Gene Expression of miR-17-3p and miR-92 in Women with Acute Toxoplasmosis

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ABSTRACT - This study aimed to assess the gene expression of miR-17-3p and miR-92 in women with acute toxoplasmosis, and the possible use of these biomarkers for diagnosis of the disease. The study included 40 IgM seropositive women (considered as having acute toxoplasmosis) and other 40 IgM seronegative women (as controls) were selected to represent the study population. Total mRNA was extracted from blood samples and cDNA was created. Quantitative polymerase chain reaction was used to amplify two miRNAs (miR-17-3p and miR-92) using specific primers. The gene expression of these miRNAs was normalized against a small nuclear RNA (RNU43). Median fold changes of miR-17-3p normalized against RNU43 was 1.9 times (range 0.0 to 21.4 times) in cases compared to 1.1 times (range 0.1 to 13.2 times) in control with a significant difference (p = 0.038). Likewise, the median fold changes of miR-92 normalized against RNU43 was 3.3 folds (range 0.0 to 19.9 folds) in cases compared to 1.7 folds (range 0.0 to 16.9 folds) in control with a significant difference (p = 0.019). However, both miRNAs showed poor diagnostic values in discriminating acute toxoplasmosis form healthy controls. These data indicate that gene expression of each of miR-17-3p and miR-92 is upregulated in women with acute toxoplasmosis compared to controls, However, none of them, separately or in combination, can be used for detection of acute toxoplasmosis.

Keywords: Toxoplasma, miR-17-92cluster, gene expression

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INTRODUCTION

Toxoplasma gondii is an obligate intracellular parasite that causes diverse pathological effects in human and another warm-blooded vertebrate (Dubbey and Beattie, 1988). Toxoplasmosis, caused by T. gondii, is a worldwide parasitic disease that is widespread in Asia, Africa, South America, and Europe (Sun et al., 2013; Fan et al., 2012; Sauer et al., 2011). The parasite can cause severe disease especially in immunocompromised patients as well as in pregnant mothers (Bossiet al., 1998; Gao et al., 2012). Moreover, toxoplasmosis may cause serious consequences in newborn babies, such as hearing and sight impairment and neurological symptoms (Gilbert, 2009).
Early treatment of pregnant women could reduce the incidence of sequelae in infected infants (McAuley, 2014). Detection of early-stage toxoplasmosis is a key measure in reducing toxoplasmosis-related health damage. Most infections in humans are asymptomatic but at times the parasite can produce devastating disease (Mittal and Ichhpujani, 2011).

Until recently, there is no available vaccine against this parasite. Furthermore, there is a growing body of evidence indicating the development of drug resistance by *T. gondii* against the most common known effective drugs such as clindamycin, spiramycin, azithromycin, Pyrimethamine (PYR) and sulfadiazine (SDZ) (Montazeri *et al*., 2018). Thus, screening and early detection of the disease, especially in pregnant women, is the most effective method for control of the disease. Most available tests for diagnosis of toxoplasmosis are serological-based assays for detection of specific anti-toxoplasma antibodies. However, almost all these tests do not have enough sensitivity and specificity (Liu *et al*., 2015). Therefore, seeking for alternative tests or markers becomes a paramount issue to ensure rapid, easy and inexpensive screening.

MicroRNAs (miRNAs) are 21–25 nucleotide noncoding RNA molecules that play important roles in different physiological and pathological processes of human body (Zhao *et al*., 2019). Although, there is a large number with a growing list of miRNAs, it was found that the expression of certain miRNAs is influenced by various conditions including the presence of infectious agents such as *T. gondii* (Xue *et al*., 2013). Xiao *et al* reported that miR-132 was upregulated in *T. gondii* infected mice and was associated with changes in dopamine receptor signaling (Xia *et al*., 2014).

Being rapidly growing branch of biological sciences, the simultaneous detection of a panel of miRNAs using specific chips may become a routine work in different laboratories worldwide. This will put these molecules in front of many traditional diagnostic methods and entirely change the concept of diagnosis especially when diagnosis involves a gray zone as in toxoplasmosis.

Therefore, this study aimed to investigate gene expression of miR-17-3p and miR-92 in acute toxoplasmosis and to evaluate the diagnostic value of miR-17-3p and miR-92 in the context of discrimination between toxoplasma-infected and healthy women.

