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ORIGINAL ARTICLE

Gene Expression Variations of *mirRNA US5-1* in Cytomegalovirus and *mirRNA181a* in Humans and Their Use as Prognostic Factors for the Risk of Miscarriage in Women in Diwaniyah Governorate, Iraq

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Key words:
mirRNAUS5-1, mirRNA 181a, miscarriage, Human cytomegalovirus (HCMV)

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ABSTRACT

Background: *Human cytomegalovirus (HCMV) can cause serious complications in pregnant women and their fetuses.* ***Objective:*** *the aim of this works is to study the gene expression role of miRNAUS5-1 and miRNA181a in women with HCMV-associated miscarriages.* ***Methodology:*** *From January 2024 and September 2024, 50 healthy participants and 50 patients with miscarried were enrolled in this study at of the Maternity and Children's Hospital. The real-time polymerase chain reaction (RT-PCR) test was used to test viral microRNA and human microRNA to detect genetic differences in their expression in women with HCMV-associated miscarriages.* ***Results:*** *Aborted women with CMV levels of miRNA-181a were substantially higher ($P<0.05$) than those of control participants, while the mean expression of mir-US5-1 gene in women who had miscarriages due to CMV was 5.49 ± 1.20, this gene was not expressed in the healthy control group* ***Conclusion:*** *Our results proved that there was an important immunological role of gene expression in women with HCMV-associated miscarriages.*

INTRODUCTION

Cytomegalovirus (CMV) comprises a spectrum of clinical manifestations, extending from mild indications to tissue invasion, leading to both direct and indirect consequences. The traditional assessment is that CMV reactivation and the resulting medical sickness are primarily attributed to disease-associated immunodeficiency. However, prior evidence from human has demonstrated that inflammatory signals can trigger the expression of latent viral genes[1].

The term "miscarriage" is the term used to describe the unplanned termination of a pregnancy before week 24[2.] Miscarriage is a major risk complication of pregnancy, with an incidence of 2-6% of couples who are able to conceive[3]. Reasons of recurring failure comprise physiological abnormalities, anatomical and environmental features, certain infections such as cytomegalovirus, blood clotting disorders, autoimmune illnesses, and genetic factors for instance chromosomal abnormalities[4,5].

It is one of the most common DNA viruses, because it spreads easily through bodily secretions and fluids, with HCMV infection rates extending from 65% to 80% in developing countries and from 80% to 100% in developed nations among pregnant women[6,7].

MicroRNAs, also known as miRNAs, are tiny, single-stranded RNA molecules with about 22 nucleotides, non-protein-coding, and are transcribed by RNA polymerases II and III. MicroRNA regulates vital activities comprising immune response, growth, metabolism and hematopoiesis, which play a significant role in preserving regular physiological purposes. In addition, microRNA productions is essential part in cell proliferation, cell death, cancer development and disease diagnosis[8]. There are also virally encoded miRNAs (v-miRs). Studies have shown that there are more than 250 v-miRs and they play critical roles in viral diseases[9]. Viral miRNAs may evolve to regulate mRNA expression based on necessity through cleavage and non-cleavage patterns. For example, for viral replication, merely one miRNA is requisite to control the main mRNA, and it might survey a cleavage pattern. However, there are many v-miRs that control viral mRNA, such as miR-BART-1p encoded by EBV, which follows a non-cleavage pattern[10]. Numerous studies have shown that miRNAs production is character in viral pathogenesis through regulating host genes modified by miRNAs, promoting viral replication, and modifying the host immune system's resistance to the virus[11].

Mir181a also influences the immune system before birth, in addition to its dynamic effects throughout life. In immune cell processes, *miR-181a* shares many common targets with these cells. It is elaborate in the growth, stimulation, and proliferation of T cells. In natural killer (NK) cells, miR-181 stimulates the growth of hematopoietic progenitor cells (HPCs)[12]. This kind of miRNA has numerous characters in the progress, differentiation, activation, enlargement, and influencing functions of immune cells[13]. While miR-US5-1 of CMV targets endoplasmic reticulum aminopeptidase1, it contributes to immune evasion. CMV has approved multiple approaches to operate host immune reactions. Among these strategies, one of the most fascinating is the expression of viral microRNAs, or miRNAs. The existing imperfect picture of the communication between viral miRNAs and host immunity points to the need for a better characterization of host genetic factors[14]. Recent searches have elucidated the character of miRNAs in human cytomegalovirus (HCMV) and the potential mechanism of infection. It was discovered that seven miRNAs encoded by HCMV target putative immune evasion mediators and facilitate viral replication. It has also been proposed that miR-US5-1 dramas a significant role in HCMV immune evasion[15].

