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Circulatory microRNAs in helminthiasis: Potent as diagnostics biomarker, its potential role and limitations

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Infections caused by helminths are responsible for severe public health problems and economic burden on continental scale. Well-timed and precise diagnosis of helminth infections is critical for taking by appropriate approaches for pathogen control. Circulating miRNAs are stable diagnostic tool for different diseases found in a variety of body fluid. As diagnostic biomarkers in infectious diseases, miRNAs detection in body fluids of helminth infected hosts is growing promptly. Uncovering miRNAs is a relatively new tool, used for early-stage detection of helminth infection from experimental or non-invasive clinical samples. miRNAs can be detected in body fluids such as serum, saliva, urine, and tissues of helminth infected host, mainly blood offering important benefits for diagnosis accurately. In this review, we discuss different characteristics of helminth parasite-derived circulating and EV miRNAs, supporting its potential uses in for helminth diagnosis and treatment efficiency.

KEYWORDS

circulatory microRNAs, biomarker, helminths, diagnosis, vesicle

Introduction

Parasitic helminths, are classified into the phylum Platyhelminthes (including trematodes and cestodes) and the phylum Nematoda (nematodes) (1). The most common parasitic infection in humans is caused by soil-transmitted helminths (STHs), filarial worms (causing agents of onchocerciasis and lymphatic filariasis) and schistosomes. According to research, STHs such as *Trichuris trichiura* and *Ascaris lumbricoides*, and hookworms, infecting over 1.5 billion people globally (2).

Schistosomiasis is a parasitic infection caused by the genus *Schistosoma*. Currently, it is existent in 78 countries, with 700 million people at risk of containing schistosomiasis (3–6). Foodborne trematodes are included *Clonorchis*, *Opisthorchis* and *Fasciola* which causes Clonorchiasis, Fascioliasis and Opisthorchiasis in human. According to WHO food borne trematodes are important causes of disability with an estimated annual total of 200,000 illnesses and more than 7,000 deaths per year (2). The World Health Organization (WHO) has already devised a strategy for controlling and eliminating helminth-related neglected tropical diseases. The most important helminth infection control strategy is mass drug administration (7).

MicroRNAs (miRNAs) are small, endogenously expressed, non-coding RNA transcripts with unique sequences that target mRNAs for post transcriptional regulation (8, 9). miRNAs have been identified in a wide range of organisms including helminths (10). Many miRNAs play important role in biological processes such as cell proliferation, growth, metabolism, and signal transduction (11). miRNA dys-regulation has also been linked to a variety of diseases, including non-communicable diseases like cancer, diabetes, kidney disease, and a variety of infectious diseases. Since last decade, the presence of miRNAs in various body fluids such as urine, serum, and plasma has been identified (12, 13). Circulating miRNAs are an effective and potential non-invasive biomarker in the pathology and prognosis of a wide range of morbidities, including infectious diseases. miRNA profiling in pathological conditions vs. healthy controls subjects can help predict disease stage (14). Extracellular vesicles (EVs), which are small membrane-bound secreted vesicles, play a role in many biological processes. EVs are present in all bodily fluids like saliva, urine, blood, serum and cerebrospinal fluid (15). In this review we demonstrated that circulating and EV derived miRNAs could be a new class of biomarkers for helminth detection and prognosis. We have briefly reviewed circulating miRNAs as diagnostic and prognostic markers for helminth infections in the following sections.

MicroRNAs in biological fluids

Several experimental studies have found extracellular/circulating miRNAs in biological fluids like plasma and serum (16), cerebrospinal fluid (17), saliva (18), breast milk (19), urine, tears, bronchial lavage, colostrum, peritoneal fluid, seminal fluid (20), and ovarian follicular fluid (21). Extracellular miRNAs exist in two populations in biological fluids. The first is found in vesicles like exosomes, micro-vesicles, and apoptotic bodies (22) while the second is associated with proteins, specifically AGO2 (23). There has been some debate over the relative abundance of these two populations. Researchers have discovered that the most of extracellular miRNAs are associated with AGO2 rather than

micro-vesicles/exosomes (23), while other study discovered that extracellular miRNAs are predominantly existent in exosomes in serum and saliva of humans (18).

