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EXPRESSION OF MicroRNA-132 AND MicroRNA-155 AS A DIAGNOSTIC BIOMARKER FOR ACUTE TOXOPLASMOSIS

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ABSTRACT : MicroRNAs (miRNAs) have been recently established as a key regulator of the host response to infection by the parasite *Toxoplasma gondii*. This study designed to evaluate the gene expression of two microRNAs, miRNA-132 and miRNA-155 in women with acute toxoplasmosis as a possible diagnostic biomarker of the disease. RNA was extracted from blood samples and cDNA was created. MiRNA-132 and miRNA-155 expression levels were assessed in 14 women with acute toxoplasmosis (IgM seropositive) and 6 IgM seronegative women (as control group) by Quantitative polymerase chain reaction (qPCR) using small nuclear RNA (RUN43) as an internal control. Results showed overexpression of miRNA-132 and miRNA-155 in women with acute toxoplasmosis compared to controls with median fold changes of (3) and (4945), respectively and significant difference (p = 0.044) and (p = 0.010). These data indicate that both miRNAs might be valuable diagnostic biomarkers in discriminating acute toxoplasmosis form healthy controls.

Key words : MicroRNA-132, microRNA-155, diagnostic biomarker, acute toxoplasmosis.

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INTRODUCTION

Toxoplasma gondii is a pathogen that causes Toxoplasmosis. The parasitic disease seen in different counteries of the world (Sun *et al*, 2013). In healthy people, most *T. gondii* infections are asymptomatic,but the parasite can cause serious diseases in immunocompromised patients and pregnant women (Gao *et al*, 2012). Acute infection of pregnant mothers causes severe congenital complains such as mental retardation, hearing and sight impairment, and epilepsy in the fetus (Olariu *et al*, 2011).

T. gondii ability to change from the tachyzoite(rapidly replicating stage) to the bradyzoite (dormant tissue cyst stages) made it resistant to host immunity and to treatment. Thus, it is very important to detecte toxoplasmosis in early-stage because it is the practical way in reducing disease and reduce the incidence of sequelae in infected infants by early treatment of infected pregnant women (McAuley, 2014). Furthermore, there is growing 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 is the most effective way of controlling it. The most available tests for the diagnosis of toxoplasmosis are serological-based assays that identify specific anti toxoplasma antibodies. However, almost all these tests have limited sensitivity and specificity (Liu *et al*, 2015). Therefore, seeking alternative tests or biomarkers represents a challenge to ensure rapid and easy screening.

MicroRNAs (miRNAs) included about 21–25 nucleotide non-coding RNA molecules which have an important roles in different pathological and physiological processes in the human body (Zhao *et al*, 2019). Previous research indicated that the expression of several miRNAs such as miRNA-17-92 gene cluster, miRNA-132, miRNA-146a, miRNA-155 and miRNA-23b is influenced by the presence of infectious agents such as *T. gondii* (Xu *et al*, 2013). The identification of a panel of miRNAs using specialized chips may become standard work in various laboratories. Many traditional diagnostic approaches will be replaced, and the idea of diagnosis

will be completely changed, especially when the diagnosis involves a gray zone, as in toxoplasmosis. The goal of this study was to investigate miRNA-132 and miRNA-155 gene expression in women with acute toxoplasmosis in order to assess the diagnostic relevance of these microRNAs in toxoplasma-infected women.

MATERIALS AND METHODS

Subjects

Between April and October 2020, a case-control research was conducted. There were 20 serumc samples from pregnant women in all, which were separated into two groups. Six serum samples made up Group I (healthy). Using an ELISA test, all samples were found to be toxoplasmosis-free. Group II consisted of 14 serum samples that were toxoplasmosis seropositive and both groups the ranged in age from 20 to 45 years.

