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Gene expression of human and human cytomegalovirus miRNA and their immunological impact in miscarried women

A Thesis

**Submitted to the Council of College of Science
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in Partial Fulfillment of the Requirements for the
Degree of Doctorate of Philosophy in Biology.**

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"Praise be to **God** for granting me the patience, willpower, and wisdom to complete my research. My academic journey, with its days, hours, fatigue, and energy, has come to an end. What remains is the fruit of success and excellence.

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Thank you so much to everyone **who stood by me**"

Dedications

I faced many difficulties throughout my academic career, but I
overcame them with steadfastness, thanks to God and His grace

At every stage of life, there is someone who deserves thanks

To the one who taught me to give, my dear father

To the secret of existence, my beloved mother

To my soulmate, my dear husband: To the one who guided me toward
my goals and restored my confidence in my ability to progress. You
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throughout my studies. I dedicate this research to you

To those who rejoiced in our success and grieved at our failure with
heart, blood, and sincerity: To my brothers, sisters, and relatives. To
those who have stood by me with their good wishes for my success and
the support of friends and loved ones

To those for whom I continue to work, my dear children, I hope to
always be a good role model and guide for you

I dedicate this research to all of you.

Athraa

Summary

Summary

Microbes significantly impact public health, including the opportunistic pathogen known as human cytomegalovirus, direct infection of the uterus and fetus can lead to serious complications in pregnant women and their fetuses potentially leading to miscarriage, therefore, this study aimed to highlight the importance of human and human cytomegalovirus (HCMV) miRNA in miscarried women and to identify immune biomarkers such as certain pro-inflammatory cytokines : TNF- α , IFN- γ , IL-8, and IL-6, the presence of HCMV in the cervix increases the risk of preterm birth or spontaneous abortion through the release of certain inflammatory cytokines.

The study was conducted on women who visited the Maternity and Children's Teaching Hospital, in addition to some medical laboratories and outpatient clinics in Diwaniyah Governorate during the period from 15 January 2024 to 15 December 2024 , the number of participants was 160 women (110 miscarried women and 50 healthy pregnant women as a control group)

Blood samples were collected and tested for HCMV using a rapid diagnostic test (cassette), the results showed that 50 (45.5%) of miscarried women tested positive for HCMV while 60 (54.5%) of miscarried women as well as all healthy controls had negative test results for HCMV , after a rapid test the infection was verified using an enzyme-linked immunosorbent assay (ELISA) to confirms for IgG and IgM ,all miscarried women with HCMV 50 (100.0%) exhibited positive results for IgG and 20 (40.0%) of miscarried women exhibited positive results for IgM, whereas all healthy pregnant women 50 (100.0%) demonstrated negative results for both IgG and IgM.

The ages of miscarried women infected with HCMV ranged from 15 to 45 years, with the mean age was 28.82 ± 6.27 ,the highest percentage

Summary

was in age group of 21-25 years, 16 (32.0%), while the lowest percentage was in age group of 41-45 years, 4 (8.0%).

As for the distribution of residence, the abortion group included 35 patients (70%) from urban areas and 15 patients (30%) from rural areas, while the control group included 40 healthy pregnant women (80%) from urban areas and 10 healthy pregnant women (20%) from rural areas.

The results of the study showed that the number of total pregnancies of patients with less than 4 pregnancies 24 (48.0%) and 26 (52.0%) of patients with 4 pregnancies or more, while the results showed most healthy women with less than 4 pregnancies 38 (76.0%), furthermore, the number of miscarriages of patients was 29 (58.0%) of HCMV-positive patients had one miscarriage, 17 (34.0%) of HCMV-positive patients had two miscarriages and only 4 (8.0%) of patients had three or more miscarriages.

