Endocervical miRNA-142 Expression in Patients with Pelvic Inflammatory Disease Positive for *Chlamydia trachomatis*

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Abstract

Background: The most common cause of sexually transmitted diseases that can lead to pelvic inflammatory disease (PID) and infertility is *Chlamydia trachomatis*. Chlamydial infections are characterized by multifocality and polymorphism changes. Chlamydia causes inflammation in the adult urethra and cervix with the possibility of serious complications, and can cause perinatal infections in infants. Highly conserved single-stranded noncoding RNAs called micro-RNAs (miRNAs), which control gene expression, have now been identified as significant effect in disease conditions. MiRNAs play important regulatory roles in various biological processes for example, differentiation, development, proliferation, apoptosis, cell cycle control, and metabolism. Changes in miRNA expression have been identified in many diseases, such as cardiac and autoimmune disorders, schizophrenia, and cancer. In this cross-sectional pilot investigation, samples were taken from control women who were not infected and from symptomatic women who were infected with *C. trachomatis*. **Objectives:** This work investigates the changes in the host miRNA-142 expression profile during chlamydial infection and not infected control group women **Materials and Methods:** The study included 200 female patients who were sampled, including endocervical swab from each female patient. Patients ranged in age from 20 to 45 years. A control group of 25 healthy ladies was also used. *Chlamydia trachomatis* was determined by conventional polymerase chain reaction (PCR) whereas miRNA-142 was detected by RT-qPCR. **Results:** All 200 collected endocervical swab specimens were diagnosed by the gynecologists as having PID. From all 200 collected endocervical swab specimens, it was found that only 22/200 (11%) were positive for *C. trachomatis* and the current study shows that the expression of the micro-142 gene elevated in *C. trachomatis* patients when compared to the control group, with the expression of the gene increasing more than 40% when compared to the control group. **Conclusions:** This study emphasizes the relevance of *C. trachomatis* infection in the female from 20 to 45 years in population of Iraq in Hilla city between February and August 2022. It also demonstrates the diversity of miRNA-142 expression involved in genital infection.

Keywords: *Chlamydia*, endocervical, miRNA, pelvic inflammatory disease, sexually transmitted infections

Introduction

The main cause of sexually transmitted infections is the obligatory intracellular bacterium *Chlamydia trachomatis*. The most common cause of pelvic inflammatory disease (PID) in the globe is *Chlamydia trachomatis*.[1] Taking into account the antigenic differences in the major outer membrane protein, *C. trachomatis* strains are categorized into three biovars: the trachoma biovar (serovars A–C); the genital biovar (serovars D–K); and the lymphogranuloma venereum biovar (serovars L1–L3). Although prototype serovar L2 strains are typically employed in investigations on host cell-C. trachomatis interactions, genital strains of *C. trachomatis* are responsible for the majority of infections.[2] Multiple sequelae can result from *C. trachomatis* infection among women, the most serious of which include PID, ectopic pregnancy, and infertility.[3]

Noncoding micro (22-nucleotide) RNAs are one promising biomarker (micro-RNAs or miRs). These are long-lasting, extensively dispersed compounds that are

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linked to inflammatory responses to human infections. In both animal models and *Chlamydia*-infected humans, miRNAs have been demonstrated to have a role in the pathogenesis of the *chlamydial* infection.[4] Micro-RNAs were associated with virulent but not avirulent strains of *chlamydial* infection cervical miR-142 expression on day 4 correlates with pathology on day 35 in *Chlamydia* infection.[5] miR-142 and/or miR-147 may be a marker for the signs or symptoms of a *Chlamydia* infection.[6] Obesity and excessive weight affect not only overall health but also reproductive health.[7] Folic acid is one of the important vitamins required for embryonic growth and development, as well as preventing the occurrence of congenital malformations.[8]

Materials and Methods

Subject of the study

Case–control analysis was used in this study (retrospective). Patients admitted to the Gynecology and Obstetrics outpatient clinics of two hospitals in Babylon Province provided clinical samples between February and August 2022. 25 endocervical swab were taken from healthy individual as control group.

A total of 200 female patients' endocervical swab samples were obtained for the research. The ages of the patients spanned from 20 to 45. These ladies were diagnosed with PID by their gynecologists based on symptoms, signs, an ultrasound of the abdomen and pelvis, and the existence of risk factors, and all of which met the criteria for PID outlined in the national recommendations for the condition.[9] All the 200 endocervical swab samples were collected according to Abdella *et al.*[9]

Ethical approval

The necessary ethical approval was obtained by verbal consent from patients. This work was authorized by the publication ethics committee of the College of Medicine in Babylon Province, Iraq (reference: BMS/0203/016; date: June 18, 2014).

