positives, and uses multivariate statistical analysis and comparison with databases for identification purposes. In the area of biomedical research, the evolution of schistosomiasis diagnostics has been advancing with technological platforms capable of diagnosing the disease at different stages of infection [77]. However, this evolution is mostly evident in low-endemic or imported-schistosomiasis areas inhabited by high-income dwellers.

3.7.1. Liquid chromatography – mass spectrometry (LC-MS)

LC-MS offers better sensitivity, speed, and versatility and uses small volumes compared to ^{1}H NMR and the 'gold standard' techniques. It can also comparatively mine more metabolites using a variety of column technologies as long as traditional and standard sample preparation techniques are used [78]. Column chemistry, coupled with the high resolution with

which ions are mass analyzed, and the use of appropriate metabolite mining software, in combination with multivariate statistical tools, simplifies the identification of metabolites. LC-MS applied to untargeted mining of metabolites is also capable of elucidating the biochemical variations associated with metabolic perturbation due to infection. Metabolites associated with infection can also be used as biomarkers for diagnostic and prognostic purposes [79]. For instance, a comprehensive LC-MS-based metabolomic analysis of serum from mice infected with *S. japonicum* has been reported [80]. This technique enabled detection of the infection one week post-infection as opposed to three weeks for the traditional, 'gold-standard' methods of detection. LC-MS-based metabolomics has been able to detect many changes in metabolic pathways which were induced by *S. japonicum*, including glycolysis, protein metabolism, DNA/RNA synthesis, phospholipid metabolism, and depression/energy metabolism. In addition, 17 metabolites were also identified which were downregulated in the first week of infection, and which were also related to the time-course of schistosomiasis progression. These metabolites were also identified as potential biomarkers of worm burden. Since the decrease in the 17 metabolites was exceptional during the first week post-infection, they could provide an early diagnostic tool for schistosomiasis. Another study comparing the metabolic profiles of male and female *S. japonicum* worms collected from mice five weeks post infection using LC-MS/MS has been reported [81,82]. Here, metabolic profiles were used to differentiate between male and female worms in the two types of mice. Additionally, an examination of human urine and plasma metabolites in individuals affected by urogenital schistosomiasis and its associated bladder pathologies using LC-MS has also been reported [83]. This study profiled metabolites as biomarkers of *S. haematobium* infection. Prior to this, another LC-MS study profiled urinary estrogen metabolites and examined self-reported infertility in women who had been infected with *S. haematobium* [84]. The study was carried out in female residents of a region in Bengo province in Angola, where *S. haematobium* is endemic, and estrogen metabolites were mined in a non-targeted fashion. In addition, potential biomarkers, such as catechol-estrogen/DNA adducts, were followed. The same research group investigated estrogen-like metabolites and DNA adducts in urogenital schistosomiasis and bladder cancer caused by *S. haematobium*, using LC-MS [85]; some of these could serve as biomarkers for the diagnosis and/or prognosis of schistosomiasis caused by *S. haematobium*. It was concluded, however, that these biomarkers require further investigation and confirmation in future studies[86]. This study was done after observing increased estradiol levels and postulating that schistosomiasis could be responsible for the production of estradiol-related molecules, which were subsequently mined using LC-MS.[87] Other models using mice for the LC-MS mining of metabolites affected by schistosomiasis have been reported [88]. Urine metabolites resulting from infection of animals with *S. mansoni* were analyzed with UPLC-MS and compared with those obtained using capillary electrophoresis (CE). To normalize the comparison, the same sets of multivariate analysis tools were employed. Recently, two patents have been registered, one for a serum biomarker, i.e. phosphatidylcholine/palmityl choline, and the other for urine biomarkers, i.e. xanthuric acid, naphthalene sulfonic acid, and heptanoyl carnitine, for early diagnosis of *S. japonicum* [80,89]. These patents describe screening methods based on mouse models and their application in fabricating devices to detect early *S. japonicum* infection in serum or urine. It has also been demonstrated, using LC-MS, that infection with carcinogenic helminth parasites caused by *S. haematobium* produces metabolites that induce the formation of DNA-adducts [90]. LC-MS has been used to identify steroid hormone metabolites and related DNA-adducts emanating from the parasite in early stages, as well as eggs of *S. haematobium* in urine from people infected with urogenital schistosomiasis [91].

Table 1 summarizes the instrumentation, ionization mode, columns, data filtration techniques, and statistical evaluation that were reported in the literature cited in this review for deciphering the metabolomic changes that occurred before and after infection with schistosomiasis.