Anti-toxoplasma IgM and IgG Antibodies detection

Anti-toxoplasma IgM and IgG antibodies were detected in serum samples from all of the women in the study using an ELISA kit (Demeditec, Germany). The standard curve linear regression equation was developed using the concentrations of the standards and their corresponding optical density (OD) values and the OD was then utilized to compute the concentrations. An IgM titer of more than 10 IU/mL is considered positive, according to the manufacturer's guidelines.

RNA extraction

MicroRNA was extracted Using Thermo Scientific, USA. Then concentration and purity of RNA were measured by Quantus Fluorometer.Extrected RNA was stored at -80°C until used.

Steps of RT-PCR

Two steps Reverse Transcription-PCR were applied for the expression of miRNA-132 and miRNA-155. The kit (Promega, USA) was used to extracte the RNAs and then to converte it into cDNAs and we used the reverse transcription primers, which was specific for each miRNA (Macrogen) (Table 1). All other steps were done as mentioned by Jihad and Naif. (2020). The miRNAs was measured by the relative quantitative method using the comparative Ct formula:

Folding = 2^-ΔΔCT, where

ΔCT= Target gene (CT)- Reference gene(CT)

ΔΔCT = Patients (ΔCT) - Control (ΔCT).

Statistical analysis

SAS was used to analyze the data (Statistical Analysis System - version 9.1). To assess significant differences between means, one-way analysis of variance (ANOVA) and the least significant differences (LSD) test were used was to assess the significan difference.

RESULTS AND DISCUSSION

The findings revealed a differential expression pattern of two microRNAs (miRNA-132 and miRNA-155) in patients with acute toxoplasmosis. After normalization with the U6 gene, the results of miRNA-132 expression data revealed a significant increase (P0.01) (Fig. 1) in miRNA-132 levels in patients compared to controls.

These findings agreed with result obtained by Xiao *et al* (2014), who observed a two-fold increase in miRNA-132 expression in human neuroepithelioma cells infected with different strains of Toxoplasma (Type I, Type II and Type III). The rise was linked to dopamine receptor signaling in infection neuropathological processes. Furthermore, an increase in miRNA-132 expression was observed in the peritoneal cells of the infected mice with toxoplasma. In addition, macrophages, neutrophils and dendritic cells were found in the mentioned cell population of infected mice, suggesting that miRNA-132 is involved in the host immune response aganst the parasite (Hill *et al*, 2012).

MiRNA-132 is a neurim miR, a type of microRNA that regulates both neuronal and immune system processes and is thought to operate as a mediator between the two systems (Soreq and Wolf, 2011). It is involved in several neural processes, including neurogenesis, neuronal differentiation, outgrowth, sprouting and synaptic plasticity, in addition to spine density (Hansen *et al*, 2010) and its dysregulation has been linked to a variety of brain illnesses (Miller *et al*, 2012). Furthermore, it was shown

Table 1 : Primer for each MiRNA.

Gene	Primer	Sequence (5' > 3')
MiRNA-132	RT Forward	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACC AGAGCCAAC CGACCA TGGTTGTAACAGTCTACAGCCA
MiRNA-155	RT Forward	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACC AGAGCCAAC TGTTAA GTTTGGCTCCTACATATTAGCA
RNU43	RT Forward Universal Reverse	GTTGGCTCTGGTGCAGGGTCCGAGGTATTCGCACC AGAGCCAACAATCAG GTGAACTTATTGACGGGCG GTGCAGGGTCCGAGGT