Circulating miRNAs as diagnostic biomarkers

miRNA levels in the circulatory system may be altered by pathology in a relevant tissue. miRNAs, for example, may be inactively released during cell necrosis or vigorously secreted in vesicular structures during the course of liver disease (24). The small size and the establishment of a miRNA-protein complex, and their fusion into exosomes or other extracellular vesicles all contribute to their high stability in biofluids (EVs) (25). Because of the striking correlation between the status or progression of various diseases and miRNA dysregulation, circulating miRNAs have been regarded as promising targets for diagnostic biomarkers (25, 26). Circulating miRNAs have been studied for their diagnostic and predictive potential in a variety of diseases, such as cancer, liver damage, and viral infections (27, 28). A large number of miRNAs have been known in parasitic helminths, representing their importance in post-transcriptional regulation. During active helminthic infection, tissue and circulating miRNAs are dysregulated in mammalian hosts. Because helminth worm-derived miRNAs are released into the circulatory systems of mammalian hosts, they have the potential to be novel intervention targets and diagnostics for helminthiases. miRNAs are favorable therapeutic targets for parasitic helminth pathology, such as schistosome-induced liver fibrosis (10).

Furthermore, circulating miRNAs are highly stable under adverse conditions such as long-term storage, boiling, low or high pH, and multiple freeze-thaw cycles (13, 29). Previous study detected *Mycobacterium avium* subspecies paratuberculosis in bovine serum samples, and revealed that the circulating miRNA profile of samples stored for 10–15 years at -20°C was quite similar to that of fresh serum samples (stored for <1 year at -80°C) (30). Another research examined the miRNA stability in serum and plasma from healthy dogs after varying amounts of time at room temperature, and the authors demonstrated that miRNAs were highly stable when stored at room temperature for 1 h but not for 24 h (31). Because of the above-mentioned characteristics of circulating miRNAs, they have unique interest of researchers as potential diagnostic biomarkers for a variety of diseases.

Role of microRNA in various disease

miRNAs play a variety of biological roles in various processes within living organisms (32, 33). i.e., angiogenesis, metastasis, invasion, growth, differentiation, and apoptosis (34,

35). miRNAs play critical role in the initiation and advancement of various carcinomas (36, 37). A wide range of biomarkers, including microRNAs (miRNAs), tPA, and von Willebrand factor, could be used as diagnostic and therapeutic biomarkers in stroke therapy (32). Circulating miRNAs are a type of miRNA that can be used as biomarkers for prognosis, diagnosis, and treatment (38). In serum and plasma samples, intact, cell-free miRNAs that are resistant to nuclease digestion have recently been discovered. These miRNAs are circulating and quantifiable, suggesting that they could be used as noninvasive, sensitive diagnostic or progression biomarkers in a variety of diseases. MiRNAs in circulation as biomarkers for monitoring therapy response and distinguishing normal from breast cancer cells (39). A study found that circulating miR-155 levels were higher in the serum of breast cancer patients (40). According to research, miR-155 could be an encouraging biomarker for breast cancer diagnosis, as well as a predictor of treatment response (41). Circulating miRNAs in plasma and serum come into direct contact with blood cells. In 2008, the initial report on the changed levels of circulating miRNAs in hematologic cancers was conducted in patients with diffuse large B cell lymphoma (DLBCL) (12). In this study, miR-155, miR-21 and miR-210 were found to be significantly higher in cancer patients' sera than in healthy control subjects (42). Patients with chronic lymphocytic leukemia (CLL), had significantly altered circulating miRNAs (43). Seven plasma miRNAs (miR-223, miR-150, miR-92a, miR-19b, miR-320, miR-17, and miR-484,) were reported to be extremely abundant in all CLL samples tested. Furthermore, changes in circulating miR-195 or miR-20a levels were found to be the best classifiers for distinguishing CLL patients from healthy control subjects (43). Circulating miRNAs were also found in patients with liver cancers; six serum miRNAs (miR-92a, miR-1, miR-375, miR-25, miR-206, and let-7f) were found to be significantly up-regulated in hepatocellular carcinoma (HCC) samples compared to healthy controls. Furthermore, three of the six miRNAs (miR-25, let-7f, and miR-375) were shown to be capable of clearly distinguishing HCC cases from healthy control subjects (44). In a recent study, over 20 miRNAs (including miR-92a) were found to be overexpressed in the sera of HCC patients (44).