METHODOLOGY

Patients

A total of 126 individual were enrolled in this study carried out in the Maternity and Children's Hospital, as well as from a number of medical labs and outpatient clinics in the Diwaniyah Governorate from January 2024 and September 2024. Patients were divided into the followings: 76 individuals Women who experienced repeated miscarriages, and 50 healthy were included in this study as control; all healthy individuals have a negative history and clinical evidence of any other disease. Blood samples were collected by venipuncture. They were placed in a gel tube and left to clot for approximately half an hour. The serum was then centrifuged at 3,000 rpm for 15 minutes and kept in Eppendorf tubes at -20°C. This was used for ELISA tests to detect HCMV antibodies

Ethical Considerations

Approval for the study was obtained from the Institutional Ethics Committee of the College of Science at the University of AL-Qadisiya (2778/ 15/1/2024).

Rapid Diagnostic Test

This test was performed to detect the presence of specific antibodies IgM, IgG in the serum against human cytomegalovirus. The kit consists of the following components, the producing company and the country as shown in table 1.

Table 1: kit Components of Rapid Diagnostic Test of HCMV

No.	Description	Company	ountry
1	Test cassette (HCMV AB IgM/IgG)	Shandong	China
2	Disposable dropper	Shandong	China

Primers

This study used the Sanger Center miRNA database registry to select miRNA sequences and the miRNA Primer Design Tool to design the qPCR primers for Human *miR-l81a and CMV mir-US5-1*, which are used for straight recognition of them via Real-Time PCR. In dissimilarity, the NCBI-Database and Primer3 Plus proposal online were used to design the qPCR Housekeeping gene (GAPDH) in this investigation. The Macrogen Company in Korea supplied these primers, as shown in table (2).

Table 2: Primers for Human and *HCMV*

PRIMER		SEQUENCE (5'-3')
CMV miRNA universal RT		GTCGTATCCAGTGCAGGGTC CGAGGTATTCGCACTGGATA CGACACGCTC
mir-US5-1 qPCR primer	F	AACAGTGTGACAAGCCTGAC
	R	GTCGTATCCAGTGCAGGGT
PRIMER		**SEQUENCE (5'-3')**
Human miRNA universal RT		GTCGTATCCAGTGCAGGGTC CGAGGTATTCGCACTGGATA CGACACGCTC
miR- l81a qPCR primer	F	AACAGTGTGACAAGCCTGAC
	R	GTCGTATCCAGTGCAGGGT
GAPDH qPCR primer	F	AAAATCAAGTGGGGCGATGC
	R	TTCTCCATGGTGGTGAAGAC G

ELISA kits

HCMV IgG and IgM ELISA Test (kit) was used in the current study for specific recognition of HCMV IgG, IgM levels in Human blood (serum) samples, following the manufacturer's instructions (DRG Instruments GmbH, Germany).

Statistical analysis

Microsoft Office Excel 2010 and the statistical package for social sciences (SPSS) version 26 were used to collect, gather, analyze, and present the documents. Independent sample t-test was utilized to inspect mean alterations between any two groups. As

long as the variable is normally distributed, the one-way anova examination was utilized to inspect mean differences between more than two groups. To explore the connection between any two categorical variables, the chi-square test was employed. Any two numerical variables could be correlated using Pearson correlation, and the results were expressed as the level of significance (P) and correlation coefficient (r). P-values less than 0.05 were observed as important, and 0.01 or less was regarded as highly significant[16].

RESULTS

Rapid diagnosis of Cytomegalovirus (CMV)

The present study enrolled 126 samples from (76 patients with abortion and 50 women healthy control) to investigate cytomegalovirus (CMV) by using rapid diagnostic test (cassette) and the results was shown in (figure 1). The present results show 50 (65.8%) of patients with abortion have positive results by rapid diagnostic test. But the present results show all healthy women subjects have negative results by rapid diagnostic test.

