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New biomarkers in the diagnosis of parasitic diseases: The example of *Cryptosporidium spp*. and *Giardia duodenalis*

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ABSTRACT

Parasitic diseases are one of the most common infections in the world and cause millions of illnesses and deaths each year. In the past, most of these infections were predominantly associated with tropical or subtropical regions. However, today, factors such as changes in climate and vector ecology, significant increases in international travel, human and animal migrations, and the increase in the number of animals that serve as mechanical and biological vectors of parasites have caused some parasitic diseases to become more prominent worldwide. This situation forces scientists to search for new and rapid biomarkers for early diagnosis of parasitic diseases. Biomarkers are biological indicators used to objectively measure and evaluate an organism's normal biological processes, disease processes or responses to therapeutic interventions. Biomarkers, also defined as changes in the constituents of tissues or body fluids, provide us with various parameters for the homogeneous classification of a disease and disease-related risk factors and can contribute to our basic knowledge of the underlying pathogenesis of the disease. Today, although variables such as clinical symptoms, clinical history, travel history and geographical location of patients are important in the detection and diagnosis of parasitic diseases, the diagnosis of these diseases is primarily based on various laboratory methods (such as microscopy and molecular methods). Besides molecular techniques, the discovery of new biomarkers using tissues or biological fluids from hosts infected with parasitic agents is attracting attention. *Cryptosporidium* spp. and *Giardia duodenalis*, an intestinal protozoan pathogen, are the leading causes of growth deficiency and even death in children and diarrhea in healthy adults. Therefore, the detection of these two pathogens has become a high priority, both to prevent potential outbreaks and to prevent the devastation they can cause in sick individuals. This review aims to draw attention to new biomarkers that are or could be used in the diagnosis of *Cryptosporidium* spp. and *Giardia duodenlis* protozoan parasites.

Keywords: Biomarkers, *Cryptosporidium* spp, *Giardia duodenlis*, diagnosis, rapid tests

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INTRODUCTION

Parasitic diseases are an important public health problem, especially in developing countries, as they are found all over the world. In particular, low socioeconomic status and poor hygiene conditions increase the risk of individuals contracting parasitic infections [1]. For example, malaria is one of the parasitic diseases that causes the most deaths in the world, especially in Africa [2]. *T. gondii* infections have been associated with various neurological diseases, according to recent studies [3]. According to the World Health Organization (WHO), 12 of the 20 diseases classified as neglected tropical diseases are of parasitic origin. Intestinal parasites affect approximately 3.5 billion people worldwide, with 450 million experiencing symptomatic disease and an annual mortality rate exceeding 200,000 [4,5]. Intestinal parasites continue to be one of the important health problems in Türkiye. Epidemiological studies have shown that there is an increase in the incidence of intestinal parasites from the east to the west of Türkiye [6]. Although significant advances have been made in the diagnosis, control and treatment of parasitic diseases, failures in diagnosis and treatment continue today [7]. Furthermore, although these diseases are more common, treatment options are limited and pharmaceutical industries are known not to invest in the development of new antiparasitic drugs [8]. This situation highlights the urgent need for new biomarkers for early detection of parasitic infections, increasing diagnostic and prognostic capabilities, and monitoring the disease.

Parasitological diagnosis has long been based on the identification of life stages of parasites through morphological analysis using light microscopy and histochemical staining. Although these methods are valuable in low-income areas where parasite burden is high, they face difficulties in diagnosing parasitic agents due to the lack of experienced health workers who can make rapid and accurate diagnoses. High sensitivity and specificity rates can be achieved with molecular methods, but this method also has its difficulties. They are laborious and time-consuming methods that are prone to misdiagnosis and errors because they require experienced technicians [7,9].

Biomarkers are defined as biological indicators used to objectively measure and evaluate an organism's normal biological processes, disease processes, or responses to therapeutic interventions [10]. In the field of parasitology, as in other fields, accurate and rapid diagnosis is the most important strategy in the fight against parasitic infections. The aim of this review is to present new biomarkers for the rapid diagnosis of *Cryptosporidium* spp. and *Giardia duodenlis* infections.