DNA extraction and polymerase chain reaction protocol

DNA extraction was performed on endocervical swabs taken from patients. That was done in accordance with the instructions provided by the manufacturer, Geneaid catalogue number (GSK004, GSK100, GSK300). The acquired DNA was processed for *C. trachomatis* molecular identification using one pair of housekeeping OMP (outer membrane protein) gene conventional polymerase chain reaction (PCR) primers and stored at 2–8°C for later applications. Table 1 lists the gene sequences and the associated circumstances.

With the aid of agarose gel electrophoresis and ultra violate (UV) light, PCR amplification was verified. A 70-volt electric current was allowed to flow for 50 min. The DNA bands were observed using a UV transilluminator at 280 nm, and digital camera was used to photograph the gel.[11]

RNA extraction and protocol

After collection of endocervical swab from patients and healthy individual the total RNA was extracted according to the TRI RNA PURE KIT (Geneaid) Catalogue Numbers (TRP050, TRPD050, TRP100, TRPD100, TRP200, TRPD200) as the manufacture's protocol and then the total RNA was store at –20°C.

Gene expression of microRNA-142

The real-time qPCR reactions were performed by using specific primers targeting reference gene *GAPDH* and the target genes microRNA-142. Conversion the total RNA to cDNA and amplification of DNA was done according to instructions provided by GoTaq® 1-Step RT-qPCR System (Promega) using BRYT Green® dye, where RT-qPCR Mixture and conditions were summarized in Table 2, where the final volume of RT-qPCR reaction was 20 µL. Relative expression fold was calculated by delta method (2^-(ΔΔCt) according to Livak and Schmittgen.[12]

Table 1: Sequencing of *Chlamydia trachomatis* OMP gene primer and polymerase chain reaction conditions

Gene's name	Primer sequences (5′- 3′)	Conditions	Reference
Designation genes			
Chlamydia OMP	F-5'CCTGTGGGGAATCCTGCTGAA3' R-5'GTCGAAAACAAAGTC ACCATAGTA 3'	94°C, 5 min 1× 94°C, 30 s 56°C, 30 s 35×72°C, 30 s 72°C, 7 min 1×	[10]

Table 2: MiR-coding gene primer with RT-qPCR conditions

Genes name	Primer sequences (5′–3′)	Condition	References
MiR-142	F-5'GGTGGGTCATAAAGTAGAAAG3' R-5'GAGCAGGGTCCGAGGT3'	37°C, 15 min 1× 95°C, 10 min 1×	[12]
GAPDH-F	GTCTCCTCTGACTTCAACAGCG	95°C, 10 s	
GAPDH-R	ACCACCCTGTTGCTGTAGCCAA	60°C, 30s 40× 72°C, 30 s	

Statistical analysis

Chlamydia trachomatis frequency distribution was presented in the form of percentages and histograms; genetic analysis was performed using the chi-square (*2) test. *P* value under 0.05 is taken into consideration. The statistical analysis was carried out using SPSS 19 version. displaying information as a mean (ISD).

Results

Molecular identification of *Chlamydia trachomatis*

DNA was taken from all 200 collected endocervical swab specimens; traditional PCR was performed utilizing these DNA samples for the amplification of particular housekeeping OMP primers; according to the sequences and program given in Table 1. After that, gel