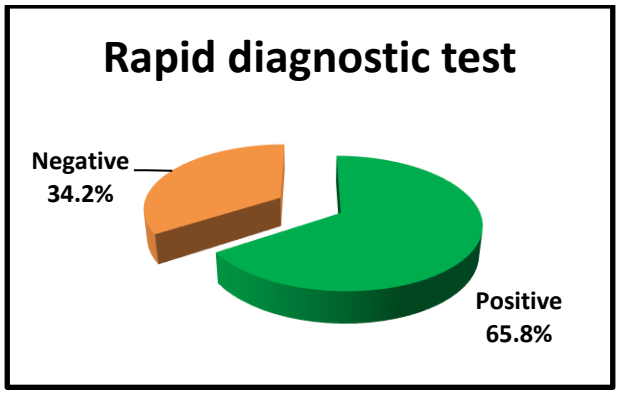

Fig.1. Bi-chart of Rapid diagnosis of Cytomegalovirus (CMV)

Detection of Cytomegalovirus (CMV) by ELISA technique

To confirm the Cytomegalovirus (CMV) infection, the suspected patients and healthy control subjects were submitted to IgM and IgG detection by ELISA technique and the outcomes are demonstrated in table (3). The fallouts display 20 (40.0%) of patients have lively CMV contagion through judgment positive outcomes for IgM. While the IgG verdicts display all of patients have active or previous CMV infection, 50 (100.0%). While all healthy control subjects 50 (100.0 %) have negative results of both IgM and IgG, and the difference was highly significant, (P< 0.001).

Table (3): Prevalence *CMV* infection according to ELISA technique in studied groups.

ELISA	*Patients n =50*	*Control n = 50*	*P value*
IgG			
Positive, *n* (%)	50 (100.0%)	0	< 0.001 ¥ HS
Negative, n (%)	0	50 (100.0%)	
IgM			
Positive, *n* (%)	20 (40.0%)	0	< 0.001 ¥ HS
Negative, n (%)	30 (60.0%)	50 (100.0%)	

GAPDH Expression Quantification via RT-PCR

The Ct assessment of GAPDH, the housekeeping gene charity in the present training is the mean of Ct value for GAPDH CMV patients group was (23.23), for the in control group stayed (22.84). Basic Assumption of Household Molecular Genes Used Studies show that their appearance persistent in the cells or tissues underneath Achievement. One of the most common household genes associated with gene expression documents is GAPDH (Barber et al., 2005). It considered the expression of 1718 genes by qRT-PCR, which used GAPDH as a locus gene in 72 types of regular humanoid tissue. Figures (2) show GAPDH amplification diagrams and dissociation curves.

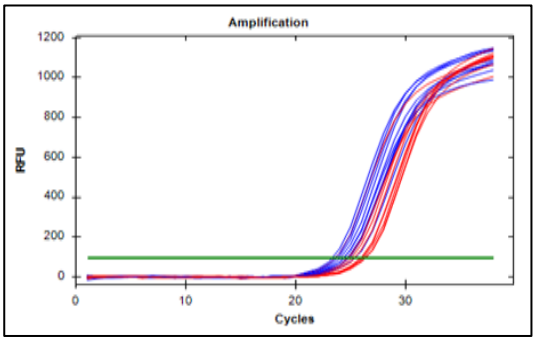

Fig.2. The Real Time PCR amplification plots of housekeeping (GAPDH) gene expression patient and healthy control testers. The blue plots patients and the red plots (control).

Real time PCR Quantification of miRNA-181a Expression

In the CMV patients, the mean Ct value of miRNA-181a amplification was (24.25). Although the control group's Ct values were mean (26–49), the control group's mean Ct values were higher than the CMV group's. For every sample, a duplicate of each quantificative PCR reaction was conducted. Each run included non-template and non-primer controls in addition to CMV and control samples. This was necessary to specify the calibrator and to achieve the statistical scheming for every group. Each run's plots,

including the dissociation curves and amplification plots, were captured. The amplification plots and dissociation curves for miRNA-181a are displayed in Figure (3).

