Bioinformatics evaluation of the homologues of *Schistosoma mansoni* biomarker proteins of bladder cancer in other *Schistosoma* species

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Abstract

Schistosomiasis remains a public health problem in developing countries. An ideal diagnostic test capable of detecting parasites as early as possible after the onset of infection is therefore highly desired. The identification of biomarker proteins associated with active infection and immune response may constitute the basis for the development of a successful diagnostic test. The aim of this study is to contribute to the development of protein biomarkers for schistosomiasis using a bioinformatics approach. The homologues of 36 previously identified urine biomarker proteins from a *Schistosoma mansoni* database, were identified in other *Schistosoma* species using SMARTBLAST and analyzed for similarities and differences using multiple sequence alignment. Of the 36 *S. mansoni* biomarker proteins, 29 had homologues with both *S. haematobium* and *S. japonicum* or either of *S. haematobium* and *S. japonicum*. Most of the 29 *S. mansoni* biomarker proteins aligned with their homologues with many conserved regions. However, some vital biomarker proteins like venom allergen-like proteins, which had been proposed as a putative drug and vaccine target, showed more semi conserved regions in which the amino acids had similar shape but weakly similar properties. The predictions of 12 markers found in all three species also show that treatment of infections may possibly benefit from the investigational drug Arteminol and specific nutraceuticals or supplements. Experimental evaluation is needed to confirm the potential of the proteins as biomarkers for early diagnosis of schistosomiasis and associated bladder cancer.

Keywords: Schistosomiasis; Protein Biomarker; Homologues; bioinformatics

INTRODUCTION

Schistosomiasis, also known as bilharzia, is a disease caused by blood-dwelling trematodes belonging to the genus *Schistosoma*. The genus *Schistosoma* contains six species that are of major pathological importance to man, *Schistosoma haematobium*, *S. mansoni*, *S. japonicum*, *S. mekongi*, *S. intercalatum*, and *S. guineensis* (French *et al.*, 2018). Of these species of flatworms, *S. haematobium*, *S. japonicum* and *S. mansoni* are the main schistosome parasites of man (Masamba *et al.*, 2016). Schistosomiasis remains one of the most prevalent neglected tropical diseases especially in Nigeria, which supposedly has the greatest number of infected people worldwide (Dawaki *et al.*, 2016). Despite the gains in the health care delivery of the past decades, schistosomiasis has prevailed as a health challenge in the tropic and the subtropics. Schistosomiasis is a chronic parasitic infection responsible for nearly 280,000 deaths per year with 200 million people worldwide being infected (Cai *et al.*, 2017). According to the World Health Organization (WHO, 2015), schistosomiasis is second to malaria alone amid the vector-borne diseases in terms of public health and remuneration importance in the tropics. An early diagnostic test would enable more rapid treatment and would interrupt the transmission cycle of the parasite and the progress of the disease (Kardoush *et al.*, 2016). However, the current diagnostic standards for schistosomiasis all depend on the detection of eggs. In addition, as the disease is asymptomatic in its early stages, clinical examinations cannot confirm infection (Weifang *et al.*, 2018).

Human bladder cancer is the fifth to the seventh most common cancer in Western countries. In many tropical and subtropical areas, however, it is the first among all types of cancer, mainly due to the endemic parasitism (Gulia *et al.*, 2017). The urinary form of schistosomiasis, known as urogenital or urinary schistosomiasis is caused by *S. haematobium* and is widespread in Africa and the Middle East (WHO, 2015). Epidemiological evidence indicates that *S. haematobium* is associated to squamous cell carcinoma of the bladder. In spite of various control measures and eradication methods that have been in use, schistosomiasis still prevails as one of the most debilitating parasitic diseases, typically affecting the poor and the less privileged predominantly in sub-Saharan Africa (Masamba *et al.*, 2016). Efforts to control and eradicate schistosomiasis rely on praziquantel, the only drug available for treatment. However, treatment with praziquantel may sometimes fail, and this may be due to possible drug resistance. There were also case reports of travelers who were infected during their stay in endemic areas and were treated with praziquantel, but resulted in treatment failure (Secor and Montgomery, 2015). Vaccine

development against this disease has experienced more failure than successes, (although there is currently a vaccine trial going on for schistosomiasis in Africa (Molehin, 2020); therefore, the identification of molecular targets that can induce protective immunity is highly desirable.

The use of biomarkers in basic and clinical research as well as in clinical practice has become so commonplace that their presence as primary endpoints in clinical trials is now accepted almost without question. The identification of biomarker proteins associated with active infection and protective immune response may constitute the basis for the development of a successful vaccine and could also indicate new diagnostic method (Schmitz-Drager *et al.*, 2015). Over the last decade, the number of omics resources used to study this neglected disease has increased considerably, mainly due to advances associated with the development of detection/analysis techniques and mass spectrometry equipment (Hwang *et al.*, 2018).

