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**Abstract**

Currently, cystic echinococcosis (CE) follow-up is a serious concern among surgeons. MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs which are present in human body fluids in a highly stable form. Recently, it is observed that Echinococcus granulosus expresses a large number of miRNAs in its developmental stages. The current study aimed at evaluating the capacity of parasitic miRNAs to serve as plasma biomarkers for hydatid cysts before and after CE surgery. Hydatidosis patients were identified using radiological and histopathological examinations. Following RNA extraction and cDNA synthesis, the expression levels of parasite-derived miRNAs including egr-miR-71 and egr-let-7 were quantitatively evaluated using real-time polymerase chain reaction (RT-PCR) in 30 hydatid cyst-infected individuals before surgery and an equal number of healthy controls. Then, three- and six-month follow-ups were performed after cystectomy. To analyze parasite-derived miRNAs, the relative fold change between uninfected and infected samples was determined and normalized to hsa-miR-16-5p as the housekeeping internal control. RT-PCR demonstrated that egr-miR-71 and egr-let-7 were specifically amplified in all the plasma samples from the infected individuals with hydatid cyst; yet they were significantly down-regulated at three and six months post-surgery ($P<0.05$). The egr-miR-71 had a higher level of expression in larval stage compared with egr-let-7. The results of the current study indicated that hydatid cyst-derived miRNAs including egr-miR-71 and egr-let-7 can be detected in human plasma. Considering the changes in the expression levels of these miRNAs after three and six months, it seems that these miRNAs, especially egr-miR-71, could serve as novel promising biomarkers for the early diagnosis and monitoring of hydatidosis.



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| **Keywords** | MicroRNA; Echinococcus granulosus; Follow-up; Plasma; qRT-PCR |



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**Dear Editor,**

It is a great pleasure for me to send you one of my researches titled " **Parasite-derived microRNAs in plasma as novel promising biomarkers for the early detection of hydatid cyst infection and post-surgery follow-up** " for consideration and processing for publishing in "**Acta Tropica**".

Present study is the first description of circulating miRNAs from hydatid cyst in humans. The current study reported that hydatid cyst-derived miRNAs including egr-miR-71 and egr-let-7 can be detected in human plasma. According to the changes in the expression levels of these miRNAs after three and six months, it was hypothesized that these miRNAs can be used as novel potential biomarkers for the early diagnosis and monitoring of hydatidosis and help to better understand the functional roles of miRNAs in host-parasite interactions.

This study was carried out through international cooperation. My contributing authors have participated in the study and concur with the submission and subsequent revisions submitted by the corresponding author.

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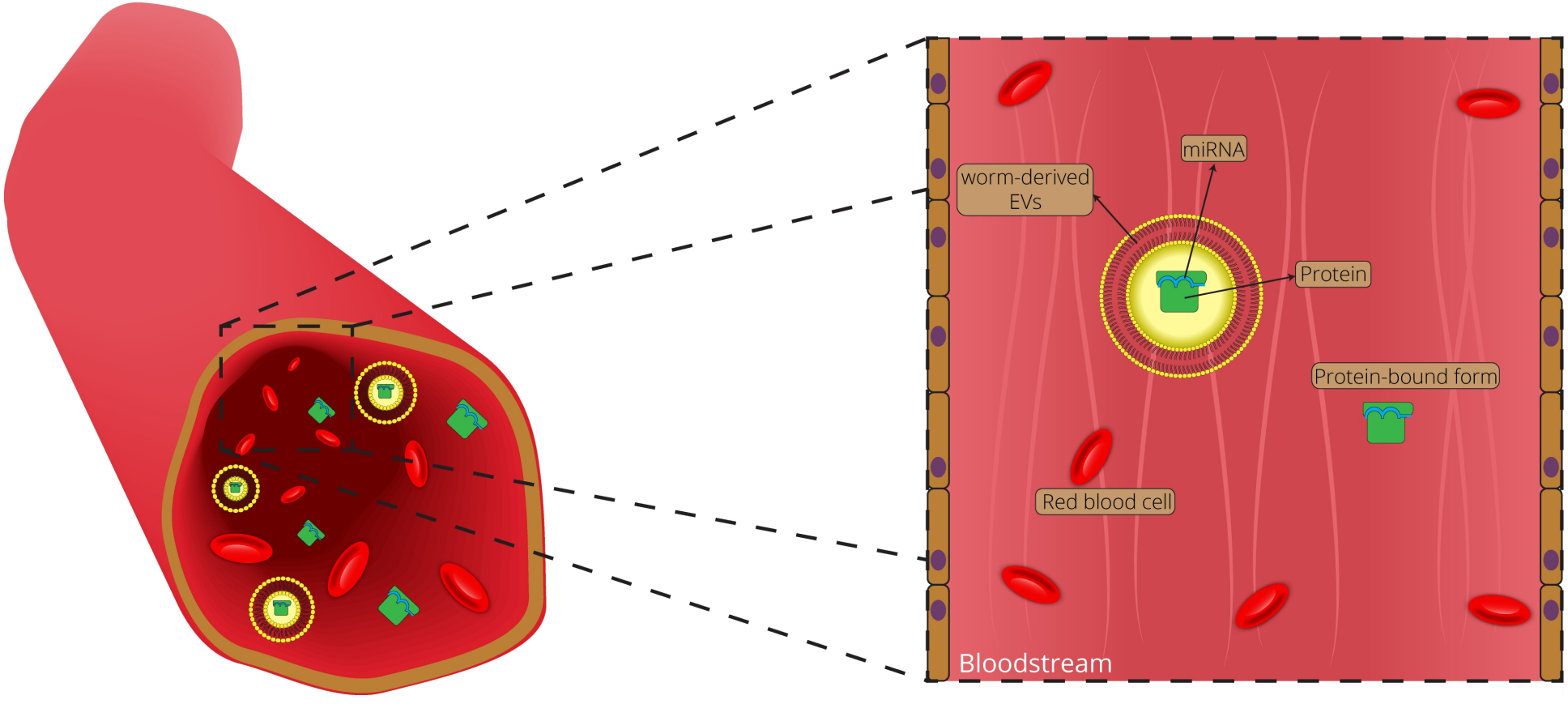
Sincerely yours
Mahmoud Mahami-Oskouei, PhD

**Highlights:**

● RT-PCR demonstrated that egr-miR-71 and egr-let-7 were specifically amplified in all the plasma samples from the infected individuals with hydatid cyst.