New Biomarkers for Cryptosporidiosis

Cryptosporidium species are common protozoan parasites that can cause serious problems in the gastrointestinal system of animals and humans. *Cryptosporidium parvum* is a zoonosis that infects many domestic and wild animals, including humans. Calves younger than 8 weeks of age are the most susceptible hosts to this *Cryptosporidium* species, which is often associated with acute diarrhea, morbidity, and mortality. Animals infected with zoonotic subtypes of *Cryptosporidium* can spread the disease to other animals and humans. The detection of other *Cryptosporidium* species in unweaned calves has attracted attention. [11,12]. The use of microscopy in the diagnosis of *Cryptosporidium* species is an inexpensive method and is considered as the reference standard in the field of parasitology. However, due to the lack of specific morphological differences among *Cryptosporidium* species, molecular tests using genetic markers such as, COWP, gp60, hsp70 and SSU rRNA are often considered the gold standard for species or genotype identification [13]. Also, in the modern era, the increased use and availability of molecular tests and analyses have significantly enabled faster and more accurate diagnoses and identification of parasitic infections. Yet, as with many parasitosis, the lack of rapid and reliable molecular diagnostic methods for *Cryptosporidium* species poses new major challenges [14].

Nowadays, in addition to the conventional and molecular methods frequently used in the diagnosis of *Cryptosporidium* species, a study by Chappell et al. [12] draws attention when looking at the current literature. According to this study; It was stated that *Cryptosporidium* species are one of the few protists that can use indole to synthesize tryptophan, while the decrease in fecal indole levels was interpreted in favor of *Cryptosporidium*. Among the effects of indole; regulation of epithelial barrier integrity and bacterial microbiota has been shown. It has also been reported that oral administration of indole to healthy mice increases intestinal integrity and that indole added to cell cultures produces an anti-inflammatory effect. Thus, it has been shown that fecal indole levels can be evaluated as a precaution against *Cryptosporidium* infection in humans or in individuals exposed to the disease and that fecal indole can be used as a biomarker.

Oriá et al. [15] demonstrated the importance of the Myeloperoxidase (MPO) enzyme in order to identify new biomarkers that may be associated with gut-brain axis dysfunction in children suffering from the malnutrition/infection vicious cycle. While it has been stated

that MPO is a well-known tissue factor associated with neutrophils that is released during enteropathy and can lead to intestinal-derived brain inflammation, it has been shown that serum MPO and serum amyloid A (SAA) levels, which are markers of systemic inflammation, are increased in mice infected with *C. parvum* and exposed to malnutrition, thus MPO is a potential biomarker.

Recently, studies focusing on the role of microRNAs (miRNAs) in the pathogenesis of infectious diseases and their potential to be used as biomarkers have increased. miRNAs are regulatory RNA molecules that modulate target gene expression and play important roles in various physiological and pathological processes such as cell growth, differentiation, proliferation and apoptosis. Recently, the discovery of miRNAs as novel biomarkers in serum samples has brought a new approach to the screening of serum samples. Because of several factors such as the easy accessibility of biological body fluids, their comparatively low cost and the availability of multiple sampling and monitoring, there is great interest in their use as biomarkers compared to the use of tissue to avoid the risk of biopsy [16,17,18]. Parasitic infections have also been shown to alter host miRNA expression.

Ulusan Bagci and Caner [17], infected ileocecal adenocarcinoma cells with *Cryptosporidium* and examined miRNA expression profile in these cells. In the study in which the expression levels of 10 miRNAs were found to be higher than in the control group, it was stated that miRNAs would be useful in the etiopathogenesis and prognosis of infections. Başak et al. suggested that *Cryptosporidium* may be a potential pathogen for colorectal cancer in humans and stated that the host *Cryptosporidium* interaction causes the expression of a number of miRNAs to change as a result of *Cryptosporidium* controlling the defense mechanism, thus, miRNA profiles in infected cells can be used as possible biomarkers in cancer diagnosis [19].

Using mass spectrometry imaging, Anschütz et al., showed that they could visualize this intracellular parasite even in a host infected with only one *Cryptospordium* oocyst [20].

Luka et al. developed a biosensor to detect *Cryptosporidium* oocysts in environmental samples and showed that this immunosensor is simple, easy to manufacture and cost-effective for sensitive and rapid diagnosis of *Cryptosporidium*. The electrochemical biosensor developed will eliminate the need for trained technicians and specialized laboratories, has high sensitivity and specificity, and is a step forward for the rapid and on-site detection of *Cryptosporidium* oocysts in water samples [21].