3.7.2. Gas chromatography-mass spectrometry (GC-MS)

When mining small molecule biomarkers, GC-MS is best suited for the analysis of volatile metabolites after appropriate sample preparation techniques to minimize matrix effects and maximize metabolite recovery [92]. GC-MS is also less cumbersome than LC-MS and yields chromatographic separation with high resolution and theoretical plate numbers. Thus, it is more suitable for use as a complementary method to LC-MS. For instance, a GC-MS study pursued the mining of metabolites in urine samples confirmed to be infected with *S. haematobium* compared with urine samples from non-infected subjects [93]. GC-MS has also been used to characterize the avian schistosoma *Trichobilharzia ocellate* [94], as compared to *S. mansoni*. In addition, sera from *S. mansoni* and *S. haematobium*-infected humans as well as from controls have been elucidated using frequency-pulsed electron-capture gas–liquid chromatography (FPEL-GLC) and mass spectrometry for carboxylic acids and amines [94]. Serum samples were collected from residents of Kerdasa and Tamooh villages in the vicinity of Cairo, Egypt. Several amines postulated to be linked to schistosomiasis were identified in the chloroform extracts of these samples. Notwithstanding the above, the literature shows scant research on the search for small-molecule biomarkers for schistosomiasis. Perhaps this could be attributed to a perceived 'lack of volatiles' in samples that are predominantly aqueous in nature or to minimal interest due to the neglected disease status of schistosomiasis.

4. Metabolite mining methods – comparative analysis

Table 1 summarizes the instrumentation, methods, ionization modes, columns, data filtration, and statistical evaluation techniques that have been employed in schistosomiasis metabolomics involving separation science and mass spectrometry for small molecules. Table 1 also emphasizes that there is very little literature on the metabolomics of schistosomiasis using either LC-MS or GC-MS. This underscores the tag attached to this disease, i.e. 'a neglected tropical

Table 1. Instrumentation, methods, ionization modes, columns, data filtration and statistical evaluation techniques used for schistosomiasis metabolomics.

Instrumentation	Method	Ion Creation	Column	Data Filtration	Statistical Evaluation	ref
Agilent 1290 Infinity LC- 6530 Accurate Mass Q-TOF MS	UHPLC-MS	ESI	ACQUITY UPLC HSS T3	MassHunter XCMS	SIMCA-P PLS-DA PCA ANOVA	[59]
Agilent 1290 Infinity LC- 6538 UHD and Accurate-Mass Q-TOF MS	UHPLC-MS	ESI	ACQUITY UPLC HSS T3	MassHunter MassHunter Mass Profiler Professional	PCA PLS-DA OPLS-DA	[60]
Agilent 1290 Infinity LC- Agilent 6538 UHD and Accurate-Mass Q-TOF MS	UHPLC-MS	ESI	ACQUITY UPLC HSS T3	MassHunter MassHunter Mass Profiler Professional	PCA PLS-DA OPLS-DA	[61]
Thermofisher Accela- Q-Exactive orbitrap	UHPLC-MS	ESI	HypersilGold	XCMS CAMERA	Mann Whitney Kruskal-Wallis PCA PLSDA SAM Random Forest trees	[62]
Finnigan Surveyor Plus HPLC – Thermofinnigan LCQ XP MAX	LC-MS	ESI	LiChroCART C18	XCalibur Comparison with known standards	the chi-square test with Yate's correction or with Fisher's exact, two-sided test, student t test	[63–67]
Waters Equity UPLC – QtoF	UHPLC-MS	ESI	Equity C18	MarkerLynx™ Application Manager ApexTrack™	SIMCA-P PCA PLS-DA OPLS-DA	[68]
waters ACQUITY UPLC-I Class System – Waters SYNAPT G2-Si HDMS	UPLC-MS	ESI	ACQUITY UPLC C18BEH	Masslynx Progenetics QI	PCA PLS-DA OPLS-DA ANOVA	[69,70]
Thermo Fischer Scientific LTQ Orbitrap XL mass spectrometer	LC-MS /MS	ESI	ACE Equivalence 5 C18	-	-	[71]
-	GC-MS	EI	-	-	-	[72]
Hewlett Packard 5890 gas chromatograph – VG 30–250 mass spectrometer	GC-MS	EI Chemical ionization	CP Sil 19 CB column	-	-	[73]
Perkin-Elmer Model 3920 gas chromatograph – Finnigan mass spectrometer 4023			OV-101	Modular Software System (MS 16 revision B)	Comparison with known standards	[74]

disease,' which is the case despite affecting over a quarter of a billion people globally. The most commonly used ionization mode is electrospray ionization (ESI) for LC-MS and electron ionization (EI) for GC-MS. Future studies could focus on interrogating other ionization techniques, such as atmospheric pressure chemical ionization (APCI). This is well suited for small-molecule ionization using LC-MS and soft ionization, such as field desorption, in addition to chemical ionization for GC-MS. These techniques need to be probed for the analysis of abundant metabolites during ionization. There is a need to explore other data filtration software packages, in addition to XCMS, in order to increase the chances of biomarker discovery. This applies to multivariate statistical tools that revolve around PCA and PLS-DA.