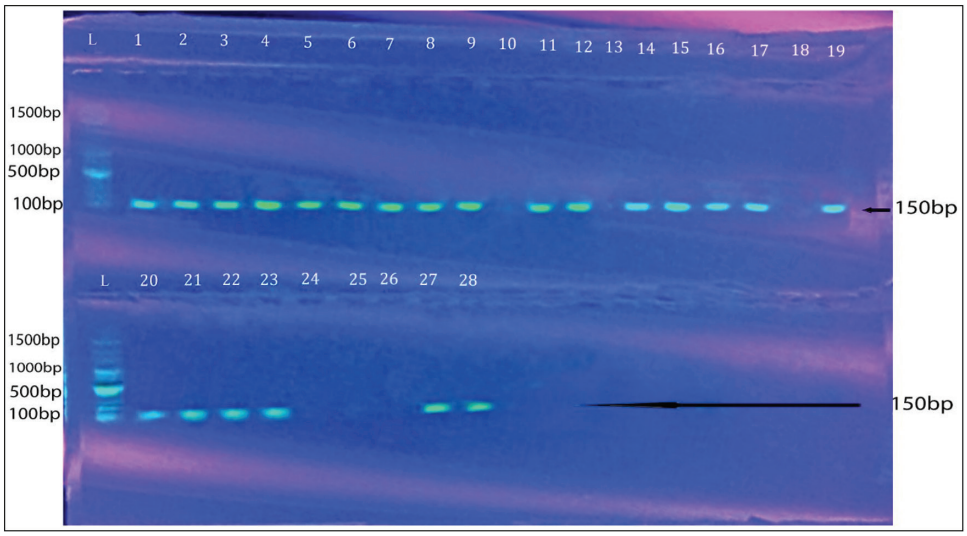

Figure 1: After staining with red safe stain, OMP polymerase chain reaction products were electrophoresed on a 1.5% Agarose gel at 70 V for 50 min. 1500 bp ladder, L; lane (1, 2, 3, 4, 5, 6, 7, 8, 9, 11, 12, 14, 15, 16, 17, 19, 20, 21, 22, 23, 27, and 28). Endocervical swabs from patients with pelvic inflammatory illness tested positive for *Chlamydia trachomatis*. The amplicons are 150 bp in size

Table 3: microRNA-142- fold gene expression among control and patients versus the reference gene (GAPDH)

Groups	No.	Expression levels (2 ^ -(ΔΔCt))		SE
		Mean	SD	
Control	25	1.69	2.45	0.55
Patient	32	42.52	29.77	5.43
P value		<0.0001*		

* Represent a significant difference at $P \leq 0.05$

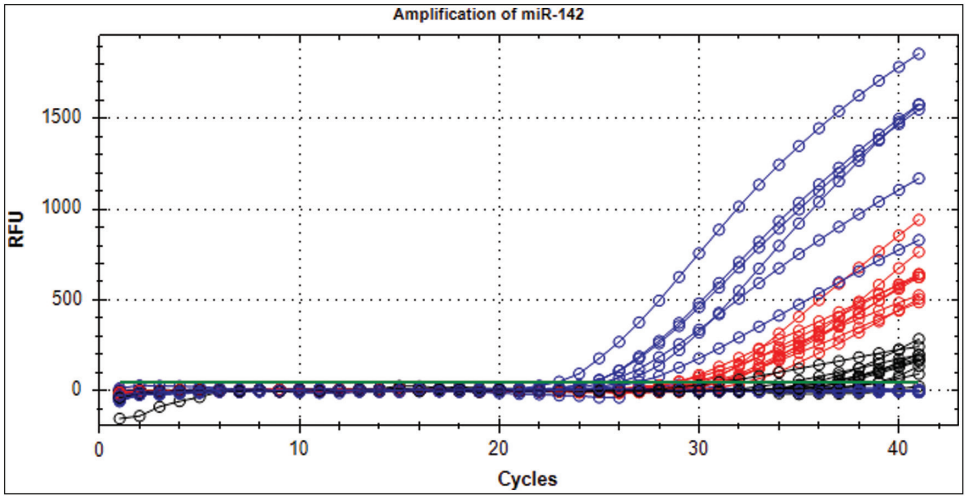

Figure 2: microRNA-142 gene expression level. This is the first run for 15 samples. Blue lines represent amplification of Reference gene (GAPDH). Red lines represent amplification of samples for Patients. Black lines represent amplification of control samples. RFU = relative fluorescence units

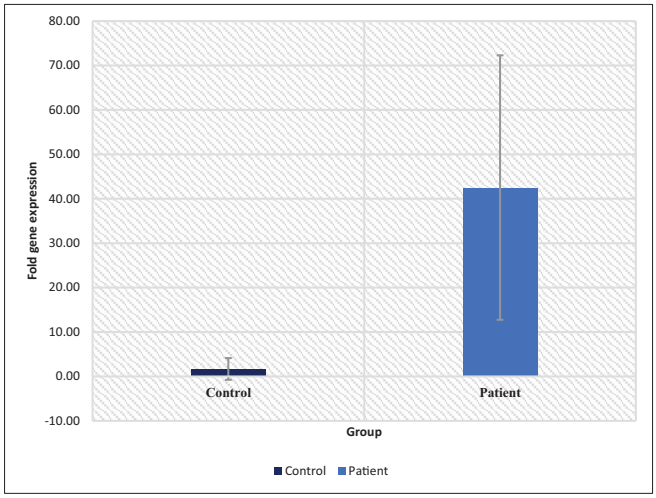

Figure 3: microRNA-142-fold gene expression among control and patients versus the reference gene (GAPDH)

electrophoresis revealed that, when compared to an allelic ladder, only 22/200 (11%) of the 200 samples produced the specific 150 bp DNA fragment, as shown in Figure 1.

