UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE CIÊNCIAS BÁSICAS DA SAÚDE
CURSO DE ESPECIALIZAÇÃO EM MICROBIOLOGIA CLÍNICA

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VESÍCULAS EXTRACELULARES EM DOENÇAS PARASITÁRIAS: DA PATOGÊNESE À FUTURAS FERRAMENTAS DIAGNÓSTICAS

Porto Alegre
2023

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Trabalho de conclusão de curso de especialização apresentado ao Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Especialista em Microbiologia Clínica.

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Porto Alegre

2023

CIP - Catalogação na Publicação

Almeida de Menezes, Saulo
Vesículas extracelulares em doenças parasitárias: da patogênese à futuras ferramentas diagnósticas / Saulo Almeida de Menezes. -- 2023.
78 f.
Orientadora: Tiana Tasca.

Trabalho de conclusão de curso (Especialização) -- Universidade Federal do Rio Grande do Sul, Instituto de Ciências Básicas da Saúde, Microbiologia Clínica, Porto Alegre, BR-RS, 2023.

1. Vesículas extracelulares. 2. Biomarcadores. 3. Exossomos. 4. Microvesículas. 5. Protozoários. I. Tasca, Tiana, orient. II. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

AGRADECIMENTOS

Aos meus pais, Vanuza e Vilmar, agradeço por todo amor que me dedicam e por toda educação que me proporcionaram. Em especial minha Mainha, por sempre torcer e acreditar em mim quando decido embarcar em novas empreitadas. Agradeço aos meus irmãos, Paulo Vitor, Paulo Vannuryo e Samuel, por todo apoio, amizade e companheirismo durante minha trajetória.

À minha querida orientadora Profª. Dra. Tiana Tasca, pelo incentivo, ensinamentos, convívio, confiança e, especialmente, pela amizade construída. Tu és um exemplo de ética e profissionalismo que consegue transmitir todo amor pelo que faz por meio da tua empolgação constante. Obrigado pela paciência e por estar sempre acreditando em mim.

Às amigas queridas do Curso de Especialização em Microbiologia Clínica – Manoela, Joana, Daniela, Raquel, Michele, Cristiane, Mariana, Noara e Rejane. Obrigado pela troca de conhecimentos, momentos de descontração e compartilhamento de frustrações principalmente nesta etapa de finalização do nosso curso.

Aos queridos amigos do Grupo de Pesquisa em Tricomonas – Fernanda, Giulia, Graziela e Magno. Obrigado por todo apoio, troca de conhecimentos, cafés da tarde e companheirismo. Obrigado também por todos os conselhos úteis, bem como palavras motivacionais e puxões de orelha. Tudo teria sido muito exaustivo sem vocês por perto.

À Universidade Federal do Rio Grande do Sul, especialmente ao Instituto de Ciências Básicas da Saúde e ao Curso de Especialização em Microbiologia Clínica, por me oportunizar um ensino de excelência.

Por fim, a todos aqueles que contribuíram, direta ou indiretamente, para a realização deste trabalho, o meu sincero agradecimento.

RESUMO

As doenças parasitárias continuam sendo de grande preocupação para saúde pública, especialmente entre indivíduos de países subdesenvolvidos com baixo status socioeconômico. De acordo com a Organização Mundial da Saúde, 12 das 20 doenças classificadas como Doenças Tropicais Negligenciadas têm origem parasitária e afetam desproporcionalmente crianças e mulheres, trazendo consequências econômicas, sociais e de saúde para mais de 1 bilhão de pessoas em todo o mundo. Historicamente, o diagnóstico parasitológico tem sido baseado na identificação morfológica por meio da microscopia de luz e colorações histoquímicas. No entanto, existem desafios no fornecimento de diagnósticos rápidos, confiáveis e robustos para várias parasitoses, e além disso a seleção do teste mais adequado ainda é um obstáculo importante no combate a essas doenças. Descobertas recentes demonstram que organismos parasitários, células hospedeiras infectadas e células hospedeiras estimuladas por antígenos parasitários podem liberar vesículas extracelulares que desempenham papel-chave na interação parasito-hospedeiro, na evasão imunológica e nos processos relacionados à infecção do hospedeiro. As vesículas extracelulares são estruturas delimitadas por membrana que são liberadas no espaço extracelular e carregam diversas biomoléculas, incluindo proteínas, lipídios, ácidos nucleicos e metabólitos, influenciando diretamente as células-alvo. Essas estruturas têm recebido atenção considerável dada a sua importância na interação patógeno-hospedeiro e potenciais aplicações na descoberta de biomarcadores diagnósticos. As vesículas extracelulares podem ser detectadas em vários fluidos corporais, incluindo sangue, saliva, líquido amniótico e urina. Compreender a composição das vesículas extracelulares e suas alterações durante o processo infeccioso, pode ser utilizado no desenvolvimento de biomarcadores para o diagnóstico precoce de infecções, assim como identificar alvos imunológicos. Portanto, esta revisão de literatura tem como objetivo elucidar o papel das vesículas extracelulares de protozoários nas interações parasito-hospedeiro e sumarizar seu conteúdo molecular, fornecendo informações para aquisição de novas ferramentas que possam ser utilizadas no diagnóstico de doenças parasitárias como a malária, doença de Chagas, leishmaniose, tricomoníase, giardíase, amebíase e encefalite amebiana granulomatosa.