● The results of this study showed that two hydatid cyst derived miRNAs, egr-miR-71 and egr-miR-let-7, were significantly down-regulated after three and six months' post-surgery and remove the cyst.


miRNA
worm-derived EVs
Protein
Protein-bound form
Red blood cell
Bloodstream

# Parasite-derived microRNAs in plasma as novel promising biomarkers for the early detection of hydatid cyst infection and post-surgery follow-up

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## ABSTRACT

Currently, cystic echinococcosis (CE) follow-up is a serious concern among surgeons. MicroRNAs (miRNAs) are small, endogenous, non-coding RNAs which are present in human body fluids in a highly stable form. Recently, it is observed that *Echinococcus granulosus* expresses a large number of miRNAs in its developmental stages. The current study aimed at evaluating the capacity of parasitic miRNAs to serve as plasma biomarkers for hydatid cysts before and after CE surgery. Hydatidosis patients were identified using radiological and histopathological examinations. Following RNA extraction and cDNA synthesis, the expression levels of parasite-derived miRNAs including egr-miR-71 and egr-let-7 were quantitatively evaluated using real-time polymerase chain reaction (RT-PCR) in 30 hydatid cyst-infected individuals before surgery and an equal number of healthy controls. Then, three- and six-month follow-ups were performed after cystectomy. To analyze parasite-derived miRNAs, the relative fold change between uninfected and infected samples was determined and normalized to hsa-miR-16-5p as the housekeeping internal control. RT-PCR demonstrated that egr-miR-71 and egr-let-7 were specifically amplified in all the plasma samples from the infected individuals with hydatid cyst; yet they were significantly down-regulated at three and six months post-surgery ($P<0.05$). The egr-miR-71 had a higher level of expression in larval stage compared with egr-let-7. The results of the current study indicated that hydatid cyst-derived miRNAs including egr-miR-71 and egr-let-7 can be detected in human plasma. Considering the changes in the expression levels of these miRNAs after three and six months, it seems that these miRNAs, especially egr-miR-71, could serve as novel promising biomarkers for the early diagnosis and monitoring of hydatidosis.



**Key words:** MicroRNA, *Echinococcus granulosus*, Follow-up, Plasma, qRT-PCR.

## 1. Introduction

Cystic echinococcosis (CE) or hydatidosis is a cosmopolitan cyclo-zoonotic parasitic infection, rendered by the metacestode stage of *Echinococcus granulosus*, which infects several organs, particularly the liver (60–70%) and lungs (10–25%) (McManus et al., 2003; Mandal and Mandal, 2012). Hydatidosis is one of the most important diseases and categorized among seven neglected endemic zoonoses (Craig et al., 2007). CE is globally distributed and found in every continent except Antarctica, but its prevalence is higher in the regions with developed sheep and cattle industry, comprising Oceania, Europe, China and Central Asia, parts of Africa, and the Americas (Deplazes et al., 2017). The overall seropositivity of CE among general population in the Middle East estimated 7.44% (Galeh et al., 2018). The seroprevalence of human hydatidosis in the Iranian general population is estimated about 5.4% (Mehrabani et al., 2014). Although the signs and symptoms of CE are variable, specific parameters including cyst size, viability, and anatomic position are significant to develop symptoms (Moro and Schantz, 2009). The diagnosis of CE in humans is based on ultrasound imaging, immunodiagnostic methods, and clinical symptoms (Zhang et al., 2011). Parasite-specific antibodies are detected by different serological tests, but the antibodies against *E. granulosus* are only detected in advanced-stage hydatidosis (Zhang et al., 2011; Yamano et al., 2014). Currently, CE follow-up is a serious concern among surgeons and detection of recurrent and relapsing cysts is commonly neglected following cystectomy processes. Therefore, there is an urgent need for identification of parasite-specific biological markers with high sensitivity and specificity to follow-up after treatment.

MicroRNAs (miRNAs), are small, endogenous, non-coding RNAs that depending on the target genes play a pivotal role in the regulation of gene expression of a wide array of biological processes including immune response, inflammatory reaction, and tumorigenesis (Negrini et al.,