In this study, a quantitative analysis of RT-PCR analyzed Expression of miRNA-181a and comparison of its expression among, speciously healthy grouping and the CMV group. The change in gene appearance was calculated by means of a relation quantitative measurement. This is based on the normalization of the Ct values for scheming ΔCt and represents the variance between the average Ct values of the miRNA-181a augmentation replica for every case and case of GAPDH.

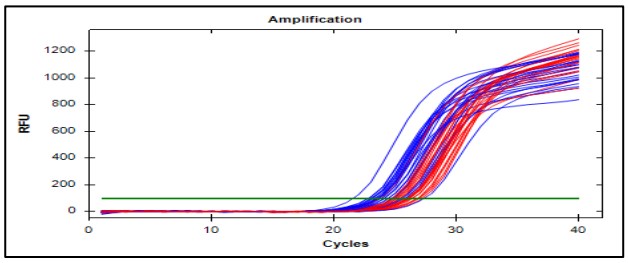

Fig.3. The Real Time PCR amplification plots of miRNA 181a gene expression patient and healthy control samples. The blue plots patients and the red plots (control).

In scheming the relation expression of *miRNA-181a gene* in both groups the 2-ΔCt outcomes stood functional. The comparison of *miRNA-181a gene expression* between aborted females have CMV and healthy controlling subject has been accepted out and the results stayed confirmed in table (4). The mean of *miRNA-181a* gene expression were 9.07 ± 1.40 and 1.0, in aborted women with CMV and healthy controlling issue separately; the flat was greatly important upper than in aborted females have CMV in appraisal with healthy controlling (P < 0.001)

Table 4: Appraisal of mean of *miRNA-181a gene expression* between patients and healthy controls

miRNA-181a expression	**patients *n* = 50**	**Control *n* = 50**	***P***
Mean± SE	**9.07 ± 1.40**	**1.0**	**< 0.001 † HS**
Range	0.12 – 31.39	0.09-6.76	

RT-PCR measurement of the expression of mir-US5-1

The CMV patients had a mean Ct value of 7.57 for mir-US5-1 amplification. In contrast, the control group's Ct values were mean (zero). For every sample, a duplicate of each quantificative PCR reaction was conducted. Each run included non-template and non-primer controls in addition to CMV and control samples. This was necessary to specify the calibrator and to achieve the statistical designs for each group. Each run's plots, including the dissociation curves and amplification plots, were captured. The dissociation curves and amplification plots for mir-US5-1 are displayed in Figure (4)

In this study, a quantitative analysis of RT-PCR analyzed Expression of *mir-US5-1* and comparison of its appearance between, deceptively healthy group and the group CMV. The change in gene appearance stood calculated by a relation quantitative measurement. This is based on the standardization of the Ct values for scheming ΔCt and represents the variance between the average Ct values of the *mir-US5-1* augmentation replica for every case and case of GAPDH

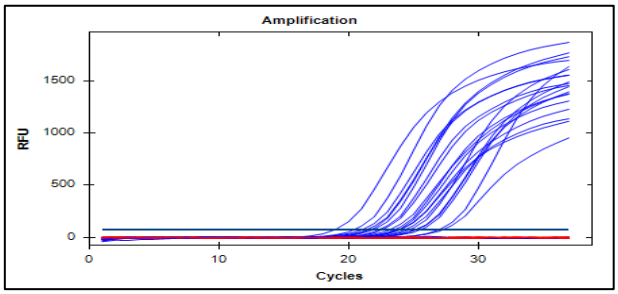

Fig.4. The RT-PCR augmentation plots of *mir-US5-1 gene* expression patient and healthy control samples. The blue plots patients and the red plots (control)

In scheming the relation expression of mir-US5-1 gene in both investigation groups the 2-ΔCt results was applied. The comparison of mir-US5-1 gene expression between aborted women with CMV and healthy controlling subject has been approved out and the fallouts were proved in table (5). The mean of mir-US5-1 gene expression in aborted women with CMV was 5.49 ± 1.20 but this gene not expressed in healthy control.