Palavras-chave: Vesículas extracelulares; biomarcadores; exossomos; microvesículas; protozoários.

ABSTRACT

Parasitic diseases remain a significant public health concern, particularly among individuals in underdeveloped countries with low socioeconomic status. According to the World Health Organization, 12 of the 20 diseases classified as Neglected Tropical Diseases are of parasitic origin and disproportionately affect children and women, bringing economic, social, and health consequences to more than 1 billion people worldwide. Historically, parasitological diagnosis relied on morphological identification through light microscopy and histochemical staining. However, challenges persist in providing rapid, reliable, and robust diagnoses for various parasites, and selecting the most appropriate test remains a major hurdle in combating these diseases. Recent findings have revealed that parasitic organisms, infected host cells, and host cells stimulated by parasitic antigens can release extracellular vesicles that play key roles in host-parasite interactions, immune evasion, and in processes related to host infection. Extracellular vesicles are membrane-bound structures discharged into the extracellular space that can carry diverse biomolecules including proteins, lipids, nucleic acids, and metabolites, directly influencing target cells. Given their significance in host-pathogen interactions and potential applications in diagnostic biomarker discovery, these structures have gained considerable attention. The extracellular vesicles can be detected in various bodily fluids, including blood, saliva, amniotic fluid, and urine. Understanding the composition of extracellular vesicles and its alterations during infection can be harnessed to develop biomarkers for early infection diagnosis and identify immunological targets. Therefore, this literature review aimed to elucidate the role of protozoa extracellular vesicles in host-parasite interactions and summarize their cargo, providing information for acquisition of novel diagnostic tools in malaria, Chagas disease, leishmaniasis, trichomoniasis, giardiasis, amebiasis, and granulomatous amebic encephalitis.

Keywords: Extracellular vesicles; biomarkers; exosomes; microvesicles; protozoa.

SUMÁRIO

1 INTRODUÇÃO

As doenças parasitárias são um grupo de enfermidades causadas por helmintos, protozoários e ectoparasitos, que possuem grande impacto nos trópicos e subtrópicos, principalmente entre pessoas com baixo nível socioeconômico. Apesar dos avanços no conhecimento e na prática médica, as doenças parasitárias continuam sendo um fardo significativo para a saúde global[1]. É estimado que a malária tenha causado a morte de cerca de 627 mil pessoas em 2020, sendo 96% dos casos na África e 80% em crianças menores de 5 anos[2]. Além da malária, 12 das 20 condições classificadas pela Organização Mundial da Saúde (OMS) como Doenças Tropicais Negligenciadas (DTNs) são de origem parasitária. As DTNs afetam desproporcionalmente crianças e mulheres e trazem consequências econômicas, sociais e sanitárias para mais de 1 bilhão de pessoas ao redor do mundo e merecem especial atenção dos órgãos de saúde pública[3].

A malária é uma doença causada por protozoários do filo Apicomplexa que pertencem ao gênero *Plasmodium* e são transmitidos através da picada da fêmea de mosquitos do gênero *Anopheles*. Existem seis espécies capazes de infectar o ser humano, incluindo *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium knowlesi*, *Plasmodium malariae* e *Plasmodium cynomolgi*, que passam por 10 ou mais estágios morfológicos durante seu ciclo evolutivo. No hospedeiro, apenas um pequeno número desses estágios provoca sintomas clínicos e a grande maioria dos pacientes infectados produz poucos (ou nenhum) sintomas. Os sintomas da doença incluem, por exemplo, febre, anemia, coma e é resultado da biologia do parasito em conjunto com a resposta fisiopatológica humana[4,5].

As leishmanioses são um complexo de doenças causadas por mais de 20 espécies de protozoários do gênero *Leishmania*. Cerca de 700.000 a 1 milhão de novos casos de leishmaniose ocorrem anualmente no mundo, resultando em até 30.000 mortes. Além disso, estima-se que 350 milhões de pessoas vivem em regiões de risco em 98 países[6]. A transmissão dos parasitos é mediada por mosquitos flebotomíneos e pode seguir um ciclo antroponótico ou zoonótico que também varia conforme a região geográfica. O surgimento dos sintomas na leishmaniose é dependente da interação complexa entre a espécie de *Leishmania* e a resposta imune do hospedeiro. A infecção pode ser assintomática ou ainda se manifestar como doença cutânea, mucocutânea ou a forma visceral que pode ser letal se não tratada[7,8].