Identification of biomarker proteins is important in early diagnosis of both schistosomiasis and schistosomiasis associated bladder cancer and has been evaluated in recent studies (Onile *et al.*, 2017); it may also have application in drug and vaccine development for the treatment of the disease. These biomarker proteins were found using *S. mansoni* database, which is the best curated schistosome species. It is therefore important to know if homologues of these biomarker proteins could be found in other schistosome species, especially in *S. haematobium* and *S. japonicum*. In this present study, we searched for homologues of the thirty-six *Schistosoma mansoni* biomarker proteins found in recent studies in *S. haematobium* and *S. japonicum*. This could be used for early diagnosis of schistosomiasis and schistosomiasis associated bladder cancer. We also attempted to identify proteins common to all *Schistosoma* species which could provide targets for developing drugs or vaccines that can be simultaneously effective against all species of the parasite.

Molecular techniques to detect schistosome infections have been developed to facilitate early diagnosis, but these are expensive and suffer from sampling limitations (Yumin *et al.*, 2018; Weerakoon *et al.*, 2018). Serologic assays to detect antibodies against schistosome antigens, however, have proven useful in the clinical diagnosis of schistosomiasis. Zheng *et al.*, (2012) strongly suggest that SjSP-216, a highly expressed gene in the young worm stage, could serve as a potential biomarker for the early immunodiagnosis of *S. japonicum* infections in vertebrate hosts

The aim of the study is to contribute to the development of protein biomarkers for schistosomiasis diagnosis using a bioinformatics approach. This will be achieved by identifying the homologues of the 36 biomarker proteins from *Schistosoma mansoni* evaluated in recent study (Onile *et al.*, 2017) in other *Schistosoma* species using bioinformatics tools. These biomarker protein sequences from *S. mansoni* will be compared with those from other *Schistosoma species* to visualize similarities and differences.

RESULTS

A total of 36 *S. mansoni* biomarker proteins (Table 3.1) were used as query for the homology analysis with *S. haematobium* and *S. japonicum*. Out of these, identical proteins were retrieved for twenty-nine proteins (Table 4.1).

Homology Determination

Of the 36 *S. mansoni* biomarker proteins (Table 2), 29 had identical proteins with both *Schistosoma haematobium* and *S. japonicum* or either of *S. haematobium* and *S. japonicum*. The biomarker proteins showed varying identities with *S. haematobium* and *S. japonicum* whole genome shotgun sequence while some of these proteins showed no significant similarity with either *S. haematobium* or *S. japonicum* whole genome shotgun sequence.

Multiple Sequence Alignment

There were conserved regions in the Calreticulin autoantigen homolog (C4Q5I7) of *S. mansoni*, *S. haematobium* and of *S. japonicum* (AAC00515.1) showing that the amino acids in the protein sequences have strongly similar properties. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues (Figure 1).

Putative actin-1 of *S. mansoni* (G4VLW2) and the homologue Actin 5C (CAX75500.1) of *S. japonicum* shows conserved regions as well as regions of complete identical nucleotide sequence (Figure 2). Phosphoglycerate hydratase (enolase) showed conserved regions as well as regions of complete identical nucleotide sequence in most of the positions in the alignment (Figure 3)

Most of the 29 *S. mansoni* biomarker proteins aligned with their homologues, giving rise to a shared identical conserved region. However, some vital biomarker proteins like venom allergen-like proteins which had been proposed as putative drug and vaccine target (Huang *et al.*, 2017), showed more of semi conserved regions in which the amino acids had similar shape, but had weakly similar properties (Figure 4).

Potential Therapeutic targets

For homologues identified consistently in all three species, 10 had potential targets known or being investigated in humans (Table 3). Such compounds included modified nucleotides, phosphatidylcholines, arteminol, and supplemental elements, among others.

Table 1: The 36 identified *Schistosoma mansoni* biomarker proteins retrieved from Onile *et al.*, 2017 and their predicted function.

Protein ID	Schistosoma Protein	Predicted Function
C4Q4S5	Tubulin alpha chain	Structural/ GTPase activity
C4Q5I7	Calreticulin autoantigen homolog	Binding
C4QBN1	Histone H4	Binding
G4LWI2	Heat shock protein HSP60	Heat shock protein
G4LYN4	ADP-ribosylation factor, ARF	Transporter
G4M1M0	DNA polymerase	Binding and catalytic
G4V6R4	Putative Rab9	Binding
G4V8L4	Putative heat shock protein 70	Binding/Heat shock protein
G4V9I0	Putative heat shock protein 70 (HSP70)	Binding/ Heat shock protein
G4V8L4	Putative heat shock protein 70	Binding/ Heat shock protein
G4VAC9	Putative uncharacterized protein	Unknown
G4VAD2	Elongation factor 1- alpha	Binding/GTPase activity
G4VAW0	Serine/threonine kinase	Binding
G4VB75	Cytoplasmic dynein light chain	Structural/Motor
G4VB79	Voltage-gated potassium channel, KCNQ	Transmembrane transporter
G4VDD2	Eukaryotic translation initiation factor 5A	Binding
G4VG19	Phosphoglycerate kinase	Kinase
G4VG20	Phosphoglycerate kinase	Kinase
G4VGA0	Sodium/potassium-transporting subunit alpha	ATPase Transporter
G4VH98	Putative fimbrin/plastin	Binding
G4VHN3	ATP synthase subunit beta	Transporter/binding
G4VIM7	cAMP-response element binding protein related	Binding
G4VKT8	Putative ATP synthase alpha subunit vacuolar	Transporter/ Binding
G4VLJ0	ATP synthase subunit alpha	Transporter/Binding
G4VLJ8	Fidgetin like-1	Binding and catalytic