The number of women who miscarried due to HCMV and were infected with COVID-19 was 29 (58.0%), while the number of women who miscarried and were infected with HCMV but not infected with COVID-19 was 21 (42.0%), the number of healthy pregnant women infected with COVID-19 was 26 (52.0%), while the number of healthy pregnant women not infected with COVID-19 was 24 (48.0%).

Enzyme-Linked Immunosorbent Assay was used to determine the levels of cytokines in the serum of both patients and healthy controls, immunological analysis results showed that tumor necrosis factor alpha (TNF- α) levels in the serum of women who had miscarried infected with HCMV were higher than in healthy controls (172.72 ± 8.99 and 61.74 ± 8.93 respectively), it also showed that women who had miscarried and were infected with HCMV had higher levels of interferon-gamma (IFN- γ) than the healthy control group with levels of 53.87 ± 4.81 and 36.65 ± 6.80 .

Summary

Furthermore , the level of IL-8 was higher in women who had miscarried and were infected with HCMV than in healthy women (127.74 ± 10.80 vs. 66.17 ± 8.26) and IL-6 levels were also higher in women infected with HCMV compared to healthy women, at 113.36 ± 6.22 vs. 35.24 ± 6.08 . The results of the study showed correlations between cytokines parameters in miscarried women infected with HCMV, there was a significant positive correlation between TNF- α and IFN- γ ($r=0.610$, $p=0.001$), TNF- α and IL-6 ($r=0.549$, $p=0.001$), TNF- α and IL-8 ($r=0.633$, $p=0.001$), IFN- γ and IL-6 ($r=0.561$, $p=0.001$), IFN- γ and IL-8 ($r=0.510$, $p=0.001$), and IL-6 and IL-8 ($r=0.681$, $p=0.001$).

The study also included investigating the gene expression of miRNA using real-time polymerase chain reaction, the mean expression of miRNA-181a was significantly higher in miscarried women infected with HCMV compared to the healthy control group and it was 9.07 ± 1.40 and 1.0, respectively and mean expression of miRNA-US5-1 in miscarried women infected with HCMV was 5.49 ± 1.20 but the this gene not expressed in healthy control, the study also showed that there is a direct correlation between the gene expression of miRNA-181a and miRNA-US5-1, in addition to their direct correlation with other parameters (TNF- α , IFN- γ , IL-8 and IL-6) in miscarried women infected with HCMV.

The current study concluded that miscarried women infected with HCMV showed higher levels of IgG and IgM antibodies compared to healthy pregnant women, as well as elevated levels of pro-inflammatory cytokines, including TNF- α , IFN- γ , IL-8, and IL-6, there was a strong positive correlation between these cytokines. Furthermore, the expression of miRNA-181a was directly correlated with the expression of miRNA-US5-1, and their expression was directly correlated with the increase in inflammatory cytokines, suggesting a common role for these molecules in HCMV-associated miscarriage.