Table (5): Comparison of mean of *mir-US5-1 gene expression* between patients and healthy controls

mir-US5-1 expression	**patients *n* = 50**	**Healthy *n* = 50**	***P***
Mean± SE	**5.49 ± 1.20**	**Zero**	**< 0.001 † HS**
Range	0 – 84.07		

DISCUSSION

The rate of development of antibodies to cytomegalovirus (CMV) during pregnancy varies widely. Depending on the virus's accessibility, invasiveness, and prevalence in the community, the increased incidence of infection may be attributed to a lack of education and awareness about the possibility of

avoiding the risk of miscarriage if there is continuous and early detection. It may also be attributed to lower socioeconomic status and low levels of education, contributing to the high prevalence of CMV Akunaeziri UA[17]. Since the virus often becomes active during the reproductive years, unlike other types of infection, and can be spread to the fetus in spite of motherly immunity, CMV contagion through pregnancy poses a significant challenge, as it leads to miscarriage in most cases , 40% of cases of CMV infection during pregnancy permission concluded the placenta and infect the fetus, which can lead to CMV disease[18]. CMV is endemic in most parts of the world, with CMV seroprevalence, which diverges geographically, ranging from 30 to 100% . Early and accurate diagnosis of infection before its effects appear, as well as minimum ranks of pathogenicity, are essential, both of which can advantage from a molecular method [19].

Previous studies in Babylon city also agreed with Khikani, et al.,[20] where the samples were serologically positive for IgG at the highest percentage (78.0%), and IgM at (16.0%). While the outcomes of our training vary from the fallouts of a study accessible via[21] in Samarra city, Another study by Al-Moussawi et al.,[22] in Babylon Governorate where the IgM rate was higher in women infected with human cytomegalovirus compared to IgG using the ELISA method. IgM antibodies may indicate a new contagion with human cytomegalovirus (HCMV) or reinfection with a novel strain. Over 70% of infections during the first trimester of pregnancy can result in miscarriage because HCMV is the most prevalent cause of congenital infectious disease. Based on pregnancy complications, the seroprevalence rates of HCMV IgM and IgG antibodies and their correlation with recurrent miscarriages may pose a serious risk[23].

The expression of *miR-181a* is different in different weeks of pregnancy. It can be suggested as a biomarker to estimate the exact time of delivery, which may reduce the side effects of miscarriage and premature birth for mother and fetus. This study indicates the essential character of miRNAs in recurring miscarriage. Our outcomes illustration that dysregulation of some miRNAs in blood is closely related with a great incidence of recurring miscarriage and suggest their use as initial diagnostic and prognostic biomarkers. The comprised investigations highpoint the involvement of miRNAs in essential signaling pathways linked to the growth and differentiation of trophoblasts, embryo activation and implantation, immune tolerance, and endometrial reception. Dysregulation of their expression performs to have severe concerns in these manners, leading to miscarriage.

The current study agreed with a study by An et al.,[24]. where miR-181a was related with an augmented danger of recurrent pregnancy loss RPL, Geng *et al.*,[25] where there was a decline in gene expression levels in a study conducted on 36 patients with RPL and 40 as a control group. Collective indication proposes that miRNAs production crucial characters in the pathophysiology of numerous reproductive illnesses Imbar and Eisenberg[26] Here, we explored whether pre-miRNA SNPs (miR-181a) are related with the hazard of RPL in a cohort of Korean females. It was more mutual in RPL sick than in controls, suggesting a strong correlation with a higher risk of RPL (all $p < 0.05$). Compared to controls, it was less common in RPL patients, indicating that these combinations have a defensive outcome (all $p<0.05$).

The results of Ardakani, et al.,27 displayed that the expression of mir-181a augmented as the usual time of birth advanced with ($p < 0.001$). Results of An, et al., 28 suggest that the four A higher risk of primary ovarian insufficiency is linked to microRNAs. Combination analysis showed that people with primary ovarian insufficiency had significantly lower levels of miR-181a expression ($P < 0.05$).

Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) investigation of a study by Zhou, et al.[29], Shen et al.,[30] and Lisboa *et al.*,[31] revealed that the level of hcmv-miR-US25-1 from the tested CMV particles in serum was significantly elevated in CMV patients (fold alteration > 2, $P < 0.01$) compared to controls.

CONCLUSION

This study found miRNA-US5-1 and miRNA-181a to be upregulated significantly in the women having miscarried fetuses, associated with HCMV. In the healthy controls, miRNA-US5-1 was not present. These microRNAs may act as non-invasive biomarkers for the early diagnosis of the infection

Declarations:

Consent for publication: Not applicable

Availability of data and material: Data are available upon request.