**SUBJECTS AND METHODS**

**The Study Population**

This case-control study included a total of 487 women (age range 19-49 years) attending the Obstetrics and Gynecology Department at Al-Imamain Al-Kadimain Medical City, Al- Yarmouk teaching Hospital, Al-Hayat Rahebat Hospital and Ibn-Balady Children and Maternity Hospitals/ Baghdad/Iraq during December 2018 to June 2019. Those women had different complaints ranging from headache to abortion. Pregnant women and those with diabetes mellitus and autoimmune diseases were excluded from the study. Data including age, body mass index (BMI), parity and previous abortion were collected through direct interview with each participant and recorded in a preformed questionnaire.
The study was approved by the Institutional Review Board (IRB) of College of Medicine/Al-Nahrain University, and a consent form explaining the aims of the study was obtained from each woman enrolled in the study before sample collection.

**Samples**

About 5 mL of venous blood were collected from each woman; 2 mL of which was kept in the ethylenediaminetetracetic acid tube (EDTA tube) and the other 3 mL in a gel tube and left at room temperature for 30 minutes then undergone centrifugation at 3000 rpm for 5 minutes. Sera were collected in epprdorf tubes and stored at -20°C until be used.

**Detection of anti-toxoplasma IgM Antibodies in Serum Samples**

A ready commercial kit (*Toxoplasma* IgM ELISA Detoxo3 /Germany) was used for the detection anti-*toxoplasma* IgM antibodies in serum samples of all included women. According to standards concentration and corresponding optical density (OD) values, the linear regression equation of the standard curve was calculated (Figure 1), and then, the OD was used to calculate the concentrations. According to manufacturer’s instructions, IgM titer above 10 U/mL is considered positive, otherwise the result is negative. Therefore, 40 women with positive result were selected to represent patients, while other 40 women with negative result were randomly selected to represent the control group.

![Figure 1: Standard curve for anti-toxoplasma IgM antibodies](http://doi.org/10.36295/ASRO.2020.231015)
RNA Extraction and cDNA Creation

RNA extraction was performed at the same day of sample collection to avoid RNA degradation. A ready commercial kit (Accuzol™ Blood genomic RNA Extraction/ Bioneer/Korea) was used for RNA extraction from whole blood samples. The manufacturer’s protocol was followed precisely. Extracted RNA was reversely transcribed into cDNA using AccuPower Rocket Script RT PreMix/Bioneer/ Korea. This kit is ready-to-use lyophilized master mix containing all components for first strand cDNA synthesis from RNA template.

**Quantitative PCR for Measuring miR-17-3p and miR-92 Concentration**

A set of primers (Table 1) was used for amplification of miR17-3p and miR-92 in whole blood samples.

In the first run, the reaction mixture comprised of 5 µl of the RNA extract, 1 µl of 2.5 mM deoxyribonucleosidetriphosphates (dNTPs), 0.3 µM of the RT primer, and 4 µl of RT 5x buffer (10mM Tris pH 8.9, 1.5 mM MgCl2, 80 mM KCl) The PCR condition involves annealing at 16 ºC for 30 min, extension at 42 ºC, enzyme activation at 85 ºC for 5 min and holding at 4 ºC for 10 min.

In the second run, the reaction mixture consisted of 5x qPCR master mix, 1 µl MgCl2, 0.5 µl of each primer, 10 ng of cDNA. The volume was adjusted to 10 µl with nuclease-free water. The PCR condition for included an initial denaturation at 95 ºC for 5 min, 40 cycles of denaturation, and 95 ºC for 15 seconds, annealing at 55 ºC for miR-17-3p and 58ºC for miR-92 for 14 seconds and extension at 72 ºC for 20 seconds.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-17-3p-RT</td>
<td>GTTGGGCTCTGGTGCAAGGAGCTGCAGTATTCGACACCAGAGCCACCTACAA</td>
</tr>
<tr>
<td>miR-17-3p-F</td>
<td>GTTACTGGCAGTGAAGGCAC</td>
</tr>
<tr>
<td>miR-92-3p-RT</td>
<td>GTTGGGCTCTGGTGCAAGGAGCTGCAGTATTCGACACCAGAGCCACCTACAA</td>
</tr>
<tr>
<td>miR-92-3p-F</td>
<td>GTTGGGCTCTGGTGCAAGGAGCTGCAGTATTCGACACCAGAGCCACCTACAA</td>
</tr>
<tr>
<td>RNU43_RT</td>
<td>GGTCACTGTGAGGAGCCACCTACAA</td>
</tr>
<tr>
<td>RNU43-F</td>
<td>GTTGGGCTCTGGTGCAAGGAGCTGCAGTATTCGACACCAGAGCCACCTACAA</td>
</tr>
<tr>
<td>Universal Reverse</td>
<td>GTTGGGCTCTGGTGCAAGGAGCTGCAGTATTCGACACCAGAGCCACCTACAA</td>
</tr>
</tbody>
</table>