A majority of studies have shown that their dysregulation can lead to the onset and development of various diseases such as stroke, diabetes, inflammatory diseases and cardiovascular disease (45, 46). Sun et al. (47) discovered that miR-124 could be used as diagnostic and therapeutic biomarkers in stroke patients, as it is released from brain tissue plays an important role in stroke. Other miRNAs that can be used as diagnostic biomarkers in stroke patients include miR-320. miR-320 expression is significantly reduced in stroke patients. Down-regulation of this miRNA has been shown to initiate anti-apoptotic processes in CNS tissue (48). Various circulating miRNAs were tested as diagnostic biomarkers in stroke patients by Sepramaniam et al. their findings show that circulating miRNAs such as

miR-27a, miR-125b-2, miR-488, miR-422a, and miR-627 express differently in stroke patients than in healthy subjects. They revealed that these circulating miRNAs were highly expressed in stroke patients (49). Several scientists have found that miRNAs play important roles in diabetes pathogenesis (45, 50). Deregulation of several miRNAs, including miR-21, miR-15, miR-144, miR-192 and miR-150 has been linked to diabetes pathogenesis (50).

miRNAs can also be used for diagnosis of protozoan infections for humans such as Malaria, Leishmaniasis, Toxoplasmosis, and Trypanosomiasis (51, 52). In malaria infection, miRNAs facilitate invade and grow of the parasite in red blood cells via escape from immune responses and defect of opsonization by circulating macrophages (53). Chamnanchanunt et al. (54) showed lower levels of hsa-miR-451 and hsa-miR-16 in plasma and RBCs of *Plasmodium vivax* human patients than non-infected subjects. Baro et al. (55) demonstrated hsa-miR-221, hsa-miR-222, hsa-miR-24, and hsa-miR-19 were decreased in *P. vivax* patient's RBCs. Nahid et al. (56) showed *Leishmania major* could change miRNA levels in macrophages after infection of the cells with the parasite; it was identified that downregulation of hsa-let-7a expression was in 48 h after infection. In a study by Geraci et al., next-generation sequencing is applied to study the levels of miRNAs in human monocyte derived dendritic cells (DC) and macrophages (MP) infected with either *L. major* or *L. donovani* parasites. The results of the study showed that *L. donovani* has a prominent role in the upregulation of hsa-miR-21 and hsa-miR-146b-5p and proteins involved in the TGF- β signaling pathway, such as SMAD7 and TRAF6 (57).

Parasitic helminths, including trematodes, tapeworms, cestodes, and nematodes, are the most common human infectious agents in developing countries (52, 58). Approximately 1 billion people live in the slums of the continents Africa, Asia, and America, infected with one or more worms (58, 59). Therefore, identification, diagnosis, treatment, and control of worm infections should be among the most essential actions in these areas (60).

MicroRNAs; circulating miRNAs as diagnostic biomarkers for helminths infection

The emergence of parasitic helminth genome sequence data has paved the way for the identification of miRNA sequences using both experimental and computational approaches. Some miRNA sequences are conserved across species, which can be used to identify potential miRNAs through artificial intelligence (61). Documentation of miRNA populations in at least 36 parasitic helminth species (8 cestodes, 11 trematodes, and 17 nematodes, including two plant parasitic nematodes, *Bursaphelenchus xylophilus* and *Globodera pallida*) has been