Competing interests: The author(s) declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

This manuscript has not been previously published and is not under consideration in another journal.

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REFERENCES

1. Heald-Sargent TA, Forte E, Liu X, Thorp EB, Abecassis MM, Zhang ZJ, Hummel MA. "New insights into the molecular mechanisms and immune control of cytomegalovirus reactivation." Transplantation. 2020; 104(5): e118–e124.

2. ESHRE Guideline Group on RPL, Bender Atik R, Christiansen OB, Elson J, Kolte AM, Lewis S, et al. "ESHRE guideline: Recurrent pregnancy loss: An update in 2022." Hum Reprod Open. 2023; 2023(1): hoad002.
3. Quenby S, Gallos ID, Dhillon-Smith RK, Podesek M, Stephenson MD, Fisher J, et al. "Miscarriage matters: The epidemiological, physical, psychological, and economic costs of early pregnancy loss." Lancet. 2021; 397: 1658–1667.
4. Abdul-Hussain AH and Aljanaby AAJ. Receptor Activator of Nuclear Factor Kappa-B Ligand and Presepsin as Crucial Biomarkers in Women with Rheumatoid Arthritis. Egypt J Med Microbiol 2025; 34 (4) accepted.
5. Potiris A, Perros P, Drakaki E, Mavrogianni D, Machairiotis N, Sfakianakis A, et al. "Investigating the association of assisted reproduction techniques and adverse perinatal outcomes." J Clin Med. 2024; 13: 328.
6. Grunwald A, Brzuszkiewicz K, Nowak K, Satora M, Klas J, Rudziński G. "Cytomegalovirus infection in pregnant women—threats, diagnosis and treatment." J Educ Health Sport. 2022; 12(12): 187–192.
7. Nowakowska D, Studzińska M, Suski P, Paradowska E, Wilczyński J, Rycel M, et al. "Evaluation of the association between maternal HCMV viremia and the course of pregnancy and neonatal outcome." Ginekol Pol. 2013; 84(12).
8. Khayoon SQ, Al-Hadraawy SK and Aljanaby AAJ. Brain-Derived Neurotrophic Factor and Interleukin-1 Beta as Novel Biomarkers in Patients Infected With E.Coli O157:H7 and Entamoeba Histolytica. Egypt J Med Microbiol 2025; 34 (4) accepted.
9. Mishra R, Kumar A, Ingle H, Kumar H. "The interplay between viral-derived miRNAs and host immunity during infection." Front Immunol. 2020; 10: 3079.
10. Tokorodani M, Ichikawa H, Yuasa K, Takahashi T, Hijikata T. "SV40 microRNA miR-S1-3p downregulates the expression of T antigens to control viral DNA replication, and TNFα and IL-17F expression." Biol Pharm Bull. 2020; 43: 1715–1728.
11. Abu-Izneid T, AlHajri N, Ibrahim AM, Javed MN, Salem KM, Pottoo FH, et al. "Micro-RNAs in the regulation of immune response against SARS-CoV-2 and other viral infections." J Adv Res. 2021; 30: 133–145.
12. Kim C, Ye Z, Weyand CM, Goronzy JJ. "miR-181a-regulated pathways in T-cell differentiation and aging." Immun Ageing. 2021; 18(1): 28.
13. Lim CX, Lee B, Geiger O, Passegger C, Beitzinger M, Romberger J, et al. "miR181a modulation of ERK-MAPK signaling sustains DC-SIGN expression and limits activation of monocyte-derived dendritic cells." Cell Rep. 2020; 30(11): 3793–3805.e5.
14. Melaiu O, D'Amico S, Tempora P, Lucarini V, Fruci D. "Impact of natural occurring ERAP1 single nucleotide polymorphisms within miRNA-binding sites on HCMV infection." Int J Mol Sci. 2020; 21: 5861.
15. Li S, Zhu J, Zhang W, Chen Y, Zhang K, Popescu LM, et al. "Signature microRNA expression profile of essential hypertension and its novel link to human cytomegalovirus infection." Circulation. 2011; 124: 175–184.
16. Abdul-Hussain AH and Aljanaby AAJ. Chemokine (C-C motif) ligand-20 and Interleukin-15 as Novel Biomarkers in Rheumatoid Arthritis Women. Egypt J Med Microbiol 2025; 34 (4) accepted.
17. Akunaeziri UA, Magaji AF, Anyaka C, Ocheke AN. "Cytomegalovirus infection among women with recurrent miscarriages." Trop J Obstet Gynaecol. 2021; 38: 128–138.
18. Rufati N, Beadini N, Rexhepi M, Besimi F. "Potential impact of cytomegaloviruses CMV, IgG, IgM, toxoplasmosis txm and txg as potential causes of sterility and abortion in pregnant women." Acta Med Balk Int J Med Sci. 2018; 3(6): 22–24.
19. Saravi NM, Aghajani F, Najafi A, Khajavi R, Rahmani Z, Jalali H, et al. "A seromolecular study to determine the prevalence of cytomegalovirus in pregnant women referred to health centers in the north of Iran." Iran J Microbiol. 2023; 15(4): 594.
20. Khikani AN, Firat M, Al-Khafaji ZA. "Association of hcmv-miR-UL36-5P gene expression with miscarriages in women with human cytomegalovirus infection in Babylon, Iraq." Al-Rafidain J Med Sci. 2024; 7(2): 43–48.
21. Anwar SA, Al-Bayati NS. "Prevalence of Toxoplasma gondii and cytomegalovirus in sera of aborted women in Samarra city." Tikrit J Pure Sci. 2018; 22(6): 34–38.
22. Al-Mousawi HTM, Al-Hajjar QN. "Immunological and molecular diagnosis of cytomegalovirus infection between aborted and pregnant women in Babylon city." Baghdad Sci J. 2021; 18(2).
23. Vasani A, Kumar MS. "Advances in the proteomics of amniotic fluid to detect biomarkers for chromosomal abnormalities and fetomaternal complications during pregnancy." Expert Rev Proteomics. 2019; 16(4): 277–286.
24. An HJ, Cho SH, Park HS, Kim JH, Kim YR, Lee WS, et al. "Genetic variations miR-10aA>T, miR-