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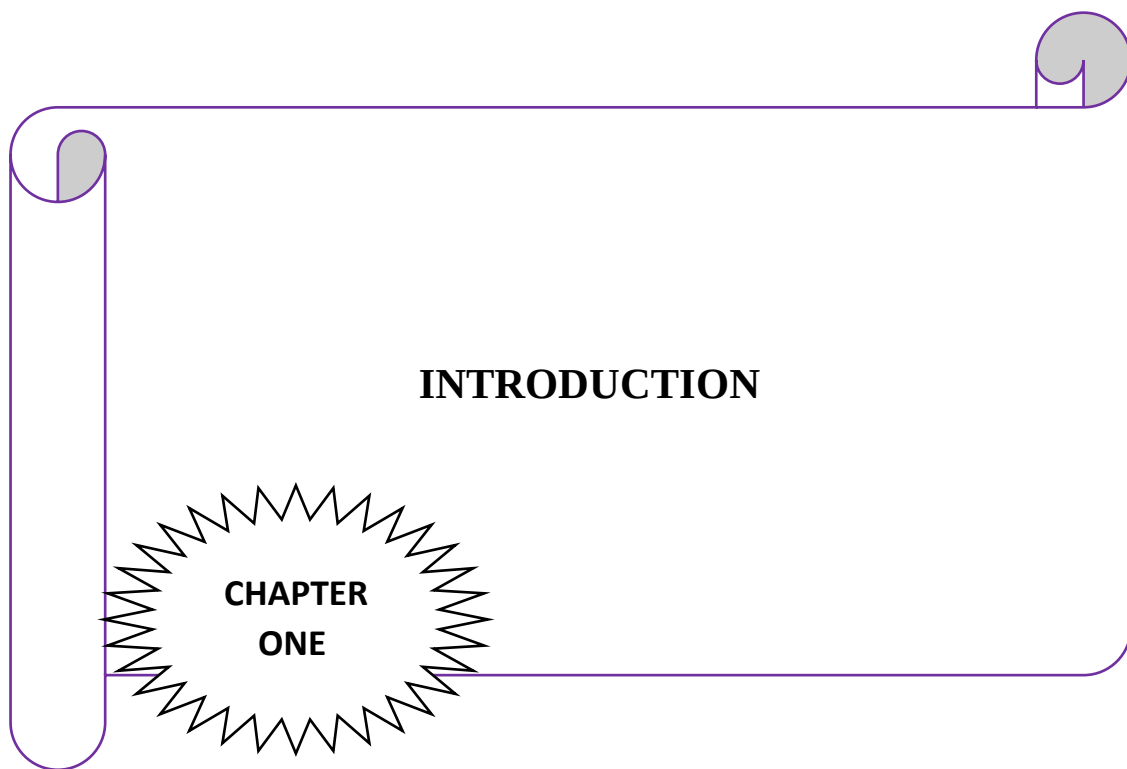
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List of Abbreviations

No.	Title
3'UTR	3' Untranslated Region
AGO	Argonaute protein
APO	Adverse pregnancy outcomes
cCMV	congenital CMV infection
CDC	Centers for Disease Control and Prevention
cDNA	Complementary DNA
CID	Cytomegalic inclusion disease
CMV	Cytomegalovirus
Ct	Cycle threshold
CTLs	Cytotoxic CD8+ T lymphocytes
dsDNA	Double-stranded DNA
E	Early
ELISA	Enzyme-linked immunosorbent assay
ER	Endoplasmic reticulum
ERAP1	Endoplasmic Reticulum Aminopeptidase 1
GADPH	Glyceraldehyde-3-Phosphate Dehydrogenase
HCMV	Human Cytomegalovirus
HHV5	Human herpesvirus 5
HIV	Human Immunodeficiency Virus
HPCs	Hematopoietic progenitor cells
IE	Immediate early
IFNs	Interferons
IFN- γ	Interferon gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
Igs	Immunoglobulins
IL-6	Interleukin -6
IL-8	Interleukin -8
ILs	Interleukins
L	Late
MHC	Major histocompatibility complex
miR-181a	MicroRNA encoded by human
miRNAs	MicroRNAs
miR-US5-1	MicroRNA encoded by HCMV
Neg.	Negative
NF-kB	Nuclear Factor kappa B or Nuclear Factor kappa-light-chain-enhancer of activated B cells
PE	Preeclampsia

Pos.	Positive
pri-miRNA	Primary miRNA
RISC	RNA silencing complex
RNA	Ribonucleic Acid
RPL	Recurrent Pregnancy Loss
RSA	Recurrent spontaneous abortion
RT-PCR	Real-time polymerase chain reaction
S1PR1	Sphingosine-1-phosphate receptor 1
SCT	Stem cell transplantation
SNPs	Single Nucleotide Polymorphisms
SOT	Solid organ transplantation
SPL	Sporadic Pregnancy Loss
TCR	T-cell receptor
Th1 CD4+	T helper type 1 CD4-positive cells
Th1 cells	T helper type 1 cells
Th2 cells	T helper type 2 cells
TNFRs	Tumor necrosis factor receptors
TNF- α	Tumor necrosis factor alpha
TORCH	(Toxoplasmosis, Rubella, cytomegalovirus and Herpes simplex virus)
UL	Unique Long
uNK	uterine natural killer
US	Unique Short
v-miRNAs	Viral microRNAs
WHO	World Health Organization