5. Schistosomiasis in the Okavango Delta communities of Botswana

5.1. Current detection surveillance and management techniques

Historically, schistosomiasis has been prevalent in tropical regions of the world, with most cases being recorded in Africa [95]. The WHO committed to controlling morbidity by 2020, and the year 2025 was set as the target for elimination of schistosomiasis as a public health threat in certain regions [96]. Most countries have developed national strategies for controlling infection using the WHO targets. In Africa, *S. mansoni* and *S. haematobium* are common types of schistosomiasis parasites [13,97]. The life cycle of these infections has been thoroughly elucidated [13,97–100].

Schistosomiasis is prevalent in the Okavango Delta region of Botswana due to the perennial and seasonal flooding of the delta [26]. For instance, in 1983, approximately 80% of schistosomiasis infections that were recorded in Botswana followed the 1977 flooding of the Okavango Delta [101]. The Okavango Delta aquatic ecosystem supports humans, animals, and plants. It is home to a variety of snails that pose a public health threat because they transmit parasites that cause schistosomiasis [102]. Traditionally, the 'gold standard' diagnostic tools described above continue to be favored in the Okavango Delta region. Although Kato Katz and urine microscopy diagnostic techniques have demonstrated high specificity, their application in low-endemic areas, which may include imported schistosomiasis cases such as the Okavango Delta, have been shown to be limited. This is because of the poor sensitivity of these techniques [103]. This diagnostic challenge makes eradication of schistosomiasis in the Okavango Delta a serious goal [37]. This is underscored by the recent mapping of the disease, which identified schistosomiasis infections as accounting for 50% of all infections among the subjects in some areas of the delta [104].

Furthermore, knowledge gaps exist in understanding the progression of schistosomiasis infection prior to egg excretion,

as well as at the chronic stage when eggs are excreted sporadically or not at all. Besides using egg excretion to classify individuals as positive, there is the possibility of using the schistosomiasis disruption effect of the biochemical flora as markers for diagnosis, but this is poorly understood [105]. It is critical with today's advanced technology to offer diagnostic techniques capable of mapping infections per origin in order to undertake focused interventions. The lack of state-of-the-art diagnostic strategies employing newer technologies for the diagnosis of schistosomiasis is not just a gap in Botswana, but also in many other developing countries. Further, insights into infection dynamics following chemotherapy are necessary for informed policy decision making. The Botswana Neglected Tropical Disease Master Plan (BNTDMP) [102] recognizes schistosomiasis as an important neglected tropical disease (NTD) that significantly impacts socioeconomically-disadvantaged and poverty-stricken communities. Consequently, the BNTDMP of 2015–20 addressed public health limitations with respect to schistosomiasis. The progress in implementing the BNTDMP has yet to be audited and publicized [102]. In 2008, a study [101] predicted that an *S. mansoni* epidemic would surface around 2020 due to high water inflow into the Okavango Delta. In fact, the high-water inflow prediction materialized in 2020, and schistosomiasis poses a serious threat to the Botswana public health system.

6. The future outlook – mass spectrometry based techniques

With regard to neglected tropical diseases, research on high-throughput technology with the capability to elucidate lower-abundance small-molecule metabolites as biomarkers for infections continues to be pursued [100]. Recent studies [83,106,107] have reported the use of small molecule metabolite biomarkers from biochemicals elicited by adult worms or eggs in rats or hamsters for the diagnosis of schistosomiasis. State-of-the-art technologies have been predicted to minimize the underestimation of the prevalence or misdiagnosis of schistosomiasis [108]. 2D separation science techniques interfaced with mass spectrometry have been reported to enhance metabolite mining capabilities [109]. This includes the development of accurate mass and high-resolution separation systems and mass analyzers that will greatly simplify metabolite identification [110]. The development of POCT systems based on *Schistosoma* biomarkers that will be mined using state-of-the-art separation and mass spectrometer systems will probably form the core of research in this area. Innovative technologies based on chemistry, electronics, and optics are also expected to accelerate the development of commercial biosensors for the diagnosis of schistosomiasis [30].