accomplished using miRNA prediction tools such as miRDeep2, mireap, and miR analyzer, or through homologous mapping. 616 pre-miRNAs from *Echinococcus multilocularis*, *Schistosoma mansoni*, *Ascaris suum*, *Echinococcus granulosus*, *Brugia malayi*, and *Haemonchus contortus* have been annotated in miRBase (10). Lin-4, the first reported nematode miRNA, was discovered in free-living *Caenorhabditis elegans* 20 years ago (62). However, the first detailed investigation of parasitic nematode miRNAs was published in 2010 for a filarial nematode *B. malayi* (63), and later extended to include other parasitic nematodes (e.g., *Trichinella spiralis*, *H. contortus*, and *A. suum*). MiRNAs have been discovered in *S. japonicum*, a trematode (64) and *S. mansoni* (65), *Fasciola gigantica*, *F. hepatica* (66), and *Eurytrema pancreaticum* (67). Although the vast majority of miRNAs are endogenous, only a small number enter the blood circulation and circulate throughout the body (68). These cell-free miRNAs have been found in urine, blood plasma, and serum (25, 69). miRNAs derived from parasitic helminths are perceptible in the biofluids of their mammalian hosts and can be used to diagnose infection, even when the parasitic species do not reside in the host's circulatory system (14). The presence of parasite-derived miRNAs in the serum/plasma of the definitive host with helminth infection has increased interest in testing worm-derived miRNAs as specific biomarkers for specific helminthic infections (10). Circulating miRNAs have recently been proposed as a new class of biomarkers for diagnosing helminth infection.

Several studies on trematodes have been conducted for identification of miRNA. Schistosomiasis a neglected tropical parasitic disease associated with severe pathology, Mortality and economic loss worldwide. Cheng et al. reported schistosome-specific five miRNAs (Bantam, miR-3479, sjamiR-8185, miR-3096 and miR-10) in rabbits infested with *S. japonicum*. Four of these five miRNAs were found to be significantly abundant in the plasma of mice infected with *S. japonicum*, implying that these miRNAs could be used as a biomarker for schistosomiasis diagnosis (70). A six miRNA candidates were validated with serum samples from a human cohort in a schistosomiasis endemic area of the Philippines, which showed that two parasite derived miRNAs (sjamiR-2b-5p and sjamiR-2c-5p) could be detected in infected individuals with a moderate diagnostic performance (68). In low-dose cercarial infection models, it was confirmed that sjamiR-277 and sjamiR-3479-3p, but not sjamiR-bantam, could be reliably detected in mouse serum, regardless of mouse strain. We were able to demonstrate a significant correlation between sjamiR-3479-3p, sjamiR-277, levels in serum and both the degree of fibrosis and egg burden in the liver using these precise methods (71). In low doses, these parasite-derived circulating miRNAs demonstrated additional potential as biomarkers for early detection of *S. japonicum* infection. According to new evidence, altered host miRNAs can be used to diagnose *S. japonicum* liver infection. Elevated levels of miR-223 in serum samples

from human, rabbit, buffalo, and mouse hosts could be used to detect *S. japonicum* infection (72). In BALB/c mice infected with *S. japonicum*, serum levels of mmu-miR-21, mmu-miR-122 and mmu-miR-34a were significantly higher (73). Furthermore, several additional studies demonstrated that parasite-derived miRNAs (miR-3479-3p, miR-277, and bantam) could detect individuals infected with *S. haematobium* or *S. mansoni* using real time qPCR in low and high infection intensity sites (74, 75). Circulating miRNAs were also identified in buffaloes infected with *F. gigantica*, a tropical liver fluke and the cause of fascioliasis; four worm-specific miRNAs, fgi-miR-87, fgi-miR-71, fgi-miR-124 and, the novel miR-1, were identified in the sera of infected animals by deep sequencing (76).