2009; Li et al., 2010). In the last five years, studies demonstrated that miRNAs can circulate in body fluids such as whole blood, serum, plasma, amniotic fluid, saliva, and urine in a cell-free form and are particularly stable in such fluids (Olivieri et al., 2017; Mitchell et al., 2008). MiRNA microarray and deep sequencing reveal that deregulation of host miRNAs takes place in parasitic infections, indicating their substantial function, especially in immune-inflammatory responses, during pathogen challenges (Jin et al., 2017; Guo and Zheng, 2017). Previous studies revealed that extracellular miRNAs alter in human serum in different diseases such as malignancies, hepatitis, and bacterial or viral infections (Zhu et al., 2017; Lu et al., 2017). On the other hand, according to recent investigations, miRNAs could be constantly detected in body fluids of humans and animals, suggesting their potential role as a biomarker for the early diagnosis of helminthic infections. For instance, sja-miR-277, miR-3479-3p, and bantam are potential diagnostic biomarkers for *Schistosoma japonicum* infection (Hoy et al., 2014; Dong et al., 2017). Additionally, it was approved that the cholangiocarcinoma induced by *Opisthorchis viverrini* can be detected by ovi-miR-192 (Silakit et al., 2014). The recently-described miRNAs specific to *Echinococcus* enhance the understanding on their role in hydatid cyst development and host-parasite interaction and as a target for early diagnosis (Cucher et al., 2011; Macchiaroli et al., 2015; Cucher et al., 2015). However, the expression profile of the hydatidosis-associated circulating miRNAs, particularly in human hosts, is not fully understood yet. The current study aimed at identifying two parasite-specific miRNAs including *E. granulosus*-miR-71 (egr-miR-71) and *E. granulosus*-let-7 (egr-let-7) in the plasma of patients with CE. It was postulated that parasite-specific miRNAs can be candidate as a general platform for specific and non-invasive diagnosis before and after CE surgery.

## 2. Materials and Methods

## 2.1. Patients, sample collection and preparation

The current study was conducted on 30 patients with hydatid cyst referred to the hospitals in the Northwest of Iran from May 2017 to April 2018. Hydatidosis patients were identified using radiological and histopathological examinations. An equal number of demographically-matched, healthy volunteers were selected as the control group. Patients with immune deficiency, autoimmunity, and transplant recipients were excluded from the study. An informed consent form and a questionnaire including the demographic characteristics (i.e., gender, age, residency, and contact with domestic animals), clinical symptoms, laboratory findings, and chemotherapy periods (in the case group) were completed by the participants. Then, 5 mL blood samples obtained from all the subjects in both groups and were drained into tubes containing sodium citrate as anticoagulant. Afterwards, the blood samples were centrifuged for 10 min at 1900*g* at 4 °C. The supernatant was collected and transferred into a new tube and then centrifuged for further five min at 3000*g* at 4 °C to pellet any debris and insoluble components. The plasma was then transferred into another tube and stored at -80 °C until RNA extraction.

## 2.2. Ethical approval

This study was approved by the research ethics committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1396.418).

## 2.3. Human plasma RNA extraction

Total RNA was extracted from the 200 µL plasma using the miRCUY$^{TM}$ RNA Isolation Kit Biofluids (Exiqon, Germany) according to the manufacturer's instruction. The extracted RNA concentration was quantified using the Nanodrop ND-1000 with A260/A280 ratios.

### 2.4. cDNA synthesis and real-time PCR

Reverse transcription were performed using miRCURY LNA™ Universal cDNA Synthesis Kit II (Exiqon) according to the manufacturer's instruction. To examine *E. granulosus*-specific miRNAs in the infected plasma, egr-miR-71 and egr-let-7 were chosen for assay by a LNA™-based PCR using total RNA from *E. granulosus*-infected human plasma. Real-time polymerase chain reaction (RT-PCR) was performed with SYBR Green Master Mix (Exiqon) and miRNA LNA™ PCR primers (Table 1) at 200 nM final concentration. Data were collected on a Light Cycler 96 system (Roche). The relative expression of egr-miR-71 and egr-let-7 between uninfected and infected samples was calculated using the $2^{-\Delta\Delta Ct}$method (Livak and Schmittgen, 2001), normalized to hsa-miR-16-5p; values for the infected plasma were compared to the ones for uninfected plasma.

### 2.5. Statistical analysis

Data analyses were performed using SPSS software (version 16.0, SPSS, Chicago, IL). Values were expressed as mean ± SD. One way analysis of variance (ANOVA) was used to calculate the significance of the results. *P* value <0.05 was considered significant level.

## 3. Results

### 3.1. Characteristics of patients with cystic echinococcosis

Thirty patients were included in the current study, of whom 16 (53.3%) were female and 14 (46.7%) male. The patients' mean age was 34.1year (standard deviation = 14.1) and 68% of them were living in rural areas and most of them had a history of exposure to domestic animals. All the female patients were housewife. Lung was the most affected organ (53.3%). The most

common clinical symptoms were related to cough (53.3%) and cough with bloody sputum (23.3%). In addition, 46.7% of abdominal pains were observed in patients with liver involvement. There was no significant relationship between gender and clinical manifestations ($P > 0.05$).

### 3.2. Detection of hydatid cyst derived miRNAs in plasma

To determine whether this parasite releases its miRNAs into patient's plasma, a comparative analysis was performed between patients infected with hydatid cyst as a case group and healthy individuals as a control group. Here, the expression of parasite-derived miRNAs including egr-miR-71 and egr-let-7 was evaluated by quantitative PCR (qPCR) using LNA-modified primers. The incorporation of LNA™ primers can increase the specificity and sensitivity of PCR. These parasite derived miRNAs displayed a significant signal over noise level in the plasma of patients with hydatidosis compared to the control group. The melting curve (Tm) analyses were 72 °C for both egr-miR-71 and egr-let-7 miRNAs (Figure. 1). Quantitative PCR results showed that these parasite derived miRNAs are present in all of the infected plasma (Figure. 2A, B).

### 3.3. The plasma levels of egr-miR-71 and egr-let-7 after three and six months' post-surgery follow-up

We collected plasma from patients infected with hydatid cyst and underwent follow up after 3 and 6 months post-surgery and analyzed by RT-PCR. The results showed that two hydatid cyst derived miRNAs, egr-miR-71 and egr-miR-let-7, were significantly down-regulated after three and six months' post-surgery and remove the cyst (Figure 3A, B).