G4VLN5	Putative uncharacterized protein	Binding
G4VLW1	Putative actin	Binding
G4VLW2	Putative actin-1	Binding
G4VM26	Putative Zinc finger protein	Binding
G4VMG4	Venom allergen-like (VAL) 3 protein	Unknown
G4VMT3	Adapter-related protein complex 3, beta subunit	Transporter
G4VP51	Putative ADP, ATP carrier protein	Structural/Transporter
G4VPE8	Putative inorganic pyrophosphatase	Binding
G4VPU8	Putative cytoplasmic dynein intermediate chain 2	Structural/Motor
G4VQ01	Uncharacterized protein	Unknown
G4VQ58	Phosphopyruvate hydratase	Binding

Table 2: *Schistosoma mansoni* proteins and their retrieved identical proteins

Identified <i>S. mansoni</i> protein	Identical <i>S. haematobium</i> protein	Identical <i>S. japonicum</i> protein
Tubulin alpha chain	No identical was protein found	1. Tubulin alpha-1 chain (CAX75502.1) 2. Tubulin alpha-1 chain (CAX75500.1)
Calreticulin autoantigen homolog Heat shock protein HSP60	Calreticulin (XP_012799776.1) 60 kDA heat shock protein mitochondrial (XP_012800213.1)	Calreticulin (AAC00515.1)
DNA polymerase	DNA polymerase zeta catalytic subunit, partial. (XP_012798609.1)	No identical protein was found
Putative rab9	Ras-related (XP_012796991.1)	Rab-7a SJCHGCO6013 (AAP06474.1) protein
Putative heat shock 70 (Hsp 70)	71 kDA glucose-regulated protein, partial (XP_012801946.1)	HSP70 (AAC00519.1)
Putative uncharacterized protein Elongation factor 1-alpha	Elongation factor 1-alpha (XP_012799780.1)	Actin 5C (CAX72714.1) 1. Eukaryotic translation elongation factor 1-alpha 2 (CAX72182.1)

			2. Eukaryotic translation elongation factor 1-alpha (CAX79121.1)
Phosphopyruvate hydratase (G4VQ58)	Enolase (XP_01293992.1)		SJCHGC00471 (AAW24521.1)
Cytoplasmic dynein light chain	Dynein light chain 2, cytoplasmic partial (XP_012797326.1)		No identical protein was found
Eukaryotic translation initiation factor 5A	Eukaryotic translation initiation factor 5A-1 (XP_012791801.1)		Eukaryotic translation initiation factor 5A (CAX71949.1)
Phosphoglycerate kinase	Phosphoglycerate kinase (XP_012797420.1)		Phosphoglycerate kinase (CAX77845.1)
Phosphoglycerate kinase	Phosphoglycerate kinase 1 (CAX77838.1)		Phosphoglycerate kinase (AAP74224.1)
ATP synthase subunit beta	No identical was protein found		1. ATP synthase F1 complex beta (AFN58251.1) 2. ATP synthase, H ⁺ transporting mitochondrial F1 complex beta (CAX75390.1)
Camp response element binding protein related	1. Hypothetical protein MS3_04489 (XP_012795970.1) 2. Hypothetical protein in MS3_10121 (XP_012801367.1)		
Putative ATP synthase alpha subunit vacuolar Fidgetin like-1	V proton ATPase catalytic subunit A (XP_012793973.1) Fidgetin-like protein 1(XP_012798816.1)		SJCHGCO9310 (AAW25271.1) protein
Putative uncharacterized protein			SJCHGC06318 (AAW25537.1) protein
Putative actin			Actin 5C (CAX69775.1)
Putative actin-1			Actin 5C (CAX69775.1)
Putative zinc finger protein	Zinc finger protein 271 (XP_012799906)		
Venom allergen-like (VAL) 3	Peptidase inhibitor 16, partial		Peptidase inhibitor 16 precursor

protein		(XP_012796982.1)	(CAX78430.1)
Adapter-related complex 3, beta subunit	protein	AP-3 complex subunit beta-2 (XP_012793674.1)	No identical protein was found
Putative ADP, ATP carrier protein	ADP, ATP carrier protein	ADP, ATP carrier protein (XP_012794736.1)	ADP, ATP antiporter protein (CAX71876.1)
Putative inorganic pyrophosphatase	Inorganic pyrophosphatase	Inorganic pyrophosphatase (XP_012798695.1)	(4QLZ-A) chain A inorganic pyrophosphatase
Putative cytoplasmic dynein intermediate chain 2	Cytoplasmic dynein 1 intermediate chain 2	Cytoplasmic dynein 1 intermediate chain 2 (XP_012801212.1)	No identical protein was found

Accession prefix

AA: reference protein sequence which undergoes continuing curation.

AAC and AAP: GenBank Protein ID for patent proteins.

CAX: EMBL (European Molecular Biology Laboratory) Protein ID.

AP_: Annotated proteins on complete genomic molecule, usually alternate assembly.