Gene expression of miRNA-142 level by using real-time polymerase chain reaction

In order to study the gene expression of microRNA-142, a total of (22) patient endocervical swab samples that were positive for Chlamydia trachomatis. RNA extract were used. Using **(ΔΔ**Ct) PCR methods, the level of expression to microRNA-142 genes in the test sample as well as in the control sample was normalized with housekeeping gene. The current study shows that the expression of the micro-142 gene elevated in *Chlamydia Trachomatis* patients with PID when compared to the control group, as shown in Table 3 and Figures 2, 3.

The relative microRNA expression levels of target genes as described previously by using PCR efficiencies and mean crossing point deviation between samples and controls, represent a significant difference at $P \leq 0.05$.

Discussion

The prevalence of *C. trachomatis* infections that lead to PID has been detect in numerous studies; one of these studies found that 8%–10% of women with chlamydial infection also had PID.[13]

Women's susceptibility to chlamydial genital infection varies by demographic, community, and exposure to a variety of risk factors. Perhaps 10%–30% of all PID cases are caused by *C. trachomatis*, which has been identified as a prominent cause of PID and female infertility globally.[14] One of the most common sexually transmitted germs is *C. trachomatis*. Chlamydial infection, which rises from the lower vaginal canal, can have major reproductive effects, such as infertility. Chlamydia was thought to be responsible for 45% of tubal infertility cases.[15]

A significant contributing factor to PID is a sexually transmitted bacterium, and as PID has been linked to an increased risk of infertility, it serves as a bridge between chlamydia infection and infertility.[16]

Furthermore, because the pathogen's intracellular position makes routine diagnosis difficult, the screening tests had to be straightforward and affordable diagnostic assays.[17]

The frequency (11%) of this study contrasts with other studies carried out in the Arabic World, which had frequencies of (5.3%) in Qatar[18] and (15%) in Saudi Arabia.[19] In addition, two separate examinations in Iran revealed that the prevalence of *C. trachomatis* was, respectively, 8.3% and 22%.[20]

The present research found that the expression of the micro-142 gene increased in *C. trachomatis* patients when compared to the control group, with the expression of the gene increasing more than 40%fold when compared to the control group, as shown in Figures 2 and 3. MicroRNA-142 was shown to be significantly up regulated in comparison to the control group.

According to Benyeogor *et al.*,[21] miRNAs were prevalent in both primary *Chlamydia* infection and reinfection, and the number of miRNAs expressed fluctuated each week following infection and reinfection.

Additional research showed that the *C. trachomatis* sign and symptom group (CTSS) had significantly higher expression levels of miR-142 and miR-147, whereas miR-155 was expressed in both the CTSS and *Chlamydia* Trachomatis not sign and symptom groups,[5] whereas miR-142 and/or miR-147 may be markers for signs or symptoms.[22] The miRNAs expression profile was different in the reproductive tissues after a reinfection, with a greater number of miRNAs expressed after reinfection and in PID. Also, the number of miRNAs expressed each week after chlamydia infection and reinfection varied, with weeks eight and one having the highest number of differentially expressed miRNAs for chlamydia infection and reinfection respectively. Ten miRNAs; mmu-miR-378b, mmu-miR-204-5p, mmu-miR-151-5p, mmu-miR-142-3p, mmu-miR-128-3p, mmu-miR-335-3p, mmu-miR-195a-3p, mmu-miR-142-5p, mmu-miR-106a-5p, and mmu-miR-92a-3p were common in both primary chlamydia infection and reinfection with high rate in reinfection and with PID.[23] Other study indicated that the expression of a specific miRNA profile associated with a virulent variant early in the infection course may be predictive of an increased risk of PID, allowing more aggressive treatment before significant pathology develops.[24]

Conclusions

Several potential biomarkers such as microRNA-142 may be considered as criteria for PID and progressive of diseases caused by *C. trachomatis*.

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Conflicts of interest
There are no conflicts of interest.

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