## 4. Discussion

MiRNA-based diagnostics attracted extensive biomarker research interest for clinical diagnosis and monitoring of human diseases (Zen and Zhang, 2012). Unexpectedly, miRNAs, particularly serum/plasma miRNAs, are resistant to RNase activity and other sever conditions such as boiling, multiple freeze–thaw cycles, low or high pH, and extended storage, which potentially explain why miRNAs are extremely stable in body fluids (Mitchell et al., 2008; Chen et al., 2008; Li et al., 2011). Helminth-derived miRNA profiling in the host body fluids is still in the early stages of research (Guo and Zheng, 2017; Meningher et al., 2016; Cai et al., 2016). Recent evidence demonstrated that tissue-specific and circulating miRNAs in mammalian hosts are deregulated during an active helminthic infection, therefore can play critical roles in host-parasite interaction (Guo and Zheng, 2017; Tritten et al., 2014; Manzano-Román and Siles-Lucas, 2012). Also, it has been shown there is that parasite-derived or parasite-specific miRNAs are existent in the serum or plasma of the infected hosts (Tritten et al., 2014; Zhang et al., 2016). Recently, it was demonstrated that the miRNA expression profile in the sheep intestinal tissue was deregulated by *E. granulosus*, which plays an essential role in resistance to hydatid disease in the early stage of infection (Jiang et al., 2016). Results of another study also indicated that *E. multilocularis* infection involved in the expression of four of ten genes important to miRNA biogenesis in the mouse liver (Jin et al., 2017). In addition, it was found that dual-specificity protein phosphatase 1 (Dusp1), as an attenuator of immune activation and a critical regulator of innate immune response, was directly targeted and suppressed by parasite-derived miRNAs, which promoted parasite survival in the host (Cáceres et al., 2013; Buck et al., 2014). To date, there are no studies that examined the profile of the two circulating miRNAs, egr-let-7 and egr-miR-71 in human host serum and/or plasma during hydatid cyst infection. Herein, it was reported that the two parasite-derived miRNAs (egr-miR-71 and egr-let-7) were stably present in the

infected human plasma in hydatidosis endemic areas, and the detection of extracellular circulating miRNAs in the plasma of hydatid cyst-infected humans was feasible ($P$<0.001).

The current study also demonstrated that the presence of helminth-derived miRNAs in patients' plasma providing a starting point to develop novel diagnostic biomarkers and monitor helminth infection. There are several evidence that parasite-derived miRNAs are present in plasma/sera of mice and humans infected with *S. mansoni* (Hoy et al., 2014). Moreover, sja-miR-277 and sja-miR-3479-3p are likely to be novel biomarkers for schistosomiasis japonica based on the evidence from a murine model (Cai et al., 2015). Another investigation demonstrated that *Dirofilaria immitis* and *Onchocerca volvulus*-specific miRNAs can be detected in the plasma of dog and human, respectively (Tritten et al., 2014). MiRNA pathway in *Echinococcus* spp. and expression profile of miRNAs in different life cycle stages of the parasite were previously described (Cucher et al., 2011). Accordingly, egr-miR-71, egr-let-7, egr-miR-1-3p, egr-miR-9-5pandegr-bantam-3p were reported as the most abundantly expressed miRNAs in the protoscoleces and cyst wall of the metacestode of *E. granulosus* (Macchiaroli et al., 2015). Besides, protoscoleces can interact with definitive and intermediate hosts, and excretory/secretory products (ESP) were released by parasites that overcome immune evasion mechanisms (Virginio et al., 2012; Carmena et al., 2004; Pan et al., 2014). Previously, it has been showed that parasite-specific miRNAs stably exist in the sera of *E. multilocularis*-infected mice (Guo and Zheng, 2017). However, it is noteworthy that there is no evidence on serum and/or plasma levels of hydatid cyst-specific miRNAs in human hosts. Since hydatid cyst can exist in any organ of the intermediate host, it is intriguing to explore how the parasite releases its miRNAs into the host bloodstream. Previous studies demonstrated that some parasites such as *D. dendriticum*, *Fasciola hepatica*, and *Schistosoma* spp. secrete extracellular structures such as

microvesicles, microparticles, and exosome-like vesicles containing parasite-derived miRNAs and proteins. They also proposed that these exosome-derived miRNAs are detectable in host tissues (Fromm et al., 2015; Bernal et al., 2014). Recently it was found that showed that the most of microRNAs in serum and saliva are encapsulated in exosomes (Gallo et al., 2012). Moreover, in several studies, similar secretory vesicles mentioned above were identified in early stages of *E. granulosus* infection (Holcman et al., 1994). In addition, in a study for the first time, showed that cestode parasites including *E. multilocularis*, *Taenia crassiceps* and *Mesocestoides corti* release extracellular vesicles into culture media and these structures contain immunodiagnostic protein cargo, surprisingly small RNAs (Ancarola et al., 2017). Therefore, based on the current study results and those of other investigations, it seems that parasites may release these miRNAs into the host bloodstream via vesicle structures such as microvesicles, microparticles, and extracellular vesicles.