XP_: Computed proteins with predicted model

AAC00515.1	MILVLICFLLSYRVSGEKVWFSETFPDEKSIDGWIQSTFNGDKQGEFKIEAGKSPVNP	60
tr C4Q5I7 C4Q5I7_SCHMA	MLSILLTLLLSKYALGHEVWFSETFPNE-SIENWVQSTYNAEKQGEFKVEAGKSPVDPI	59
XP_012799776.1	MFSALLVLLLSYALGHEVWFSETFPNE-SIEDWVQSTYNADKQGEFKVEAGKSPVDPI	59
	*: *: :*** . *:*****:* *:*:***:*. :*****:*****:***	
AAC00515.1	DLGLKTTQDARFYGIARKISEPFSNRDKTLVLQFTVKFDKTVTCGGAYIKLLGSDIDPKT	120
tr C4Q5I7 C4Q5I7_SCHMA	DLGLKTTQDARFYGIARKISEPFSNRGKTTVLQFTVKFDKTVSCGGAYIKLLGSDIDPKK	119
XP_012799776.1	DLGLKTTQDARFYGIARKISQPFNSNRDKTMVLQFTVKFDKTVSCGGAYIKLLGSDIDPKK	119
	*****:*****:*****:*****:*****:*****:*****:*****:*****:*****	
AAC00515.1	FHGETPYKIMFGPDICGMATKRIHVIFNYKGNHLIKKDIPCKDDQKTHLYTLIVRPN	180
tr C4Q5I7 C4Q5I7_SCHMA	FHGESPYKIMFGPDICGMATKKVHVIFNYKGNHLIKKEIPCKDDLKTHLYTLIVNPNK	179
XP_012799776.1	FHGETPYKIMFGKALV-----HSGKNHLIKKEIPCKDDQKTHLHTLIINPNK	167
	:** : :*:*****:***** :*****:***:***	
AAC00515.1	YEVLDNEKVESGLLEEDWNMLAPKMIIDPNKKPDDWEEYIDDPNDEKPLDWDKPKT	240
tr C4Q5I7 C4Q5I7_SCHMA	YEVLDNEKVEEGSLEDDWMLPDKIIDPNKKPDDWVDEQFIDDPDDKPKDNWDQPKT	239
XP_012799776.1	YEVLDNEKVEEGSLEDDWMLPDKIIDPNKKPDDWVDEQFIDDPDDKPKDNWKQPKM	227
	*****:*. :*:***:*. :*****:*****:*****:*****:*****:*****	
AAC00515.1	IPDMDAKKPDWDDMDGEWKRPEKHNPYKGEWSPRRIDNPYKQWKPQIDNPDYKP	300
tr C4Q5I7 C4Q5I7_SCHMA	IPDMDAKKPDWDDAMDGEWERPQKDNPEYKGEWTPRRIDNPYKGEWKPQIDNPEYKH	299
XP_012799776.1	ITDMDAKKPDWDDVMDGEWKPLKDNPEYKGEWTPRRIDNPYKGEWKPQIDNPEYKH	287
	* ***** *****: * :*****:*****:*****:*****:*****:*****	

Figure 1 Calreticulin autoantigen homolog of *S. mansoni* (C4Q5I7); calreticulin of *S. haematobium* (XP_012799776.1) and calreticulin of *S. japonicum* (AAC00515.1) in multiple sequence alignment

Legend

“*” means that the amino acid in that column are identical in all sequence in the alignment. “:” denotes conserved regions i.e. amino acid with strongly similar properties. “.” denotes semi-conserved regions. The red color shows hydrophobic residues, blue color shows acidic residues, magenta shows basic residues, green shows hydroxyl residues while grey color show unusual amino acids. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues

tr G4VLW2 G4VLW2_SCHMA	MAEEDVAALVIDNGSGMCKAGFAGDDAPRAVFPISIVGRPRHQGVVMVGMGQKDSYVGDEAQ	60
CAX69775.1	MAEEEVAALVIDNGSGMCKAGFAGDDAPRAVFPISIVGRPRHQGVVMVGMGQKDSYVGDEAQ	60

tr G4VLW2 G4VLW2_SCHMA	SKRGILTLKYPPIEHGIVTNWDDMEKIWHHTFYNELRVAPPEHPVLLTEAPLNPKANREKM	120
CAX69775.1	SKRGILTLKYPPIEHGIVTNWDDMEKIWHHTFYNELRVAPPEHPVLLTEAPLNPKANREKM	120

tr G4VLW2 G4VLW2_SCHMA	TQIMFETFNVPAMYVAIQAVLSLYASGRITGIVLDSGDGVTHTVPIYEGYALPHAILRLD	180
CAX69775.1	TQIMFETFNAPAMYVAIQAVLSLYASGRITGIVLDSGDGVTHTVPIYEGYALPHAILRLD	180

tr G4VLW2 G4VLW2_SCHMA	LAGRDLTDYMMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSALEKS	240
CAX69775.1	LAGRDLTDYLMKILTERGYSFTTTAEREIVRDIKEKLCYVALDFEQEMGTAASSSALEKS	240

tr G4VLW2 G4VLW2_SCHMA	YELPDGQVITIGNERFRCPALFQPSFLGMEASGIHETTYNSIMKCDVDIRKDLYSNTVL	300
CAX69775.1	YELPDGQVITIGNERFRCPALFQPSFLGMESSGIHETTYNSIMKCDVDIRKDLYANTVL	300

tr G4VLW2 G4VLW2_SCHMA	SGGSTMYPGIADRMQKEISALAPSTMKIKIVAPPERKYSVWIGGSILASLSTFQQMWISK	360
CAX69775.1	SGGSTMYPGIADRMQKEISALAPSTMKIKIVAPPERKYSVWIGGSILASLSTFQQMWISK	360