INTRODUCTION

CHAPTER ONE

1. Introduction

Miscarriage is a major global health problem and plays an important role in human reproduction, approximately 15% of pregnancies end spontaneously, it is defined as the loss of a fetus before the 20th week of pregnancy, miscarriage occurs in 12-15% of women over the course of their lives, approximately 5% of women lose two consecutive pregnancies, while 1% lose three or more pregnancies (Arora *et al.*,2020).

Some potential infectious diseases include opportunistic herpes viruses, the most important of which is human cytomegalovirus (HCMV), direct infection of the uterus and fetus with infectious agents can lead to placental damage, chronic endometritis, and other types of infections leading to miscarriage (Piccinni *et al.*,2021). HCMV can cause systemic infection and serious illness in people with weakened immune systems (Mohsin *et al.*,2022). It is a major contributor to prenatal and intrapartum infections which can lead to serious complications during pregnancy including miscarriage (Wang *et al.*,2025)

Human cytomegalovirus is one of the most common herpes viruses worldwide, the Centers for Disease Control and Prevention (CDC) and the World Health Organization (WHO) have confirmed that HCMV infection is very common and is transmitted between people of all ages (Al-Mousawi and AL-Hajjar, 2021).HCMV is not seasonal and is prevalent worldwide with an estimated prevalence of 83% in the general population, 86% in women of reproductive age ,in addition, the prevalence rate is higher in lower socioeconomic groups (Leruez-Ville and Ville,2017).

Since the estimated seroconversion rate in pregnant women is higher than the prevalence of HCMV in the general population, this data is important. Primary CMV infection causes severe congenital lesions and a higher risk of vertical transmission (Lanzieri *et al.*,2014) .

Women of reproductive age are frequently exposed to HCMV infection, which is more common in pregnant women than in non-pregnant women (Enders *et al.*, 2012). Pregnancy is often associated with weakened immunity and uninfected women may be more susceptible to infection with HCMV in the early stages of pregnancy, increasing the risk of infection, HCMV is responsible for more than 70% of miscarriages and congenital infections (Usta *et al.*, 2016).

The maternal-fetal border represents the site of interaction between various immune cells, including macrophages, T cells, dendritic cells and natural killer cells, successful pregnancy requires an appropriate balance between these immune cells, as recurrent spontaneous miscarriage is characterized by dysregulation of these immune components and an imbalance between pro- and anti-inflammatory immune cells (Andreescu *et al.*,2025).

Cytokines play a fundamental role in cell signaling and communication within the immune system and other physiological processes. Some of the most common cytokines include interleukins (ILs), interferons (IFNs), tumor necrosis factors (TNFs) and others (Liu *et al.*,2021a) . During pregnancy, cytokines play a crucial role in mediating immune responses ,adequate amounts of pro- and anti-inflammatory cytokines are essential for successful implantation and maintenance of pregnancy,an imbalance of (TNF- α) , (IFN-gamma) , (IL-6) and other pro-inflammatory cytokines has been observed as elevated levels may create

a hostile environment for fetal development leading to miscarriage (Liu *et al.*,2022). In addition to IL-8, it is also a pro-inflammatory cytokine and is involved in a variety of other cellular processes. It is highly expressed during pregnancy at the junction between the mother and the fetus, and has been shown to be involved in numerous activities and roles related to both healthy and pathological pregnancies (Vilotić *et al.*,2022).

Local studies indicate that spontaneous abortion due to HCMV infection is associated with an increased immune response to pro-inflammatory cytokines and may play a direct role in placental inflammation and fetal loss (Al-Kinani and Al-Kaabi, 2022).