The current study also performed three- and six-month follow-ups. The results demonstrated that egr-mir-71 and egr-let-7 were significantly down-regulated in the plasma after removing the cyst. Meningher et al. (2016) showed that two parasite-derived miRNAs, bantam and miR-3488 were detected in sera of patients with*Schistosoma* infection. QRT-PCR analysis after treatment on seven patients showed a significant reduction in the expression level of the parasite miRNA. Therefore, these parasite-derived miRNAs could serve as novel biomarkers of outcome of therapy and disease-control programs (Meningher et al., 2016). The miR-71is a conserved miRNA and highly expressed across platyhelminthes not found in vertebrates (Macchiaroli et al., 2015; Christodoulou et al., 2010; Jin et al., 2013). This miRNA is an essential factor in the regulation of germline-mediated longevity and stress response in the free living nematode *Caenorhabditis elegans* (Boulias and Horvitz, 2012). In a study reported that *E. multilocularis*-

derived miR-71 (emu-miR-71) was released into the hostile host environment and affected the functions of macrophages (Zheng et al., 2016).

Recently, the miRNA target genes have been recently predicted in *Echinococcus* (Macchiaroli et al., 2017). Among these target genes, some had effects on host immune responses suggesting that miRNAs could act as immune-regulatory agents. Accordingly, miR-71 and miR-2 (miR-2a/2b/2c) families had a higher number of target genes, accounting for ~30% (211/724) of all target genes similar to *S. mansoni*-derived miR-71 and miR-2 target genes (Macchiaroli et al., 2017; de Souza Gomes et al., 2011). For instance, it was predicted that members of the transforming growth factor beta (TGFβ) signaling pathway and rho-associated protein kinase 1 (ECANG7_07875) conserved targets of miR-71. This signaling pathway serves essential roles in parasite growth and host-parasite interactions in *S. japonicum* (Huang et al., 2009). Also, armadillo importin-alpha gene (ECANG7_01054) that is potentially involved in segmentation and the mitogen-activated protein kinase (MAPK) signaling pathway was targeted by egr-miR-71 (Macchiaroli et al., 2017). Emu-miR-71 was potentially involved in protoscolex development by suppression the expression of nemo-like kinase (NLK) (Guo et al., 2017). Thus, it can be stated that egr-mir-71 may be essential to develop and survive hydatid cyst in the intermediate host.

The let-7 is one of the first reported miRNAs and found as the first known human miRNA also an essential factor in the developmental timing of *C. elegans*. This miRNA is highly conserved in human tissues (Abbott et al., 2005; Su et al., 2012). The let-7 regulates mouse insulin response by targeting several genes of the insulin-PI3K-mTor pathway, including the insulin receptor (Zhu et al., 2011). This miRNA potentially targets the vitamin D receptor (VDR) gene that its expression is correlated with the let-7 in *E. granulosus* (Macchiaroli et al., 2017). Dimerization

241 and activation of the VDR and retinoid X receptor (RXR) genes are effective in the development,
242 homeostasis, differentiation, and metabolism of *E. granulosus* (Zheng et al., 2013). VDR gene is
243 the main regulator of the c-MYC/MXD1 network and misregulation of these genes is involved in
244 human lung cancer (Großhans et al., 2005). Based on this evidence, it seems that let-7 may be
245 essential to develop and survive hydatid cyst in the intermediate host.

246 The relapse or recurrence of hydatid disease is still a major problem in patients undergoing
247 surgical treatment since the detection of cysts is ignored after surgery and associated with rupture
248 of the cysts and release of protoscoleces into the bloodstream (Rouhani et al., 2013; San Pedro et
249 al., 1992; Akyıldız et al., 2009). In the current study, follow-up was performed on patients after
250 surgery to assess the long term expression level changes of egr-miR-71 and egr-let-7. In line with
251 the current study assumptions, the expression level of egr-miR-71 was significantly down-
252 regulated three and six months after surgery and removal of the cyst. Moreover, the expression
253 level of egr-let-7 was very low compared with the primary analysis. The results of the current
254 study suggested that these parasite-derived miRNAs, especially egr-miR-71 with a higher level
255 of expression in the larval stage, can be used as a novel promising biomarker for the early
256 diagnosis and monitoring of hydatidosis in humans. The current study provided a basis for
257 further studies into the functional roles of these miRNAs in hydatid cyst pathogenesis, host-
258 parasite interaction, and parasite biology.

259 In conclusion, microRNAs of parasitic helminths provide an exciting outlook to regulate the
260 development, differentiation, drug resistance, and parasite-host interactions. Due to the sanitary
261 importance of hydatidosis in the endemic areas and limitations in the early detection of the
262 disease, the current study evaluated two parasite-derived miRNAs in the plasma of patients with
263 hydatidosis in order to introduce an accurate diagnostic tool and follow-up the patients. To the

best of authors' knowledge, this study is the first description of circulating miRNAs from hydatid cyst in humans. The current study reported that hydatid cyst-derived miRNAs including egr-miR-71 and egr-let-7 can be detected in human plasma. According to the changes in the expression levels of these miRNAs after three and six months, it was hypothesized that these miRNAs can be used as novel potential biomarkers for the early diagnosis and monitoring of hydatidosis and help to better understand the functional roles of miRNAs in host-parasite interactions.

## Competing interests

The authors declare that they have no competing interests.