miRNAs derived from various nematode species have been found circulating in host tissue and the circulatory system. Tritten et al. reported the existence of miRNA (nematode-derived) candidates in the plasma of dogs infected with *Dirofilaria immitis*, a filarial heartworm, using a veterinary model (77). Similarly, in two independent studies, a panel of *Onchocerca*-derived mature miRNAs including miR-100a/c/d, miR-81, and miR-71 were identified in the nodule fluid and the plasma from bovine infected with *O. ochengi* (78). Furthermore, miRNA candidates of potential nematode origin, such as miR-36, miR-100a/d, miR-92, and lin-4, were discovered in the plasma of baboons infected with the 'eye worm' *Loa loa* (78). miRNAs derived from nematodes, such as lin-4, miR-100a/d, and miR-71, were also discovered in the serum or plasma of people infected with *O. volvulus*, the causative agent of onchocerciasis (79). Silakit et al. revealed that miR-192 levels were significantly higher in cholangiocarcinoma (CCA) patients' serum than in healthy donors. Furthermore, hsa-miR-192 expression levels were found to be elevated in both patient-derived CCA liver tissues and *Opisthorchis viverrini*-induced CCA liver tissues from a hamster model (80). Strikingly, plasma miRNA profiling disclosed that eight miRNAs including hsa-miR-885-5p, hsa-miR-505-3p, hsa-miR-483-5p, hsa-miR-92b-3p, hsa-miR-874, hsa-miR-1307-3, hsa-miR-1275, and hsa-miR-320b, associated with Intrahepatic cholangiocarcinoma (ICC) are induced by *O. viverrini*, developing the basis of a circulating miRNA-based biomarker panel for ICC (81). A study evaluating the circulating miRNAs released by *Angiostrongylus cantonensis* (the cause of eosinophilic meningoencephalitis) as potential biomarkers of infection, found that the level of acamiR-146a in serum was significantly higher in *A. cantonensis*-infected mice compared with uninfected control animals (82).

Cestode-derived miRNA, emu-miR-277 and emu-miR-10 were found in the serum of all *E. multilocularis* infected mice. Furthermore, gene expression of mmu-miR-146a-5p, mmu-miR-107-3p, mmu-miR-103-3p, mmu-miR-339-5p, and mmu-miR-21a-3p was increased in infected mice sera while mmu-miR-222-3p was decreased (83). The study reported by Alizadeh et al. (84) that the levels of two circulating worm-specific miRNAs (egr-miR-71 and egr-let-7) were detectable in

the plasma of patients infected with *E. granulosus* compared with uninfected individuals. Here in Table 1, we summarize some circulating miRNAs of helminth infection.

MicroRNAs detect from extracellular vesicle

Extracellular vehicles (EVs), which are small membrane-bound secreted vesicles, play a role in many biological processes (89). According to mounting evidence, exosomes and other extracellular structures (e.g., microvesicles) containing specific proteins, miRNA or miRNA-like molecules are actively released by parasites (90). EVs help in pathogens spread and play a regulatory role in the host immune system. Exosomes have been isolated from several body fluids, including serum, urine and saliva (91). EV molecules as biomarkers for the diagnosis and prognosis of diseases (92). However, there is still much to be explored regarding the applications of helminth-derived EVs, such as their potential in diagnosis, as therapeutics (93). Recent studies have identified that EVs isolated and characterized from helminth parasites such as proteomic investigation of *S. mansoni* EVs revealed that these are potential vaccine candidates (94); exosomes from *S. japonicum* induce M1-type immune-activity in macrophages *in vitro* (95), *Echinostoma caproni*

and *F. hepatica* EVs are internalized into gut host cells (96), and miRNAs are related with *F. hepatica* EVs (97). Exosome components are being studied for their potential value as a source of biomarkers for cancer and other diseases (98). The discovery of *S. mansoni* EVs and the characterization of their cargo via combinatorial protein/sncRNA characterization points to the identification of an important new participant in the complex biology underlying schistosome/host interactions. Further research into the function and stability of intra- and extra-vesicular sncRNA components, as well as the role of these *S. mansoni* EVs, could lead to the development of novel schistosomiasis diagnostics or interventions (94). EVs secreted by *O. viverrini* have been shown to induce a tumorigenic phenotype and proinflammatory in human cholangiocytes (99). Notably, Bernal et al. (100) were the first to discover miRNAs in exosomes secreted by *Dicrocoelium dendriticum*, a ruminant trematode. Ancarola et al. reported seven unique miRNAs (miR-190-5p, let-7-5p, miR-4989-3p, miR-61-3p, miR-219-5p, miR-71-5p, and miR-277-3p), in *Taenia crassiceps* vesicles, but only let-7-5p was described in *Mesocestoides corti* EVs. These microRNAs are not only useful for basic cestode biology, but also for the rational search for new diagnostic targets (101). According to Zhu et al. (102) miRNA-containing EVs can be released by *S. japonicum* eggs, and the EVs can transfer their cargo to recipient cells *in vitro*. Another recent study found