MiRNAs are small, single-stranded RNA molecules that regulate biological activities such as immune response, growth, and hematopoiesis, and play an important role in maintaining normal physiological functions and diagnosing disease (Liu *et al.*,2020). Numerous studies have shown that miRNAs contribute to viral pathogenesis by regulating host genes modified by miRNAs, promoting viral replication and modulating the host immune system's resistance to the virus (Abu-Izneid *et al.*,2021).

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 (Kim *et al.*,2021). While mir-US5-1, specific for HCMV, contributes to immune evasion and facilitates viral replication (Melaiu *et al.*,2020).

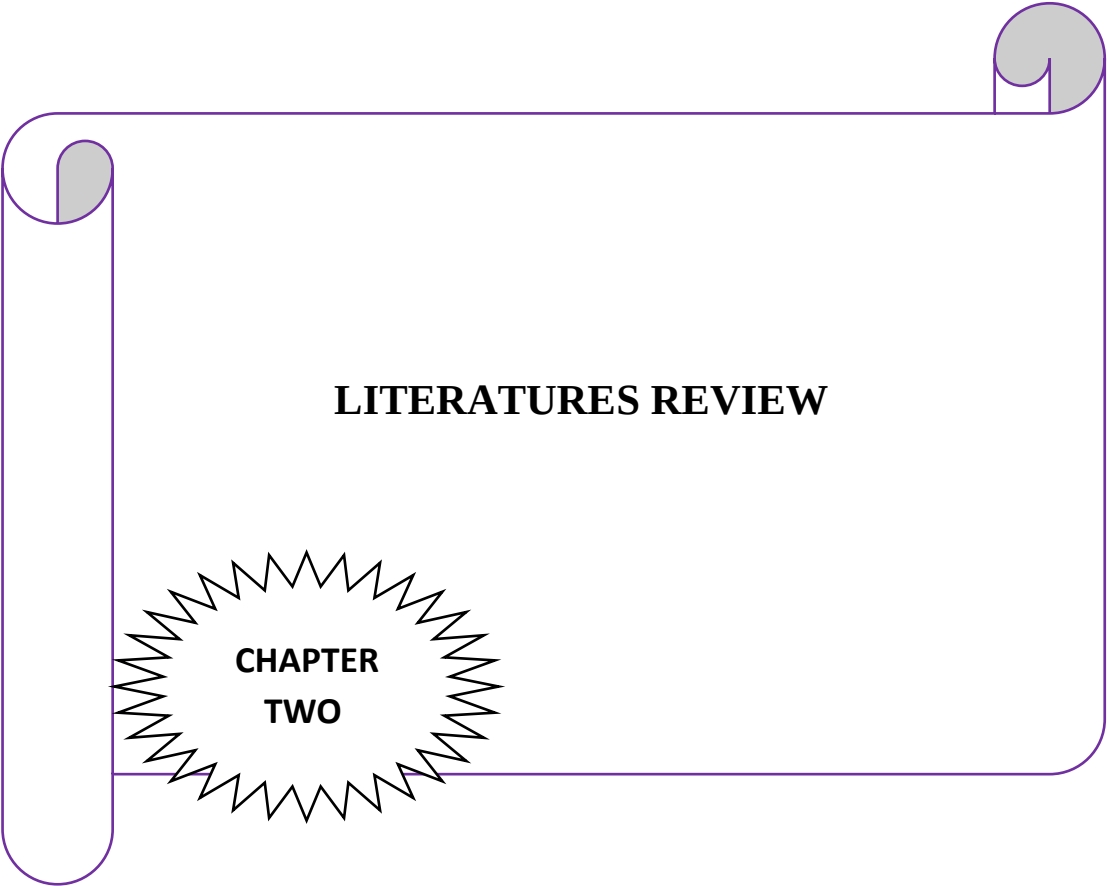
Recent studies have confirmed a close relationship between the expression of certain mirRNA molecules and the occurrence of spontaneous abortion ,these molecules act as post-transcriptional regulators that precisely influence gene expression in placental cells, increased levels of mirRNA in trophoblasts have been found to enhance apoptosis pathways and inhibit

angiogenesis, impeding proper placental development and thus increasing the risk of miscarriage (Bhardwaj *et al.*, 2025).