Figure 2 Multiple sequence alignment of putative actin-1 of *S. mansoni* (G4VLW2) and actin 5C of *S. japonicum* (CAX75500.1)

Legend

“*” means that the amino acid in that column are identical in all sequence in the alignment. “:” denotes conserved regions i.e. amino acid with strongly similar properties. “.” denotes semi-conserved regions. The red color shows hydrophobic residues, blue color shows acidic residues, magenta shows basic residues, green shows hydroxyl residues while grey color show unusual amino acids. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues

Legend

11

Table 3. Drugs and compounds active against potential multi-species markers of medically important schistosomes

Markers in all 3 species (Query)	Potential Drugs and compounds	Drug Bank ID	Status	Similarity to Human form (coverage; identity %)
Putative inorganic pyrophosphatase	DB06851; DB07291	N-(pyridin-3-ylmethyl) aniline;5-amino-1-(4-chlorophenyl)-1H-pyrazole-4-carbonitrile	E;	89;51
Putative ADP, ATP carrier protein	DB00171;DB00720;DB02426;DB03429;DB04178;DB01736;DB01077	ATP; Clodronic acid; Carboxyatractyloside; Tetrastearoyl cardiolipin; Di-Stearoyl-3-Sn-Phosphatidylcholine;[3-(Dodecanoylamino)Propyl](Hydroxy)Dimethylammonium;Etidronic acid	I, nutraceutical;A, I, vet_A;E;E;E;E;A	91;42
Venom allergen-like (VAL) 3	not found			
Putative ATP synthase alpha subunit vacuolar	DB00630;DB01077;DB06733;DB06734	Alendronic acid; Etidronic acid; Bafilomycin A1;Bafilomycin B1	A;A;E;E	98;77.97
Calreticulin autoantigen homolog	DB00025; DB00031;DB06245;DB01065;DB11093; DB11348;DB09130;DB13998;DB13999; DB13949;DB14481;DB14520	Antihemophilic factor, human recombinant; Tenecteplase; Lanoteplase; Melatonin; Calcium citrate; Calcium Phosphate; Copper; Lonocotocog alfa; Morocotocog alfa;Ferric cation; Calcium phosphate dihydrate; Tetraferic tricitrate decahydrate	A, I; A; I;A, nutraceutical, vet_A;A, I;A;A, I;A, I;A;A;A;A	84;56
Putative rab9	DB04315	Guanosine-5'-Diphosphate		100;63.7
Putative heat shock 70	DB04216; DB07045; DB09130; DB01254; DB11638	Quercetin;(2R,3R,4S,5R)-2-[6-amino-8-[(3,4-dichlorophenyl) methylamino]purin-9-yl]-5-(hydroxymethyl)oxolane-3,4-diol;Copper;Dasatinib; Artenimol	E, I; A;I	93;74.84
Elongation factor 1-alpha	DB11638	Artenimol	E, I	100;78.3
Phosphopyruvate hydratase	DB09130; DB01593; DB11638; DB14487; DB14533; DB14548; DB02726	Copper; Zinc; Artenimol; Zinc acetate; Zinc chloride;Zinc sulfate, unspecified form;2-Phosphoglycolic Acid	A, I; A, I; E, I;A, I;A, I;A, E;E	99;75
Eukaryotic translation initiation	not found			100;54.1

factor 5A					
Phosphoglycerate kinase	DB03909; DB04510; DB00709; DB09130; DB11638	Adenosine-5'-[Beta, Gamma-Methylene]Triphosphate; 3-phospho-D-glyceric acid; Lamivudine; Copper; Arteminol	E; E; A, I; A, I; E, I	100;68.97	

A: approved; Experimental, I: investigational

DISCUSSION

Schistosomiasis, a devastating and highly prevalent neglected tropical disease (NTD), is endemic in Nigeria (reviewed in Abdulkadir *et al.*, 2017; Eke et al 2020). The emergence of schistosome parasites that are resistant to the traditional treatment due to over-reliance on praziquantel demands the development of newer and more effective treatment which requires accurate diagnostic methods (Fernandes *et al.*, 2018). More needs to be known about the molecular pathology that influences the outcome of the infection by *S. haematobium* in order to develop new diagnostics, therapeutic and infection prevention strategies (Weerakoon *et al.*, 2018).

The composition of the urine microbiome in schistosomiasis and its associated functions are being tackled by genomic sequencing technology. The identification of proteins common to all species of schistosomes will provide targets for developing drugs or vaccine to control schistosomiasis and the associated bladder cancer in view of the growing concern about resistance developing to the existing therapeutic (Magalhaes *et al.*, 2016). The detection of cancer-associated biomarkers, preferably isolated from urine and blood, has therefore become important. Such biomarkers are now being developed and will provide tools that could be useful to evaluate the specific effects of long-term exposure to *S. haematobium*; cancer-specific urine biomarkers may therefore, play an important role in diagnosis of people with long-term *S. haematobium* infections (Sotillo *et al.*, 2016).