After the reaction was completed, the amplification and melting plots were checked. The threshold cycle (Ct) values was obtained for each miRNA sample and then were normalized to obtain ΔCt values which eventually used to plot relative expression values. RNU43 gene was used as internal reference.
Gene Expression Calculation

Folding = \(2^{-\Delta\Delta CT}\)

\(\Delta CT = CT_{\text{gene}} - CT_{\text{House Keeping gene}}\)

\(\Delta\Delta CT = \Delta CT_{\text{Treated}} - \Delta CT_{\text{Control}}\)

The data obtained from real time experiments were detected according to the Ct values which calculated from cycles and was proportional to the starting target copy number (logarithmic scale) used for amplification (the point that the fluorescence signal increased above baseline is the threshold cycle) which are inversely related to the amount of starting template that mean the high value of Ct refers to the low levels of gene expression or amplification gene while low Ct value indicate high level of gene expression or high copy of gene amplification.

Amplification plots appeared when the fluorescent signal from sample is plotted against cycle number; however, amplification plots include the accumulation of product through the period of qPCR experiment.

Statistical Analysis

All analyses were performed using Statistical Package for Social Sciences (SPSS) software (Version 25). Continuous variables were subjected to normality test using Shapiro Wilk test. Variables with normal distribution were expressed as mean ± standard deviation (SD) and analyzed with independent t-test. Variables with non-normal distribution were expressed as median and range and analyzed with a non-parametric Mann Whitney U-test. Categorical variables were expressed and number and percentages and analyzed with Chi square. Pearson’s correlation was used to assess the correlation between different continuous variables. Receiver operating characteristics (ROC) curve was used to evaluate the diagnostic value of \(\text{miR-17-3p}\) and \(\text{miR-92}\) in discrimination between acute toxoplasmosis and healthy controls. A p level of ≤ 0.05 was considered significant.

RESULTS

Demographic and Clinical Characteristics of the Study Population

Generally, cases and controls had comparable demographic and clinical characteristics with no significant differences. Mean age of the patients was 26.85±5.81 years and 29.13±9.42 respectively. Likewise, both groups showed very close BMI (29.12±3.9 kg/m\(^2\) and 27.88±4.2 kg/m\(^2\) respectively). Although infected women had slightly higher rate of <3 parity than controls (67.5% versus 57.5%) and higher rate of ≥ 2 previous abortions (25% versus 15%), the differences were not significant (Table 3-1).
Table 3-1: Demographic and clinical characteristics of the study population

<table>
<thead>
<tr>
<th>Variables</th>
<th>Cases (n=40)</th>
<th>Controls (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years (mean±SD)</td>
<td>26.85±5.81</td>
<td>29.13±9.42</td>
<td>0.197</td>
</tr>
<tr>
<td>BMI, kg/m² (mean±SD)</td>
<td>29.12±3.9</td>
<td>27.88±4.2</td>
<td>0.213</td>
</tr>
<tr>
<td>Parity &lt;3</td>
<td>27(67.5%)</td>
<td>23(57.5%)</td>
<td>0.356</td>
</tr>
<tr>
<td>≥3</td>
<td>13(32.5%)</td>
<td>17(42.5%)</td>
<td></td>
</tr>
<tr>
<td>Previous abortion &lt;2</td>
<td>30(75%)</td>
<td>34(85%)</td>
<td>0.201</td>
</tr>
<tr>
<td>≥2</td>
<td>10(25%)</td>
<td>6(15%)</td>
<td></td>
</tr>
</tbody>
</table>

**Gene Expression of miRNAs**

The single, sharply-defend melting curve of qPCR of *miR-17* (figure 2 upper) indicates that pure, homogeneous PCR products were produced and confirming the specificity of reaction. The amplification curve (Figure 2 lower) shows the Ct value of *miR-17-3p*.