Biosensor technology is becoming a technology of choice for resource-limited settings for the diagnosis of disease. Biomarker mining in noninvasive matrices such as urine, stool, or saliva will ultimately yield metabolites that can be incorporated into biosensors for smart phones [111]. These smartphones are expected to simplify the diagnosis of schistosomiasis and the management of infection, especially in rural areas, because they do not require trained health personnel to be used effectively. This technology will be user-friendly and non-discriminative, even for illiterate rural communities [112]. The Okavango Delta is expected to benefit from the pursuit of this and related research.

7. Expected outcomes

Efforts toward the fabrication of POCT devices based on smart phone biosensor technology will revolutionize diagnostic capability for schistosomiasis. POCT devices will provide screening tools for noninvasive samples, which will ultimately require confirmation with traditionally-classified 'gold standard' diagnostic tools. POCT devices can be added to national neglected diseases master plans for disease elimination, such as the Botswana Neglected Tropical Disease Master Plan. These devices can be fabricated in two forms: low-cost disposable dip sticks and cell phone-based biosensors. In both cases, the technology would be cheap and affordable, even in rural areas, which are the most impacted communities. A major advantage of this technology is the use of POCT devices without the need for trained technicians or health/biomedical scientists. It is anticipated that once the devices are rolled out for use, there will be no difficulty in distribution logistics other than the sensitization of the general public.

8. Conclusions

Global efforts to eliminate schistosomiasis in humans have been initiated and are now quite advanced [113]. This involves, among other places, the Okavango Delta of Botswana, where schistosomiasis is endemic. However, despite the mass drug administration of praziquantel, which focuses on treating populations, the prevalence of schistosomiasis in Africa continues to increase [114]. Accurate diagnosis of schistosomiasis has been identified as critical for the control of schistosomiasis transmission. Nevertheless, there are limitations associated with several diagnostic techniques. Despite the fact that PCR is commonly used because of its accuracy, metabolomics now seems to have taken center stage. This review discussed schistosomiasis varietal metabolites in noninvasive samples that can be used as biomarkers to fabricate simple and affordable POCT devices. Metabolomics assesses the final downstream product of gene expression and biochemical reactions and utilizes modern instrumental analytical techniques with higher sensitivity and specificity. Despite the large amount of money required to assemble a modern separation science-mass spectrometry laboratory to enable a metabolomics hunt for biomarkers of schistosomiasis, it is hoped that African countries can pull resources together through regional platforms, such as the Southern African Development Community (SADC), to come up with centralized facilities where this type of research can be pursued. Not only will this ameliorate financial constraints, but it will also tap into the pool of human resources required to carry out this type of work. The aim, once schistosomiasis biomarkers have been successfully mined, would be to fabricate multi-biomarker biosensors for neglected tropical diseases for smartphones and similar technologies. These multi-biomarker biosensors that can handle noninvasive samples for diseases such as schistosomiasis, malaria, and sleeping

sickness would be incorporated as arrays on a single chip and inserted into smartphones to aid rural areas impacted by those diseases, even without trained health and medical personnel. This will improve surveillance and monitoring, in that interventions can be carried out before the disease reaches an advanced stage.

9. Expert opinion

Metabolomics, using advanced analytical chemistry technologies, has become increasingly popular for the assessment of metabolites in human fluids, as biomarkers and also for studying the disruption of metabolic profiles in humans. Biofluids remain the specimen of interest for biomarker mining, and for studying metabolic and catabolic perturbations associated with infection. In particular, noninvasive samples such as saliva, urine, and stool have gained popularity because of their ease of access even in the absence of medical personnel. This review has brought to the fore data acquisition, data filtration, and data analysis strategies that have the potential to extract novel biomarkers, in addition to elucidating disruptions in metabolic and catabolic pathways caused by schistosomiasis. It is expected that the identification and confirmation of schistosomiasis biomarkers will lead to the fabrication of diagnostic tools for early intervention and management of the disease, especially for impacted rural communities in tropical settings. Recent advances in smartphone biosensors have boosted the use of biomarkers for enhanced surveillance of infections, even by non-medical professionals. It is envisaged that once the biomarkers for schistosomiasis are mined and confirmed, they will revolutionize future diagnostic strategies for neglected tropical diseases (NTDs), in that multi-disease biosensors incorporating biomarkers for a range of NTDs, such as malaria and sleeping sickness, will emerge, harnessing smart phone technology. Some NTDs are preventable, and can be eliminated with early diagnosis and treatment. These smartphone biosensors will be realized through collaborative efforts between leading scientists in the next five years. Five biomarkers reported in this review have recently been mined for schistosomiasis in China, and patented [80]. This should act as a springboard for more biomarkers in the near future, and the realization of smartphone biosensor chips.

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