TABLE 1 Circulatory miRNAs detected from infected hosts.

Pathogens	Circulating miRNA	Sample	References
<i>Loa Loa</i>	miR-36, miR-92, lin-4, miR-100a/d	Baboon plasma	(79)
<i>Onchocerca ochengi</i>	miR-100a, miR-81	Cow plasma	(79)
<i>O. ochengi</i>	miR-71, bantam -a/b/c, miR-100a/c/d miR-86-5p,	Cattle nodule fluids	(78)
<i>S. japonicum</i>	miR-150-5p, miR-146a-5p and let-7d-5p, let-7a-5p	Mouse/human serum	(85)
<i>O. volvulus</i>	miR-87-3p, miR-100a/d, miR-71, bantam-a, and Lin-4,	Human plasma and serum	(78)
<i>O. volvulus</i>	ov-miR71-23nt, ov miR-100d, ov-bantam-a, ov miR71-22nt,	human serum	(86)
	bma-miR-71, bma-miR-236-1, and ov-miR-87-3p		
<i>Litomosoides sigmodontis</i>	miR-71, miR-86, bantam-a/b, miR-100a/b/c,	Mouse serum	(87)
<i>O. viverrini</i>	has-miR-192	Human Serum	(80)
<i>D. immitis</i>	miR-34, miR-71, miR-228 and miR-100a/c/d,	Dog plasma	(77)
<i>S. mansoni</i>	sma-bantam, sma-miR-3479-3p and Sma-miR-277,	Mouse/human serum	(75)
<i>S. japonicum</i>	sja- bantam, sja-miR-3479-3p and Sja-miR-277,	Mouse serum/ rabbit serum	(70, 71)
<i>O. viverrini</i>	hsa-miR-1275, hsa-miR-320b, hsa-miR-483-5p,	Human Plasma	(81)
	hsa-miR-505-3p, hsa-miR-92b-3p, hsa-miR-1307-3,		
	hsa-miR-874, and hsa-miR-885-5p		
<i>S. japonicum</i>	mmu-miR-134-5p, [mmu-let-7b-3p, mmu-miR-1194,	Mouse Plasma	(88)
	mmu-miR-1981-3p, mmu-miR-210-5p], [mmu-miR-542-3p		
	and mmu-miR-92a-2-5p]		
<i>S. japonicum</i>	mmu-miR-21, mmu-miR-34a and mmu-miR-122,	Mouse/human Serum	(71)
<i>S. japonicum</i>	sja-miR-2b-5p and sja-miR-2c-5p	human serum	(68)
<i>E. multilocularis</i>	emu-miR-227 and emu-miR-10	Mice serum	(83)
<i>S. japonicum</i>	miR-223	Human, Rabbit, Buffalo and Mouse serum	(72)

TABLE 2 MicroRNA detect from extracellular vesicle.