This study was performed to establish homology of the 36 biomarker proteins evaluated in a previous study (Onile *et al.*, 2017) in other schistosome species. It was found that protein homologues of the 36 *S. mansoni* biomarker proteins are present in *S. haematobium* and *S. japonicum*. Identification of homologues of these biomarker proteins could be used for early diagnosis of schistosomiasis and schistosomiasis associated bladder cancer and could also provide targets for developing drugs or vaccines that can be effective against all species of schistosomes.

The multiple sequence alignment was done by aligning *S. mansoni* biomarker proteins with their respective identical proteins retrieved. The basic information provided by the multiple sequence alignment is the identification of conserved sequence regions which is useful in designing wet laboratory experiment to test and modify the functions of these proteins, in predicting the function and structure of these proteins.

Calreticulin-like protein of *S. mansoni* which is a Ca²⁺ binding/storage protein, being found in a number of different animal taxa, and has been considered as a novel antigen for the detection of anti-*S. mansoni* antibodies (Wang *et al.*, 2018). It has homologues in both *S. haematobium* and *S. japonicum* with many identical amino acids and conserved regions when aligned (Figure 1).

Putative actin -1 has been identified as a possible drug target for the treatment of schistosomiasis as a strong association between actin and *S. mansoni* adult worm surface membranes has been confirmed (Fernandes *et al.*, 2011). Studies have described the role of actin in enhancing the activity of praziquantel (PQZ) treatment of schistosomiasis. It is suggested that PQZ intercalates in the surface membrane lipid bi-layers, thereby inducing tegumental changes that leads to antigen exposure, including actin. This study showed that putative actin-1 protein has a homolog in other *Schistosoma* species, with many identical regions and conserved regions (Figure 2).

Phosphopyruvate hydratase is homologous to enolase protein in *S. haematobium* (Figure 3), and is implicated in autoimmune diseases; its utilization by pathogens while invading host tissue is well documented. Therefore, it is a key player in understanding the host–parasite interaction, and it offers a target for chemo- and immunotherapy (Liu *et al.*, 2014). Phosphopyruvate hydratase, elongation factor 1-alpha and putative actin-1 protein have been identified as eggshell protein markers that can induce cellular or antibody responses and may be useful schistosomiasis diagnostic candidates. They have also been identified as putative *S. mansoni* biomarker proteins (Onile *et al.*, 2017). They all have homologues in both *S. haematobium* and *S. japonicum* with many conserved regions and semi conserved regions in the multiple sequence alignment (Figure 4.10).

HSP 60 is a molecular chaperon (Ludolf *et al.*, 2014) which has a homologue in *S. haematobium*, though no significant similarity was found between the protein sequence and *S. haematobium* whole genome shotgun sequence; but no protein similar to HSP 60 was found in *S. japonicum* though it has significant similarity with *S. japonicum* whole genome shotgun sequence. The alignment of HSP 60 with its homologue in *S. haematobium* showed many conserved regions and regions where all the amino acids in the sequences were identical.

Venom allergen-like (VAL) proteins are associated with excretion/secretion products and extracellular environment of the parasite; they have been used as a trial vaccine against hookworm infections in humans (Wilbers *et al.*, 2018). The VAL protein families are abundant in different helminth species including gastrointestinal nematodes, where they are known to carry

out several roles in the infective activities of parasites (Tribolet *et al.*, 2015). Venom allergen-like (VAL) 3 was identified among the proteins secreted in the tunnels formed by destruction of epidermal cells by cercariae, during the first two hours of penetration of human skin. Later, the protein was found to have the ability to bind lipids in vitro and complement the sterol export phenotype of yeasts in vivo (Fernandes *et al.*, 2018). In this study, we showed that venom allergen-like protein has homologues in both *S. haematobium* and *S. japonicum* but with few conserved and semi-conserved regions (Figure 3).

Elongation factor 1-alpha, phosphopyruvate hydratase and histone-4 were all identified as potential *Schistosoma* biomarkers (Onile *et al.*, 2017). These proteins have been identified previously in purified eggshell fragments of *Schistosoma mansoni* and also figured as schistosome antigens which may induce cellular or antibody responses (Dvorak *et al.*, 2016). These eggshell markers may be very useful schistosomiasis diagnostic candidates rather than vaccine candidates, since such a vaccine would be likely to target the eggs and further encourage granuloma formation and pathology rather than priming the immune system against the parasite. These proteins all have homologues in other *Schistosoma* species with enormous conserved regions

Elongation factor 1-alpha has homologues in both *S. haematobium* and *S. japonicum* with many regions where the amino acids in the sequences are identical and many conserved regions occurring in the alignment of the proteins.

Lastly, of interest were the potential targets compounds for the homologues consistent in all three species. Although information from the drugbank database is often the result of testing/current testing on humans, some of the predictions could be of future use. For instance, Artesimol, which occurs often in Table 3, is being investigated for malaria treatment, as it is partly related to the Artemisinins which have been previously in small scale trials for parasitic disease treatment (<https://clinicaltrials.gov/ct2/show/NCT02653898>). A modified phosphatidylcholine was also a potential target for an ATP carrier protein (Table 3). This may make biological sense as we previously reported that changes in phosphatidylcholine metabolism was a hallmark of schistosomiasis (Adebayo *et al.*, 2018). The nutraceutical elements are also interesting as they are cheaply available if they do have any benefits.