**miR-17-3p**

Data of miRNA expression were subjected for normality test and had been found to be non-normally distributed. Thus, Mann Whitney U test was used to compare the medians of miRNA expression between cases and controls. There was a significant difference in upregulation of *miR-17-3p* expression between the two groups. The median fold changes of *miR-17-3p* normalized against RNU43 was 1.9 folds (range 0.0 to 21.4 folds) in cases compared to 1.1 folds (range 0.1 to 13.2 folds) in control.
Figure 2: miR-17-3p expression using qPCR technique. Melting Curve for serum miR-17-3p is presented as a single, sharply-defined melting curve with a narrow peak (upper figure). The amplification curve showing the Ct values for miR-17-3p (lower figure).

MiR-92

Almost similar results were obtained for miR-92. The miR-17amplicon shows a single peak, representing a pure, single amplicon (Figure 3 upper). The amplification curve (Figure 3lower) shows the Ct value of miR-92.
Figure 3: miR-92 expression using qPCR technique. Melting Curve for serum miR-92 is presented as a single, sharply-defined melting curve with a narrow peak (upper figure). The amplification curve showing the Ct values for miR-92 (lower figure).

Also, there was a significant difference in upregulation of miR-92 expression between the two groups. The median fold changes of miR-92 normalized against RNU43 was 3.3 folds (range 0.0 to 19.9 folds) in cases compared to 1.7 folds (range 0.0 to 16.9 folds) in control.

**Diagnostic Values of miR-17-3p and miR-92**

Receiver operating characteristic curve was used to evaluate the diagnostic value of miR-17-3p in the context of discrimination between *Toxoplasma*-infected cases and healthy controls (Figure 3). The area under the curve (AUC)
was 0.636, 95% CI=0.513-0.758, p= 0.038. The sensitivity and specificity of the test at cut off value of $\text{miR-17-3p}=1.75$ folds were 0.525 and 0.744 respectively indicating poor diagnostic value.

A slightly better discriminative value was obtained in this miRNA. The AUC was 0.653, 95% CI=0.531-0.775, $p=0.019$. The sensitivity and specificity of the test at cut off value of $\text{miR-92}=2.05$ folds were 0.675 and 0.615 respectively (Figure 4) indicating poor diagnostic value too.

Figure 4: Receiver operating curve for a combination of $\text{miR-17-3p}$ and $\text{miR-92}$ in the context of discrimination between *Toxoplasma*-infected and healthy women.

Linear regression test between the two miRNAs in cases (Figure 5) revealed linear adjusted $R^2$ of 0.228, while this value was 0.495 in controls (Figure 6).
Figure 5: linear regression between miR-17-3p and miR-92 in Toxoplasma-infected women

Figure 6: linear regression between miR-17-3p and miR-92 in healthy control women

Association of miR-17-3 and miR-92 with Reproductive Characteristics

Table 3-3 shows the association of miR-17-3p and miR-92 with parity and previous abortion. Interestingly, median expression of miR-17-3p in infected women with < 3 parity was 3.0 (range 0.5 to 21.4) which was significantly higher than that in women with ≥3 parity (median 1.3, range 0.0 to 5.3). Other expression values of either miRNA-17 or miRNA-92 were compatible between different statuses of parity and abortion in both cases and controls without significant differences.