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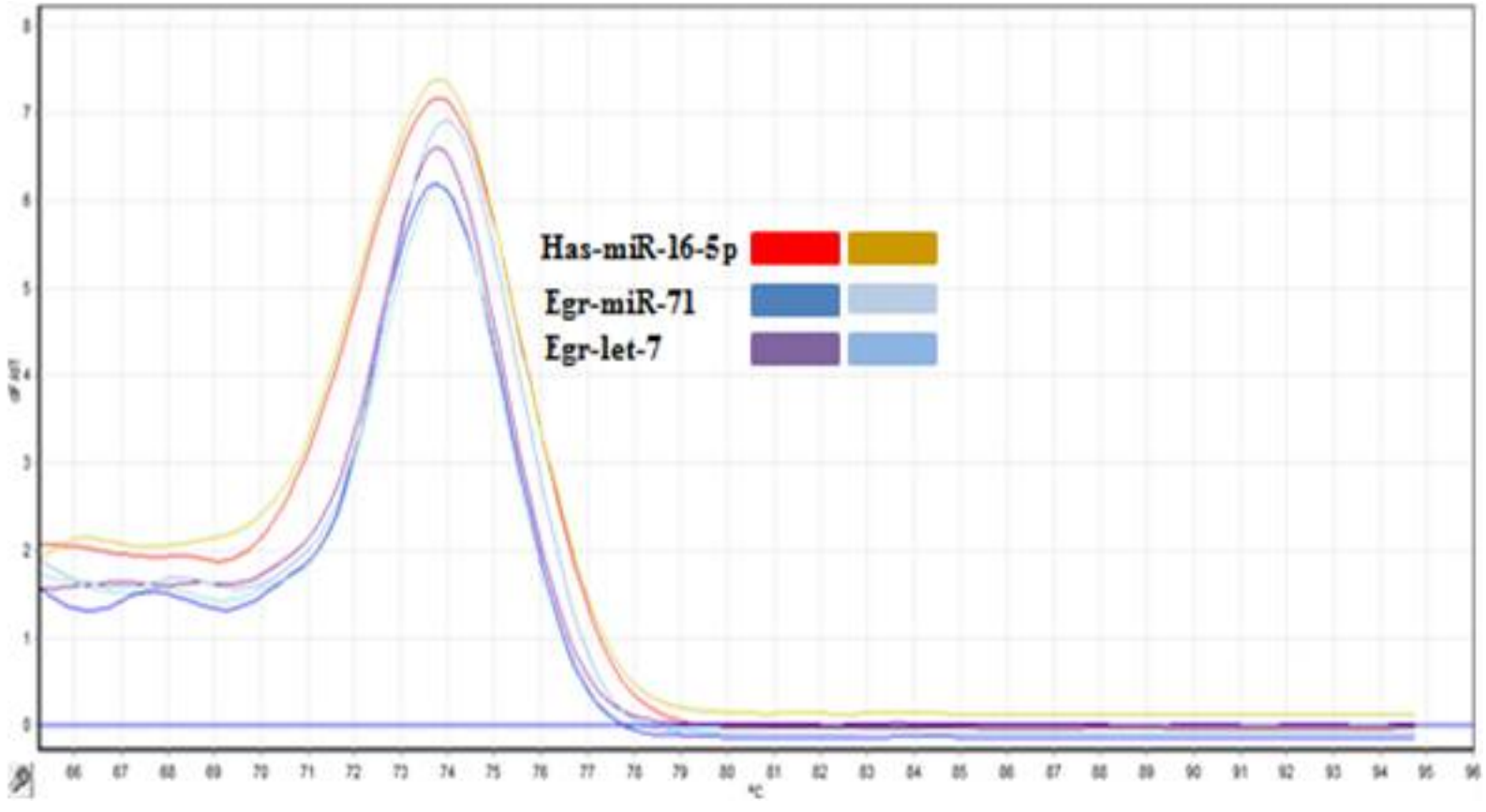
**Figure legends:**

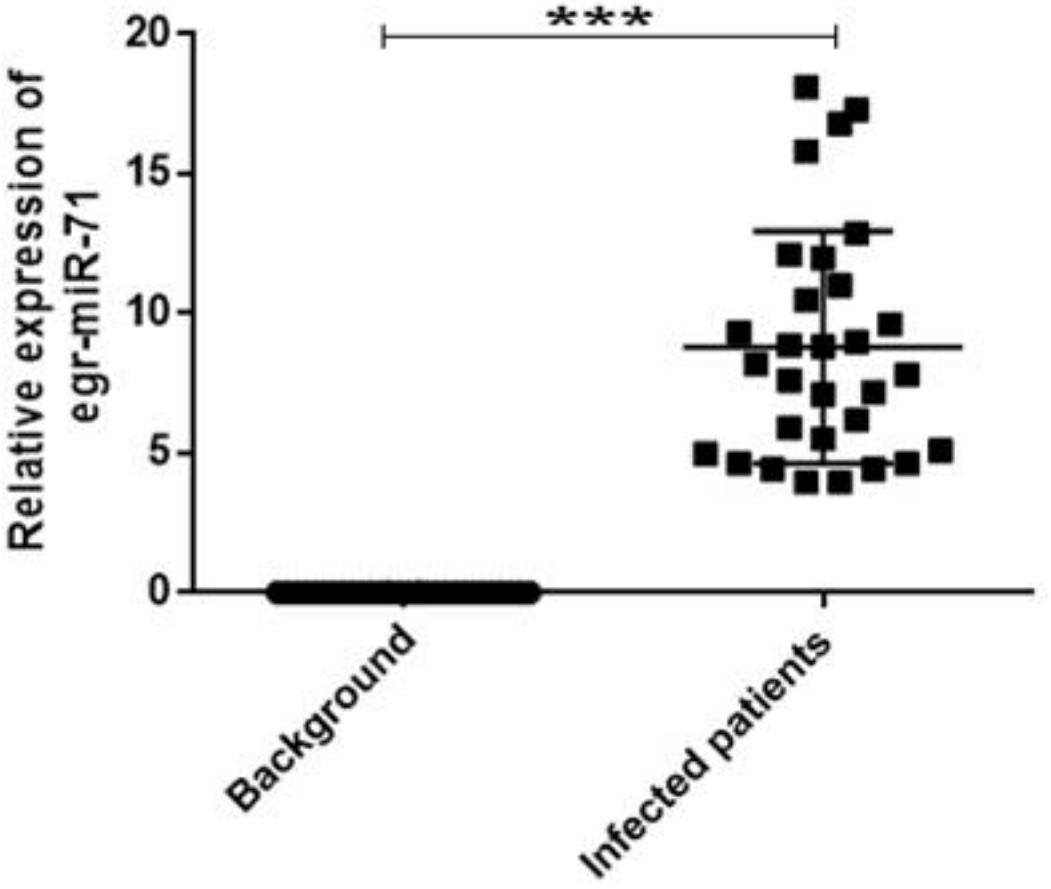
**Fig. 1.** Melting curve analyses of egr-miR-71, egr-let-7 and hsa-miR-16-5p.