Pathogens	miRNAs detected in extracellular vesicle	Sample	References
<i>S. japonicum</i>	<i>miR-125b</i> and <i>bantam</i>	Worm	(103)
<i>F. hepatica</i>	<i>miR-1</i> , <i>miR-125b</i> , <i>miR-71a</i> , <i>miR-190</i> and <i>miR-87</i>	Exosome like vesicle from adult worms	(97)
<i>D. dendriticum</i>	<i>miR61</i> , <i>miR-190</i> , <i>miR-71</i> , <i>miR-2a-3p</i> and <i>let-7</i>	Exosome from adult worms	(100)
<i>Heligmosomoides polygyrus</i>	<i>miR-7</i> , <i>miR-87</i> , <i>bantam</i> , <i>Lin-4</i> , <i>miR-71</i> and <i>miR-100</i>	Adult worms' Secretory product	(87)
<i>Trichuris suis</i>	miRNA-size RNA	L1-stage larvae Secretory product	(104)
<i>S. mansoni</i>	<i>Sma-miR-new-204</i> , <i>Sma-miR-new-176</i> , <i>Sma-miR-new-271</i> , <i>Sma-miR-new-215</i> , and <i>Sma-miR-new-270</i>	Schistosomula	(94)
<i>T. crassiceps</i>	<i>miR-71-5p</i> , <i>miR-219-5p</i> , <i>miR-4989-3p</i> , <i>miR-277-3p</i> , <i>miR-61-3p</i> , <i>miR-190-5p</i> , and <i>let-7-5p</i>	Adult worm	(101)
<i>M. corti</i>	<i>let-7-5p</i>	Adult worm	(101)
<i>S. japonicum</i>	<i>sja-miR-71a</i> , <i>sja-miR-36-3p</i> , <i>sja-bantam</i> , <i>sja-miR-2a-3p</i> , and <i>sja-miR-10-3p</i>	Egg EVs	(102)

miRNA, Y RNA, and an Argonaute protein in exosomes secreted by *Heligmosomoides polygyrus*, a rodent parasitic nematode (87). It has been discovered that these *H. polygyrus* miRNA signatures may play a role in modulating host innate immunity (87). Furthermore, miRNAs enriched in EVs secreted by *F. hepatica* have been linked to immune-regulatory function, tissue growth, and cancer (97). Here we summarize some miRNAs detected from extracellular vesicle in Table 2.

Limitation of circulatory miRNA biomarker

As we discussed above, circulating miRNAs are becoming potential non-invasive biomarkers for the diagnosis of helminths infection. Despite there are still challenges to overcome before clinical application. Both limitations and potentials of miRNAs should be taken into account when diagnosing helminth infections. Studies had shown that although the use of biomarkers was a practical and unique idea in diagnosing diseases, the necessary and sufficient conditions for its use must be provided, including storage conditions of samples correct selection of primers and type of miRNAs, correct data analysis, and the use of experienced people (72, 105). miRNAs can be detected using various specific and sensitive approaches, including Northern blot Analysis (106), *in situ* hybridization (107), real-time PCR (108), miRNA microarray (109) and next-generation sequencing (110), and some of them are already used as diagnostic or prognostic markers. This demonstrates their utility in both clinical and personalized medicine. However, it has several limitations, such as the high cost of the instrumentation required for thermal cycling and signal detection, and the need for well-equipped laboratories (7). miRNAs biomarkers has the potential to address the shortcomings of other diagnostic tests,

including cross-corrections, false negatives, low sensitivity, and specificity (111, 112). In addition, whereas the costs for developing diagnostic tools targeting circulating miRNA and EV components such as sncRNAs and proteins are high, expenditure could be reduced substantially if multiplex or high-through-put assays targeting multiple helminths and/or non-helminth pathogens are developed for simultaneous application (113).

Conclusion

In the current situation the helminth infection has become more prevalent, numerous strategies with high sensitivity and specificity have been developed. The diagnosis of helminth can be using diagnostic methods like PCR, qPCR, serology, and next-generation sequencing that target the miRNA and worm EVs. The detection of miRNA may be a feasible substitute to the in-use conventional measures which involve the use of multiple blood, serum, saliva, urine samples for the detection of helminth infection in any stages. Circulating miRNAs have the potential to serve as non-invasive biomarkers for the early detection of helminth infection due to their accessibility and long-term stability. Extracellular/circulating miRNAs are now recognized as important players in intercellular communication as well as biomarkers for diseases. Further research on circulating miRNA profiles would broaden helminth biomarker research and enable the development of diagnostic strategies and examinations based on a sensitive and simple test.

Author contributions

HU collected the data and prepared draft. All authors have shared in all aspects and

read and agreed to the published version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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