<table>
<thead>
<tr>
<th>Groups</th>
<th>miRNAs</th>
<th>Folds increase</th>
<th>Parity</th>
<th>p-value</th>
<th>Previous abortion</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases</td>
<td>miR-17-3p</td>
<td>3.0 (0.5-21.4)</td>
<td>0.031</td>
<td>1.95 (0.21.4)</td>
<td>0.747</td>
<td></td>
</tr>
<tr>
<td></td>
<td>miR-92</td>
<td>3.3 (0.0-19.9)</td>
<td>0.909</td>
<td>3.4 (0.19.9)</td>
<td>0.469</td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>miR-17-3p</td>
<td>0.9 (0.1-3.9)</td>
<td>0.110</td>
<td>1.0 (0.1-13.2)</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td></td>
<td>miR-92</td>
<td>1.45 (0.0-11.9)</td>
<td>0.604</td>
<td>1.7 (0.0-16.9)</td>
<td>0.242</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

The overall IgM seropositivity of Toxoplasma was 8.21%. Compared with local studies, this rate was reasonable. In Kurdistan/ Iraq, Ali et al. (2018) examined 350 women (250 aborted and 100 with normal birth) and found a total of 50 positive cases for IgM (14.29%), 40 of whom (16%) were aborted women and 10(10%) were women with normal birth. In the middle of Iraq, Darweesh et al. (2018) enrolled 58 women with abortion, 42 pregnant with previous abortion, and 50 healthy women with no pregnancy or abortion. The seroprevalence of IgM in those women were 6.9%, 4.76% and 0% respectively. In the south of the country, Al-Sadoon et al. (2018) collected blood samples from 117 female college study at Basrah University (age 19-24 years) for detection the prevalence of anti-Toxoplasma antibodies. Only two females (1.13%) were positive for IgM antibodies.

Such differences between these studies is expected because several demographic factors such as age, marriage, pregnancy, abortion and animal contact have a considerable impact on the seroprevalence of the parasite (Mizami et al., 2017)

The present study showed a significant upregulation of miR-17-3p and miR-92 expression in women with acute toxoplasmosis compared to controls. The relative median expression of these miRNAs was 1.9 and 3.3 folds, respectively in patients compared to 1.1 and 1.7 folds, respectively in controls. This is the first clinical study that...
investigates the gene expression of *miR-17-3p* and *miR-92* in the blood samples from patients with acute toxoplasmosis.

This result is, in part, in accordance with a previous study performed by Cai *et al.* (2013), who used microarray profiling and a combination of conventional and molecular approaches to investigate the levels of miRNAs in human macrophage during *T. gondii* infection. The study disclosed that several miRNAs including *miR-17-92* cluster genes were trans-activated through promoter binding of the signal transducer and activator of transcription-3 (STAT3) following *T. gondii* infection.

A year later, Cai *et al.* (2014) reanalyzed the miRNA expression profile in macrophages infected with *T. gondii* to investigate the precise mechanisms of parasite interference with the apoptotic pathway. Besides the significant increase in *miR-17–92*, the authors reported a decrease in *Bim* expression (pro-apoptotic Bcl-2 family member) in these cells compared to non-infected macrophages. Database analysis of *miR-17–92* miRNAs revealed the potential binding sites in the 3′-untranslated region (3′UTR) of *Bim* which is one of the crucial effectors of pro-apoptosis.

In another study, Zeiner *et al.* (2010) used microarray profiling and northern blotting to explore the gene expression of *miR-17-92* cluster in human foreskin fibroblasts infected with *T. gondii*. Data from microarray analysis revealed that both *miR-17* and *miR-92* families had generally higher expression rate in *Toxoplasma*-infected cells compared to non-infected cells. Of note, the expression of these miRNAs marginally increased at 6hrs post-infection, while there was a marked increase at 12 and 24hrs post-infection.

Furthermore, He *et al.* (2016) showed that *miR-17-3p* is increased in mouse spleen infected with *T. gondii* RH strain. However, knockdown of *miR-17-92* in human fibroblast feeder (HFF) cells had no marked effect on infection, parasite replication or host cell degradation (Zeiner *et al.*, 2010).

Thus, according to the present study and most previous studies, there is an upregulation in the gene expression of *miR-17-92* associated with *T. gondii* infection. This upregulation can have dramatic effects on the infected cells or even the whole body. Transgenic mouse that have been engineered to overexpress *miR-17-92* by 2-fold was found to develop lymphoproliferative disease and autoimmunity and die prematurely. Therefore, even relatively modest increases in the levels of *miR-17-92* can have profound biological consequences *in vivo* (Zeiner *et al.*, 2010).