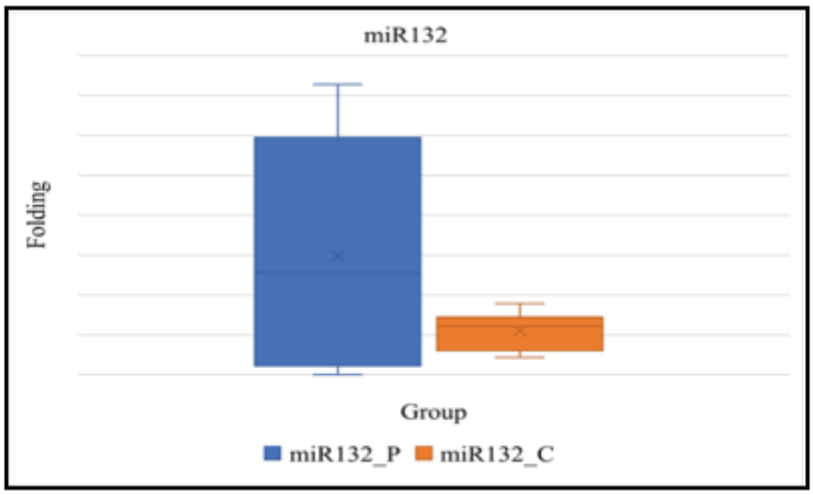

Fig. 1 : Fold expression of miRNA-132 in patients (blue) and control (brown).

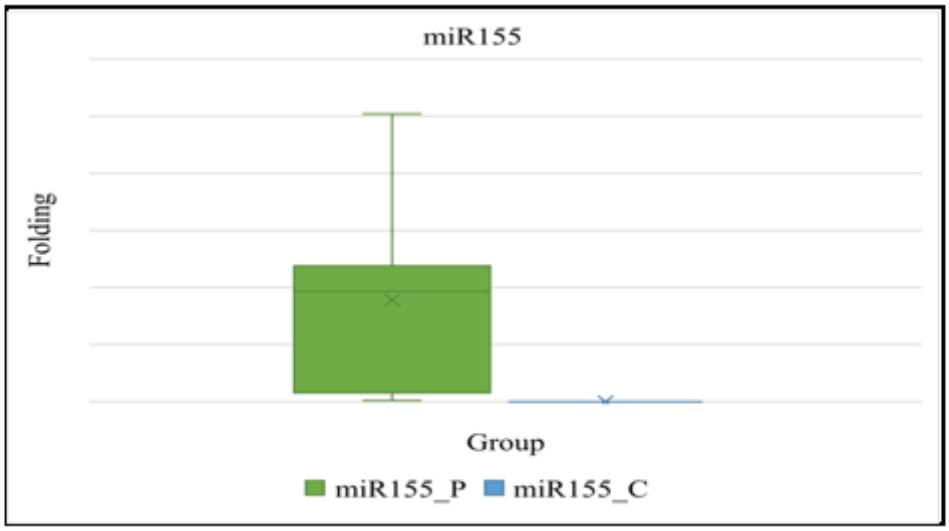

Fig. 2 : Fold expression of miRNA-155 in patients (green) and control (Blue).

that miRNA-132, via targeting the dopaminergic transcription factor Nurr1, promotes the development of dopamine neurons from mouse embryonic stem cells (Yang *et al*, 2012). Another study found a link between Toxoplasma-induced up-regulation of miRNA-132 and changes in the dopamine receptor pathway, which could help explain some neuropsychiatric symptoms like motor disabilities, mental impairment, learning difficulties, and personality changes seen in congenital toxoplasmosis and toxoplasmic encephalitis. When compared to healthy controls, the gene expression of the second microRNA and miRNA-155 were significant (P<0.01) up-regulated in patients (Fig. 2).

The findings of this investigation were consistent with those of Cannella *et al* (2014), who observed that *T. gondii* infection dramatically increased miRNA-155 levels. This induction was strain-independent. Another study found that after mice were infected with RH tachyzoites, the miRNA-155 gene was overexpressed in the spleens of the mice (He *et al*, 2016).

Apart from the innate immune response, MiRNA-155 is an immunomodulatory microRNA that affects on signal pathways for the identification of Th1 response and also acts as a regulator of T-cell and B-cell maturation (Cannella *et al*, 2014). Its derease could result in a reduction in the immunological response mediated by cellular and humeral T cells. Overexpression of miRNA-155 is also known to induce inflammation by targeting SOCS1 mRNA, increasing IFN-, and reducing TNF- (Rao *et al*, 2014).