**Fig. 2.** Hydatid cyst derived miRNAs, egr-miR-71 (A) and egr-let-7 (B) in human plasma during hydatidosis. The expression of egr-miR-71 and egr-let-7 was normalized to hsa-miR-16-5p, and fold changes were calculated as the ratio of values compared with the background in plasma of uninfected individuals. (*** $P< 0.001$)

**Fig. 3.** Egr-miR-71 (A) and egr-let-7 (B) expression in human plasma before and after surgery. Follow-up the patients performed after three and six months' post-surgery. (* $P< 0.05$, ** $P< 0.01$, *** $P< 0.001$).


Has-miR-16-5p
Egr-miR-71
Egr-let-7

A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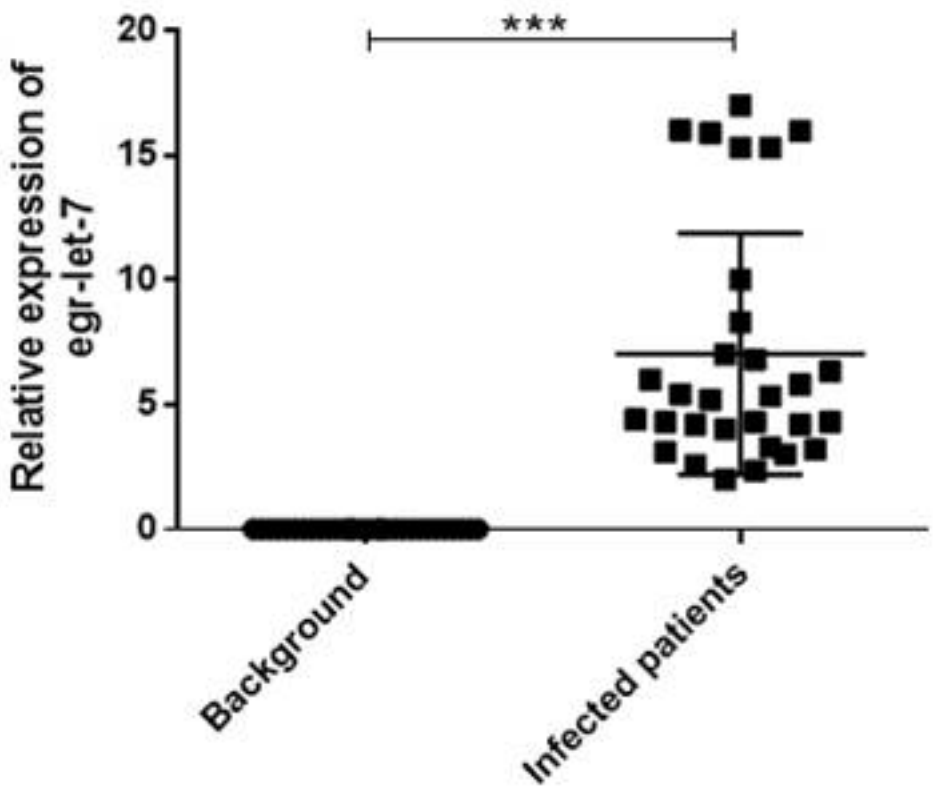
Relative expression of egr-miR-71
Background
Infected patients

**B**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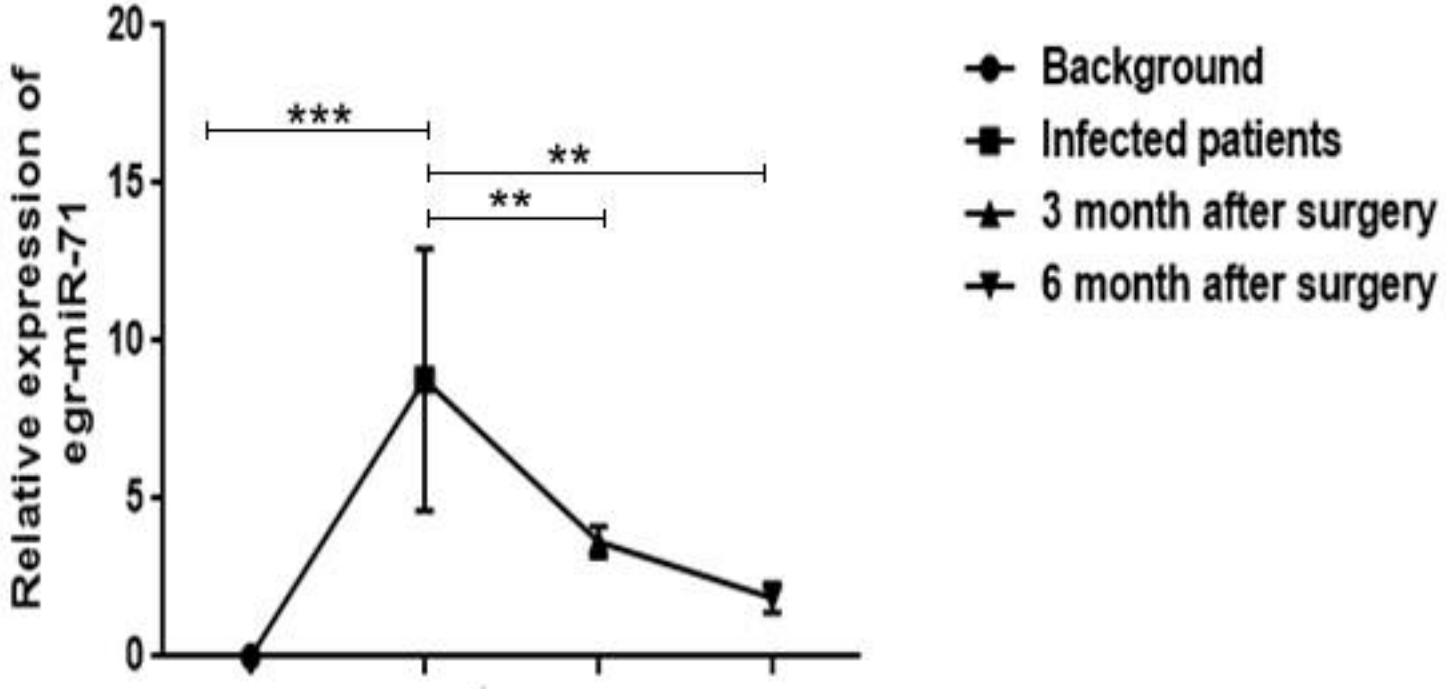
Relative expression of egr-let-7
Background
Infected patients