A doença de Chagas (DC) ou tripanossomíase americana é uma doença tropical transmitida por vetores e causada pelo protozoário *Trypanosoma cruzi*. Com o aumento do

processo migratório, a DC que antes era endêmica das américas atualmente infecta 8 milhões de pessoas ao redor do mundo[9]. Durante a fase aguda da infecção a maioria dos pacientes é assintomático, enquanto a fase crônica sintomática é observada anos depois, com cerca de 30% evoluindo para complicações cardíacas e digestivas que ameaçam a vida. A cardiomiopatia chagásica é a principal causa de cardiomiopatia não isquêmica na América Latina e afeta 30% dos pacientes infectados[10].

A giardíase é uma das principais doenças diarreicas em todo o mundo, com mais de 300 milhões de casos por ano, principalmente em países de baixa renda e em desenvolvimento[11]. A infecção é causada pelo protozoário entérico e extracelular *Giardia duodenalis* (sinônimos *Giardia lamblia* e *Giardia intestinalis*). Apesar da maioria dos casos serem assintomáticos, a giardíase pode cursar com diarreia aquosa, esteatorreia, náusea, dor abdominal, vômito e perda de peso. Além disso, a principal consequência da colonização pelo parasito é a má absorção de nutrientes que pode trazer prejuízos substanciais no desenvolvimento de crianças acometidas[12]. Pouco é conhecido sobre os mecanismos associados às síndromes pós-giardíase, mas a infecção por *Giardia* também pode resultar em síndrome do intestino irritável e alergias alimentares[13].

A tricomoníase é a infecção sexualmente transmissível não-viral mais comum no mundo, causada pelo patógeno extracelular *Trichomonas vaginalis*. Cerca de 156 milhões de novos casos são registrados por ano e apesar de não ser uma doença de notificação obrigatória sua incidência é maior que a de outras infecções que fazem parte deste grupo, como clamídia, sífilis e gonorreia[14]. Atualmente 80% das infecções são assintomáticas[15], o que viabiliza a colonização crônica do trato urogenital de homens e mulheres, trazendo complicações de saúde como infertilidade, doença inflamatória pélvica, distúrbios gestacionais e predisposição ao câncer cervical e de próstata[16]. Ainda, existe uma correlação positiva entre a infecção por *T. vaginalis* e o aumento da aquisição e transmissão do HIV[17,18].

A amebíase é uma infecção negligenciada e um grande problema de saúde pública para os países em desenvolvimento, acometendo 50 milhões de pessoas por ano e levando a cerca de 100.000 mortes[19]. O agente etiológico da enfermidade são protozoários extracelulares não-flagelados da espécie *Entamoeba histolytica* que causam quadros de diarreia em regiões com superlotação, falta de saneamento e falta de abastecimento de água potável[20]. Apesar da maior parte das infecções serem assintomáticas, o parasito pode invadir a mucosa intestinal e causar dor abdominal, diarreia, disenteria, colite invasiva e perda de peso. Além disso, alguns indivíduos manifestam os sintomas extraintestinais, como abscessos hepáticos, pulmonares ou cerebrais, o que leva a riscos graves e até fatais[21].

As amebas do gênero *Acanthamoeba* são microrganismos cosmopolitas que podem viver como organismos de vida livre em solo, água e ar, ou como parasitos quando dentro dos tecidos do hospedeiro[22]. Esses protozoários são os agentes etiológicos da encefalite amebiana granulomatosa e da ceratite por *Acanthamoeba* em indivíduos hígidos. As infecções por *Acanthamoeba* constituem-se como um sério risco à saúde humana, porque apesar da baixa ocorrência mundial apresenta alta taxa de mortalidade, principalmente entre indivíduos imunocomprometidos[23,24]. A esses fatores, soma-se a falta de recursos para o diagnóstico e tratamento direcionado para a infecção, associado principalmente ao conhecimento insuficiente da patogênese, fisiopatologia e mecanismos de resposta imune do hospedeiro contra os antígenos de *Acanthamoeba*[25].

A identificação morfológica dos estágios do ciclo de vida dos parasitos usando microscopia ótica e colorações histoquímicas tem sido a base do diagnóstico parasitológico por décadas. Contudo, apesar de altamente útil em regiões de baixa renda e com alta carga parasitária, apresenta a problemática da falta de clínicos experientes para realização de diagnósticos rápidos e precisos[26,27]. O advento da biologia molecular e sua posterior implementação no diagnóstico parasitológico possibilitou um grande aumento da capacidade de detectar e identificar simultaneamente vários organismos parasitários em amostras clínicas e nos vetores naturais. Ainda assim, a indisponibilidade de diagnósticos rápidos, confiáveis e robustos para muitas parasitoses e a escolha do teste classificado como mais adequado para outras representam grandes desafios[28]. Dentro desse contexto, vesículas extracelulares com seus papéis fundamentais na comunicação parasito-hospedeiro e propriedades imunomoduladoras, representam potenciais ferramentas para o diagnóstico de doenças parasitárias.