As a result, these two miRNAs can be employed as a biomarker in pregnant women infected with Toxoplasma to determine the risk of neuro-complications in the fetus.

Competing interests declaration

All of the authors confirmed that there is no competing or personal interests.

Informed consent

All patients and controls gave us the permission to conducte this study before it could begin.

REFERENCES

Cannella D, Brenier-Pinchart M P, Braun L, van Rooyen J M, Bougdour A and Bastien O (2014) miR-146a and miR-155 delineate a MicroRNA fingerprint associated with Toxoplasma persistence in the host brain. *Cell Rep*. **6**, 928–937.

Gao J, Zhao J, He H, Wang T, Yang B and Chen G (2012) *Toxoplasma gondii* infection in pregnant women in China. *Parasitology* **139**(2), 139–147.

Hansen K F, Sakamoto K, Wayman G A, Impey S and Obrietan K (2010) Transgenic miRNA132 alters neuronal spine density and impairs novel object recognition memory. *PLoS One* **5**, e15497.

Hill D, Gouffon S, Saxton M and Su C (2012) Differential gene expression in mice infected with distinct Toxoplasma strains. *Infect Immun.* **80**, 968–974.

Jihad N A and Naif H M (2020) Evaluation of microRNA-20, -21 and -143 expression in human papilloma virus induced premalignant and malignant cervical lesions. *Gene Reports* **20**, 100702. doi:10.1016/j.genrep.2020.100702

Liu Q, Wang D, Huang Y and Zhu Q (2015) Diagnosis of toxoplasmosis and typing of *Toxoplasma gondii*. *Parasit Vectors* **8**, 292.

McAuley B (2014) Congenital toxoplasmosis. *J. Ped. Infect. Dis. Soc.* **3**(suppl_1), S30-S35.

Miller H, Zeier Z, Xi L, Lanz A, Deng S, Strathmann J, Willoughby D, Kenny J, Elsworth D, Lawrence S, Roth H, Edbauer D, Kleiman J and Wahlestedt C (2012) MicroRNA-132 dysregulation in schizophrenia has implications for both neurodevelopment and adult brain function. *Proc Natl Acad Sci USA* **109**, 3125–3130.

Montazeri M, Mehrzadi S, Sharif M, Sarvi S, Shahdin S and Daryani A (2018) Activities of anti-Toxoplasma drugs and compounds against tissue cysts in the last three decades (1987 to 2017), a systematic review. *Parasitol Res*. **117**(10), 3045-3057.

Olariu R, Remington S, McLeod R, Alam A and Montoya J G (2011) Severe congenital toxoplasmosis in the United States: clinical and serologic findings in untreated infants. *The Pediatric Infect. Dis. J.* **30**(12), 1056-1061.

Soreq H and Wolf Y (2011) NeurimmiRs: microRNAs in the neuroimmune 766 interface. *Trends Mol Med.* **17**, 548–555.

Sun X, Lu H, Jia B, Chang Z, Peng S and Yin J (2013) A comparative study of *Toxoplasma gondii* seroprevalence in three healthy Chinese populations detected using native and recombinant antigens. *Parasit Vectors* **6**(1), 241.

Xiao J, Li Y, Prandovszky E, Karuppagounder S, Talbot C and Dawson L (2014) MicroRNA-132 dysregulation in *Toxoplasma gondii* infection has implications for dopamine signaling pathway. *Neuroscience* **268**, 128–138.

Yang D, Li T, Wang Y, Tang Y, Cui H, Tang Y, Zhang X, Chen D, Shen N and Le W (2012) MiRNA-132 regulates the differentiation of dopamine neurons by directly targeting Nurr1 expression. *J Cell Sci.* **125**, 1673–1682.

Zhao C, Sun X and Li L (2019) Biogenesis and function of extracellular miRNAs. *ExRNA* **1**(1), 1-9.