One explanation for this effect assumes an increase in *pri-miR-17-92* transcriptions in *T. gondii*-infected cells. However, the transcription of *pri-miR-17-92* has been shown to be positively regulated by E2F3 (Woods *et al.*, 2007) and c-Myc (O'Donnell *et al.*, 2005) transcription factors rather than STAT. In *Toxoplasma*-infected HFFs, the expression profile suggests a modest increase in E2F3 level (+1.4-fold), c-Myc (+1.9-fold) compared to uninfected HFFs after 24hrs post-infection. Therefore, it is possible that the *Toxoplasma*-induced upregulation of *pri-miR-17-92* expression is, at least in part, brought about by such a change in host transcription factors (Saeij *et al.*, 2007).
It is worth mentioning that the increases in miR-17-92 are a specific response to Toxoplasma infection. Zeiner et al. (2010) have demonstrated that this subset of miRNAs was affected only during Toxoplasma infection, and there were no such changes in cells infected with closely-related coccidian Neospora.

The current study evaluates the diagnostic value of miR-17 and miR-92 in the context of discrimination between acute toxoplasmosis and healthy controls. The sensitivity and specificity of miR-17-3p were 0.525 and 0.744 respectively, and that for miR-92 was 0.675 and 0.615 respectively indicating a poor diagnostic value. Even the combination of the two miRNAs did not improve significantly their discriminative value. That is because the upregulation of expression of these miRNAs, although specifically associated with T. gondii infection but not with other alike infection; however, it can occur in a large number of non-infectious diseases and conditions. For example, increased serum level of miR-17-92 was reported in some neurological diseases such as anxiety and depression-related behaviors, in certain heart pathologies like myocardial infarction, myocardial ischemia/reperfusion and cardiac aging (Guet et al., 2017). Furthermore, there is a large spectrum of malignancies that involve the upregulation of these miRNAs (Bai et al. 2019). As many of these disorders could not be excluded in neither cases nor controls of the present study, this explains the low sensitivity and specificity of these biomarkers.

In both groups (women with acute toxoplasmosis and healthy controls women) miR-17-3p showed a highly significant positive correlation with miR-92. Despite the fact that the miR-17-92 cluster is controlled by the same promoter and is stimulated by almost a similar transcriptional regulator, the polycistronic structure of this cluster allows that each subset of miR-17-92 may be expressed at different levels (Robaina et al., 2016). Moreover, specificities in half-life and degradation of individual miRNAs can influence its plasma level. Navarro, et al. (2009) found that miR-17 and miR-20a are specifically overexpressed with high Myc mRNA levels in certain types of tumors. Furthermore, Li et al. (2012) found low expression of miR-20a and high expression of miR-19a, miR-19b-1 and miR-92a-1 mRNA levels in cases with TP53 alterations in comparison with healthy subjects. Another study in primary cutaneous B-cell lymphomas found that miR-106a, miR-20a, and miR-20b overexpression associated with PTEN downregulation in patients with B-cell lymphoma (Battistella et al., 2015). However, it seems that miR-17-3p and miR-92 have much in common regarding the transcriptional regulator and plasma half-life. For example, Ballabio, et al. (2010) found downregulation of both of these miRNAs in patients with cutaneous T-cell lymphoma, while Robaina et al. (2016) showed high expression of them in pediatric Burkitt lymphoma with reactive lymph nodes.

The only significant association between miR-17-3p and miR-92 and reproductive characteristics in the current study was that the median expression of miR-17-3p in infected women with < 3 parity was 3.0 (range 0.5 to 21.4) which was significantly higher than that in women with ≥3 parity (median 1.3, range 0.0 to 5.3). Unfortunately, there is no similar study to compare this result and there is no certain explanation; however, an in vitro study revealed that
parity induces downregulation of the Wnt/Notch signaling ratio which is one of the most important regulators of miR-17-92 expression (Meier-Abt et al.,2013).

Collectively, the present data suggest that Gene expression of both miR-17-3p and miR-92 is upregulated in women with acute toxoplasmosis. However, neither miR-17-3p nor miR-92 have a diagnostic value in the context of discrimination between women with acute toxoplasmosis and healthy controls. There is a highly significant positive correlation between miR-17-3p and miR-92 in both women with toxoplasmosis and healthy controls

**REFERENCES**


Zhao C, Sun X, Li L. Biogenesis and function of extracellular miRNAs. ExRNA 2019;1:38