CONCLUSION

Homology of the identified *S. mansoni* biomarker proteins was established in both *S. haematobium* and *S. japonicum*. This will help in early diagnosis of schistosomiasis and associated bladder cancer, although wet laboratory experiments are strongly indicated to serve as

confirmation to the results of this study. This study can also help to provide targets for drugs or vaccines that can be simultaneously effective against all species of schistosomes.

MATERIALS AND METHODS

Data collection

Protein sequences of the 36 schistosome biomarkers were retrieved from the Uniprot database <https://www.uniprot.org/> in FASTA format. *Schistosoma haematobium* and *S. japonicum* whole genome shotgun sequences were retrieved from GenBank <https://www.ncbi.nlm.nih.gov/genbank/> as nucleotide and they were translated into protein format using EXPASY translating tool <https://web.expasy.org/translate/>.

SMARTBLAST

The 36 *S. mansoni* biomarker proteins sequence in FASTA format were SMARTBLASTed <https://blast.ncbi.nlm.nih.gov/smartsblast/smartsBlast.cgi> to find protein sequences of other organism that are similar to the query protein sequence. The FASTA formats of protein sequences identical to each of the thirty-six protein were retrieved and used for multiple sequence alignment.

Homology determination methods

The retrieved biomarker protein sequences were BLASTed against *Schistosoma haematobium* and *Schistosoma japonicum* translated whole genome shotgun sequence to search for homologous sequences. Sequence thus obtain were then aligned using BLASTP <https://blast.ncbi.nlm.nih.gov> to show the region of similarity.

Multiple Sequence Alignment

Multiple alignments of protein sequences are important in studying sequences and providing basic information about conserved sequence region. Each of the thirty-six *S. mansoni* biomarker protein sequences were aligned with similar *S. haematobium* and *S. japonicum* protein sequences found when each of the protein was Smart Blasted. Multiple sequence alignment for each protein was done using Clustal Omega <https://www.ebi.ac.uk/Tools/msa/clustalo/> which is a multiple

sequence alignment program. The input protein sequences which were aligned were all in FASTA format.

Potential Therapeutic targets

Markers with homologues in all three species were used as query on the drugbank database(drugbank.ca) in order to identify potential therapeutic targets which were already in use in humans or being investigated.

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AAC00515.1	MILVLICFLLSYRVSGEKVWFSETPFDEKSIDGWIQSTFNGDKQGEFKIEAGKSPVNP	60
tr C4Q5I7 C4Q5I7_SCHMA	MLSILLTLLLSKYALGHEVWFSETPFNE-SIENWVQSTYNAEKQGEFKVEAGKSPVDPI	59
XP_012799776.1	MFSALLVLLLSEYALGHEVWFSETPFNE-SIEDWVQSTYNADKQGEFKVEAGKSPVDPI	59
	*: *: :*** . *:*****:* *:.*:***:.*:*****:*****:***	
AAC00515.1	DLGLKTTQDARFYGIARKISEPFSNRDKTLVLQFTVKFDKTVTCGGAYIKLLGSDIDPKT	120
tr C4Q5I7 C4Q5I7_SCHMA	DLGLKTTQDARFYGIARKISEPFSNRGKTTVLQFTVKFDKTVSCGGAYIKLLGSDIDPKK	119
XP_012799776.1	DLGLKTTQDARFYGIARKISQPFNSNRDKTMVLQFTVKFDKTVSCGGAYIKLLGSDIDPKK	119
	*****:*****:*****.* *****:*****:*****:*****:*****.	
AAC00515.1	FHGETPYKIMFGEDICGMATKRIHVIENYKGNHLLIKKDIPCKDDQKTHLYTLIVRPNS	180
tr C4Q5I7 C4Q5I7_SCHMA	FHGETPYKIMFGEDICGMATKRIHVIENYKGNHLLIKKDIPCKDDQKTHLYTLIVRPNS	179
XP_012799776.1	FHGETPYKIMFGKALV-----HSGKNHLLIKKEIPCKDDQKTHLHTLIINPNNK	167
	****:***** : .*:*****:***** ****:***:.*:*	
AAC00515.1	YEVLDVNEKVESGLLEEDWNMLAPKMIDDPNDKKPDDWEEYIIDDPNDEKPLDWDPKT	240
tr C4Q5I7 C4Q5I7_SCHMA	YEVLDVNEKVEEGSLEDDWMLPFPKIDDPNDKKPDDWVDEQFIDDPDDKKPDNDQPKT	239
XP_012799776.1	YEVLDVNEKVEEGSLEDDWMLPFPKIDDPNDKKPDDWVDEQFIDDPDDKKPDNDQPKM	227
	*****.* *:.*:.* * ***** :*:*****:.* :.*:*	
AAC00515.1	IPDMDAKKPDWDDMDGEWKRPKHNPEYKGEWSPRIENPKYKGQWKPAQIDNPDKP	300
tr C4Q5I7 C4Q5I7_SCHMA	IPDMDAKKPDWDDMDGEWEPQKDNPEYKGEWTPRRIDNPKYKGWKPQIDNPDKH	299
XP_012799776.1	ITDMDAKKPDWDDVMDGEWKPLKDNPEYKGEWTPRRIDNPKYKGWKPQIDNPDKH	287
	* ***** *****: * *.*****:*****:*****:*****:*****:*	