New Biomarkers for Giardiasis

Giardia duodenalis (synonymous with *G. intestinalis* and *G. lamblia*) is one of the most common pathogens causing diarrhea worldwide. Although it is a self-limiting and treatable disease in healthy individuals, it has raised public health concerns in communities due to its partial contribution to the 1.6 million diarrheal deaths reported in 2016. [22,23].

G. duodenalis is found in human and animal environments. Therefore, it is of significant clinical and economic importance not only for humans but also for the environment, livestock and companion animals. This requires an integrated One Health approach for comprehensive control of giardiasis. *G. duodenalis* was included in the WHO Neglected Diseases Initiative in 2004 [23].

Giardiasis is diagnosed by microscopy of cysts or trophozoites in fecal samples. Although microscopy is considered the gold standard in the diagnosis of giardiasis, limiting factors such as intermittent cyst excretion in infected hosts, the number of stool samples examined and the expertise of technicians affect the success of microscopy [24]. Therefore, it is nowadays noted that there is a need for re-examination of existing methods and new discoveries to provide patients with accurate diagnosis and effective treatment. Thus, the search for specific biomarkers for giardiasis has become very important due to the need for improved diagnostics and therapeutics.

Currently, rapid antigen detection test, non-enzymatic immunochromatographic and immunofluorescent antibody tests are used to detect *Giardia* antigens in feces. However, the identification of different antigenic profiles of isolates from different geographical regions and the occurrence of antigenic variation are disadvantages of using these tests. Also, due to the biological characteristics of *Giardia* and the poor understanding of the long-term humoral immune response after a natural giardiasis infection, serologic tests have proven to be of little value in the diagnosis of giardiasis. Therefore, the fecal antigen detection test is much preferred [25]. However, these tests should be used as complementary tests, especially in patients with negative microscopy results but persistent symptoms [23].

It has been reported that some proteins can be used as biomarkers in the diagnosis of *G. duodenalis*. These natural proteins of *Giardia* are heat shock proteins (HSPs), cyst wall proteins (CWPs), giardins, tubulins (cytoskeletal proteins), enolase-a, fructose-1,6-bisphosphate aldose (FAB), arginine deaminase (ADI), ornithine carbamoyl transferase (OCT), variant surface proteins (VSPs). Variant-specific surface proteins (VSPs) are cysteine-rich proteins found on the surface

of trophozoites. These proteins are involved in parasite escape from the host immune response and host-parasite interaction, and are also components of cellular signaling. The most characterized VSP is VSPH7, a 56kDa protein. Because it is considered to be highly immunogenic. The VSP 5G8 protein is shown as a potential candidate for vaccine development because it evokes a strong humoral immune response when injected into mice [26,27,28].

Khalaf et al., [29] investigated the effects of *G. lamblia* infection on some biomarkers such as mucin-2 protein (MUC2), ghrelin (GHRL) and obestatin (OB) and reported that the concentrations of (MUC2), (GHRL) and (OB) were significantly increased in male and female patients infected with *G. lamblia* compared to the control group. They also found a significant increase in the concentrations in the total number of patients infected with *G. lamblia* and in the control group. Thus, they suggested that the (MUC2) protein and the (GHRL) and (OB) hormones may be potential biomarkers in the diagnosis of *G. lamblia*.

Currently, LC-MS/MS and MALDI-TOF/TOF with mass spectrometry are widely used in proteomic analysis of *Giardia*, which will enable researchers to investigate the virulence of giardiasis, pathogenicity mechanisms of *G. duodenalis*, and post-translational modifications of *Giardia* proteins during inoculation, and will help identify vaccine and drug targets as well as reveal potential candidates for new diagnostic biomarkers. Because genomic sequencing to define *Giardia* assemblages has revealed previously unknown proteins associated with the *Giardia* proteome [30].

In conclusion, this review has shown that new biomarkers that have been and could be used in the diagnosis of cryptosporidiosis and giardiasis are promising. The sensitivity and specificity of current methods are affected by basic factors in the diagnostic technique, the skill of the personnel and the intervals between cysts shed from patients and carrier hosts. However, scientific studies have paved the way for further exploration to improve the accuracy of cryptosporidiosis and giardiasis diagnosis; in this case, new biomarkers have become one of the potential targets that require further study.

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Author Contributions

All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

Conflicts of Interest

There is no conflict of interest for the publication of this article.

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