A


Relative expression of egr-miR-71
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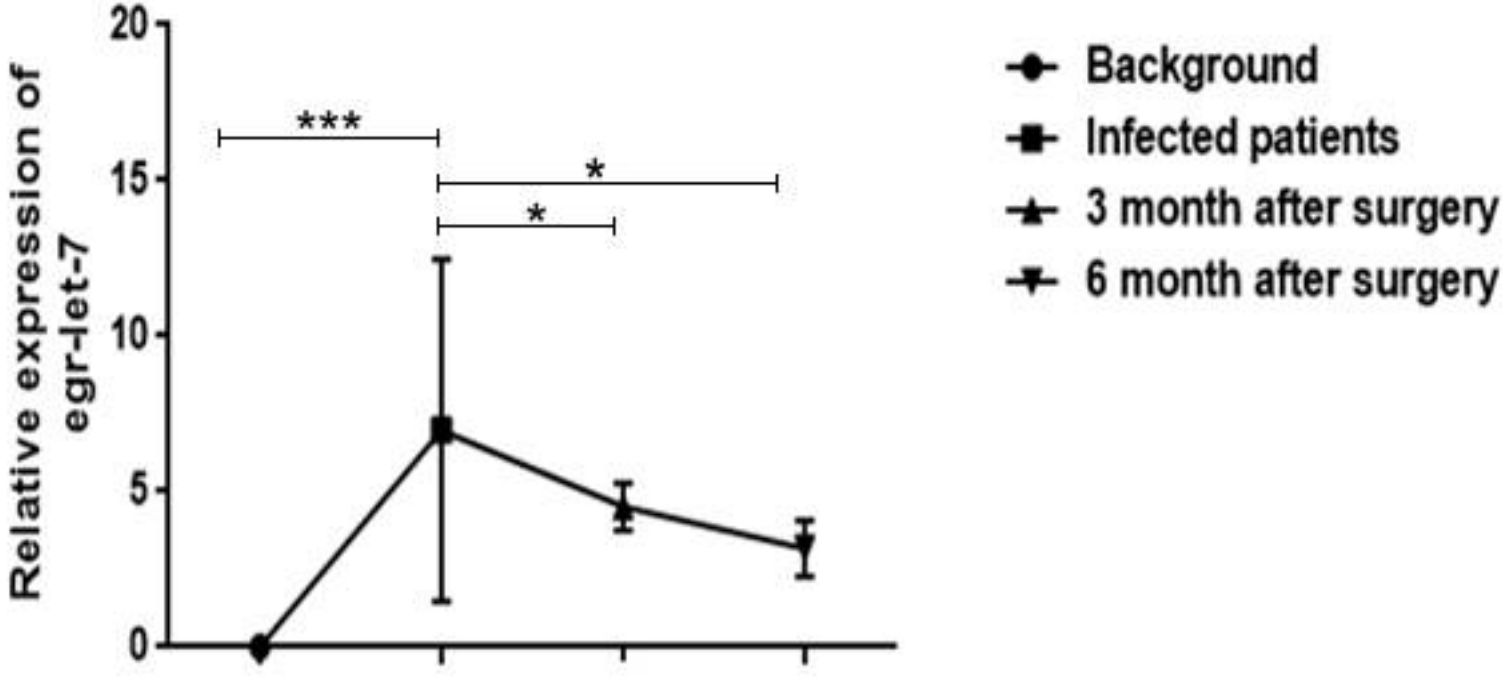
Background
Infected patients
3 month after surgery
6 month after surgery

**B**


Relative expression of egr-let-7
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Background
Infected patients
3 month after surgery
6 month after surgery

**Table 1**

Exiqon miRNA primers used in RT-PCR

| **Name** | MiScript Primer Assay | Product number |
|---|---|---|
| **LNA™ egr-mir-71** | UGAAAGACGAUGGUAGUGAGA | 2105281 |
| **LNA™ egr-let-7** | UGAGGUAGUGUUUCGAAUGUCU | 2102031 |
| **LNA™ hsa-mir-16-5p** | UAGCAGCACGUAAAUAUUGGCG | 205702 |