Figure 1 Calreticulin autoantigen homolog of *S. mansoni* (C4Q5I7); calreticulin of *S. haematobium* (XP_012799776.1) and calreticulin of *S. japonicum* (AAC00515.1) in multiple sequence alignment

Legend

“*” means that the amino acid in that column are identical in all sequence in the alignment. “:” denotes conserved regions i.e. amino acid with strongly similar properties. “.” denotes semi-conserved regions. The red color shows hydrophobic residues, blue color shows acidic residues, magenta shows basic residues, green shows hydroxyl residues while grey color show unusual amino acids. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues

tr G4VLW2 G4VLW2_SCHMA CAX69775.1	MAEEDVAALVIDNGSGMCKAGFAGDDAPRAVFP MAEEEVAALVIDNGSGMCKAGFAGDDAPRAVFP ****;	60 60
tr G4VLW2 G4VLW2_SCHMA CAX69775.1	SKRGILTLKYPIDHGVITNWDDEKIWHHTFYNELRV SKRGILTLKYPIDHGVITNWDDEKIWHHTFYNELRV *****	120 120
tr G4VLW2 G4VLW2_SCHMA CAX69775.1	TQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLD TQIMFETFNVPAMYVAIQAVLSLYASGRTTGIVLD *****	180 180
tr G4VLW2 G4VLW2_SCHMA CAX69775.1	LAGRDLTDYMMKILTERGYSFTTTAEREIVRDIKE LAGRDLTDYMMKILTERGYSFTTTAEREIVRDIKE *****;	240 240
tr G4VLW2 G4VLW2_SCHMA CAX69775.1	YELPDGQVITIGNERFRCPEALFQPSFLGMEASGI YELPDGQVITIGNERFRCPEALFQPSFLGMEASGI *****;	300 300
tr G4VLW2 G4VLW2_SCHMA CAX69775.1	SGGSTMYPGIADRMQKEISALAPSTMKIKIVAP SGGSTMYPGIADRMQKEISALAPSTMKIKIVAP *****	360 360

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Figure 2 Multiple sequence alignment of putative actin-1 of *S. mansoni* (G4VLW2) and actin 5C of *S. japonicum* (CAX75500.1)

Legend

“*” means that the amino acid in that column are identical in all sequence in the alignment. “:” denotes conserved regions i.e. amino acid with strongly similar properties. “.” denotes semi-conserved regions. The red color shows hydrophobic residues, blue color shows acidic residues, magenta shows basic residues, green shows hydroxyl residues while grey color show unusual amino acids. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues

CAX78430.1	-----MFILFALFILPNIIASDEEKDFRTTMLYLHNLHRYRNECNLKI	44
tr G4VMG4 G4VMG4_SCHMA	-----MKGEVY--DTE-----FTCEDNIKMTKG----	21
XP_012796982.1	IYMTASLTTRYQRMVPTLLLAICSMEAVF--GLNAEEEEQLMLQVHNEHRRYRSKY----	54
	: . :	
CAX78430.1	FRYPILSEEILKPLKWNKGLEEDAKKWARQCINKTEELEGRVKGKWLVGQNIIDLTTEL	104
tr G4VMG4 G4VMG4_SCHMA	INMTASSTETRYHRMEWDDELAQAAQRWSKKCNPFEEPI-GNVGKWNVGRNSVQSEL	80
XP_012796982.1	NETDIMPAAEAILQPLEWDELEAAQQRWSQKCDAYDEKPI-GSVGKFDSVGRNTAVHSEL	113
	. : * : : * : * : * : * : * : * : * : * : * : *	
CAX78430.1	YEAPKRWADGAYFYDHTNKCNNTELCNKYKRIVQRKTHVGCAGHQCENFPERKTNIVV	164
tr G4VMG4 G4VMG4_SCHMA	AEFPKCNIVVIVHVVVLLHIG---WKNRTFINTIQTSS-VVQRLGAIVTSRYVYTQIM---	133
XP_012796982.1	AEAVTYMMKESDHYDHKSDRCEPPHQCDSYKQIVQAETAYVGCYTRCPGYEYPANMLIA	173
	* . * : : * :	
CAX78430.1	-----KLN-----	189
tr G4VMG4 G4VMG4_SCHMA	-----	133
XP_012796982.1	CFYSPAVIKRQPYTDEKNCCKNVTEGQNGINEKKK	209

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Figure 4 Multiple sequence alignment of venom allergen-like (VAL) 3 protein (G4VMG4) of *S. mansoni*; peptidase inhibitor 16, partial of *S. haematobium* (XP_012796982.1) and peptidase inhibitor 16, partial of *S. japonicum* (CAX78430.1)

Legend

“*” means that the amino acid in that column are identical in all sequence in the alignment. “.” denotes conserved region i.e. amino acid with strongly similar properties. “:” denotes semi-conserved region. The red color shows hydrophobic residues, blue color shows acidic residues, magenta shows basic residues, green shows hydroxyl residues while grey color show unusual amino acids. The colors in the alignments show the locations of similarity and difference among the sequence based on the chemical nature of the amino acid residues