30cA>G, miR-181aT>C, and miR-499bA>G and the risk of recurrent pregnancy loss in Korean women." Biomedicines. 2022; 10(10): 2395.

25. Geng X, Mao G, Zhao D, Xiang Y, Wang M, Yu G, et al. "Downregulation of miR-33a/b and miR-181a contributes to recurrent pregnancy loss by upregulating S1PR1 and repressing regulatory T cell differentiation." Placenta. 2022; 121: 137–144.
26. Imbar T, Eisenberg I. "Regulatory role of microRNAs in ovarian function." Fertil Steril. 2014; 101(6): 1524–1530.
27. Ardakani E, Mazaheri M, Forouzanfar M, Mojibian M, Jafarinia M. "Crucial role of corticotropin-releasing hormone, corticotropin-releasing hormone-binding protein, miR-200c, and miR-181a in preterm delivery: A case-control study." Int J Reprod Biomed. 2023; 21(9): 715–722.
28. An HJ, Cho SH, Ryu CS, Ko EJ, Park HW, Kim YR, et al. "Genetic associations of miRNA variants (miR-10a, miR-30c, miR-181a, miR-499b) with primary ovarian insufficiency in Korean women." Maturitas. 2025; 191: 108153.
29. Zhou W, Wang C, Ding M, Bian Y, Zhong Y, Shen H, et al. "Different expression pattern of human cytomegalovirus-encoded microRNAs in circulation from virus latency to reactivation." J Transl Med. 2020; 18(1): 469.
30. Shen ZZ, Pan X, Miao LF, Ye HQ, Chavanas S, Davrinche C, et al. "Comprehensive analysis of human cytomegalovirus microRNA expression during lytic and quiescent infection." PLoS One. 2014; 9(2): e88531.
31. Lisboa LF, Egli A, O'Shea D, Isberg A, Hartmann A, Rollag H, et al. "hcmv-miR-UL22A-5p: a biomarker in transplantation with broad impact on host gene expression and potential immunological implications." Am J Transplant. 2015; 15(7): 1893–1902.