O termo genérico vesículas extracelulares (VEs) se refere a um grupo heterogêneo de estruturas envoltas por bicamada lipídica derivadas dos mais diversos tipos celulares. A produção de VEs constitui um processo bastante conservado em todos os domínios da vida, incluindo bactérias, arqueias e eucariotos[29]. Inicialmente vistas apenas como “poeira celular”, hoje sabe-se que são muito importantes na sinalização de processos celulares fisiológicos e patológicos[30]. As VEs podem carregar diferentes moléculas, incluindo proteínas, lipídios, DNA, RNA mensageiro e microRNAs, que têm implicações diretas na célula-alvo[31]. Assim, as VEs interagem com as células-alvo por contato e posterior fusão com a membrana plasmática, o que permite a integração de proteínas transmembrana e a liberação de seu conteúdo na célula-alvo[32]. As VEs podem ser encontradas em quase todos os fluidos corporais, incluindo sangue,

saliva, fluido amniótico e urina, e também podem ser coletadas a partir do sobrenadante de culturas celulares[33,34].

Diversos estudos mostram que uma quantidade substancial de moléculas parasitárias é transportada através das VEs liberadas diretamente por parasitos[35,36], células hospedeiras infectadas por parasitos[37] e células hospedeiras estimuladas por antígenos parasitários[38], tornando-as ferramentas em potencial para auxiliar no diagnóstico de doenças parasitárias. Assim, a presente revisão tem como objetivo sumarizar o conhecimento disponível acerca das VEs de protozoários de importância clínica com relação ao seu papel na interação parasito-hospedeiro e potencial para servir como biomarcadores diagnósticos relevantes e eficazes.

1.1 OBJETIVOS

1.1.1 Objetivo geral

Revisar a literatura sobre as vesículas extracelulares de protozoários de importância clínica e verificar seu potencial como possíveis biomarcadores de aplicação no diagnóstico de doenças parasitárias causadas por *Plasmodium* spp., *Leishmania* spp., *Trypanosoma cruzi*, *Giardia duodenalis*, *Trichomonas vaginalis*, *Entamoeba histolytica* e *Acanthamoeba* spp.

1.1.2 Objetivos específicos

a) Revisar o papel das vesículas extracelulares na comunicação parasito-hospedeiro das doenças parasitárias: leishmaniose, doença de Chagas, malária, giardíase, tricomoníase, amebíase e encefalite amebiana primária;
b) Compilar o conteúdo biomolecular das vesículas extracelulares dos agentes infecciosos causadores de parasitoses, a fim de servir de instrumento para desenvolvimento de novas ferramentas que atuem no diagnóstico dessas infecções;
c) Abordar a importância da aquisição de novos métodos para o diagnóstico de doenças parasitárias para controlar, direcionar a terapêutica e limitar a propagação dos agentes parasitários.

3 CONCLUSÃO E PERSPECTIVAS

Os resultados obtidos no desenvolvimento deste trabalho de conclusão de curso permitem as seguintes conclusões:

- Os parasitos se comunicam com os hospedeiros por meio de vesículas extracelulares e estas podem oferecer informações importantes sobre novas moléculas específicas como alvos potenciais para o diagnóstico de doenças parasitárias nos próximos anos;

- Apesar dos estudos fornecerem evidências de abordagens baseadas em vesículas extracelulares para auxiliar no diagnóstico da malária, doença de Chagas e leishmaniose, ainda há uma lacuna quanto à capacidade de detecção dessas estruturas em amostras de pacientes com giardíase, tricomoníase, amebíase e encefalite amebiana granulomatosa;

- O desenvolvimento de painéis diagnósticos usando vesículas extracelulares com moléculas específicas de parasitos pode favorecer a intervenção clínica, promovendo um prognóstico favorável dos pacientes;

- Este estudo serve como base para guiar futuras pesquisas de desenvolvimento de novos métodos diagnósticos destinados a controlar, direcionar a terapêutica e limitar a propagação dos agentes parasitários.

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MICROBES AND INFECTION
A Journal on Infectious Agents and Host Defenses

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ISSN: 1286-4579

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Microbes and Infection publishes articles on human pathogens or pathogens of model systems. However, articles on other microbes can be published if they contribute to our understanding of basic mechanisms of host-pathogen interactions. Purely descriptive and preliminary studies are discouraged.

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