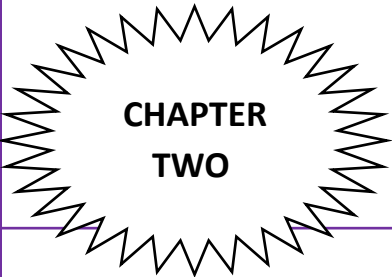
A significant increase in the expression of miRNA was observed in women with miscarriage associated with human cytomegalovirus infection, confirming the role of this viral miRNA as a mediator in inducing miscarriage (Khikani *et al.* ,2024).

Due to the lack of studies on miRNA in miscarried women infected with HCMV, the study aimed to reveal gene expression of human and human cytomegalovirus miRNA and their immunological impact in miscarried women through the following:

- 1- Diagnosis of HCMV infection in miscarried women and healthy pregnant women using the rapid test and detecting antibodies IgM and IgG using enzyme-linked immunosorbent assay (ELISA).
- 2- Determination of the level of some immune cytokines including TNF- α ,IFN- γ ,IL-8 and IL-6 in miscarried women and healthy pregnant women using enzyme-linked immunosorbent assay (ELISA).
- 3- Molecular assessment of human and HCMV miRNAs (mir-US5-1 and mir-181a) using real-time polymerase chain reaction (PCR).



LITERATURES REVIEW



CHAPTER TWO

2. Literatures Review

2.1. Miscarriage

It is the spontaneous loss of a pregnancy before the fetus reaches viability without any external intervention, this term encompasses all cases of pregnancy loss that indicate the unintended death of the fetus from the moment of conception until the 24 th week of pregnancy (Al-Memar *et al.*, 2020). About 26% of pregnancies end in miscarriage, while about 10% of clinically complete pregnancies (Kanmaz *et al.*,2019).

There are many important causes of miscarriage including genetic and uterine abnormalities, endometritis, environmental pollutants and infectious agents (Fabbri *et al.*,2011). Chromosomal abnormalities account for between 50% and 60% of miscarriages during the first trimester of pregnancy resulting from chromosomal abnormalities in the fetus. These mutations are often nonviable leading to spontaneous cessation of fetal development (Dul *et al.*, 2012).

Hormonal factors also play an important role such as lack of progesterone leads to an unstable uterine environment that is unsuitable for embryo implantation, on the other hand immune disorders lead to increased blood clotting in the placenta thus reducing oxygen flow to the fetus (Lee *et al.*,2018). Additionally an unhealthy lifestyle may increase the risk of miscarriage such as smoking, alcohol consumption, obesity (Wagner *et al.*,2018)

Severe maternal infections can also lead to viremia or bacteremia and uterine dissemination, these infections are caused by a wide range of pathogens including *Toxoplasma gondii* infection ,Rubella virus, Herpes simplex, Human cytomegalovirus and others (Abdulkhaliq *et al.*,2017).

2.2.Human cytomegalovirus (HCMV)

HCMV is a viral agent that may directly contribute to miscarriage and stillbirth, HCMV is possible or probable cause in an average of 7.1% of cases, the virus is present in a variety of samples including fetal remains, placenta, serum and dried postmortem blood spots, infection has been associated with the most prominent histologic changes such as fetal vascular thrombosis, hydrops fetalis and chronic chorionic villus inflammation and may contribute to disruption of placental function and vascular damage that impacts the continuation of pregnancy (Pesch *et al.*,2024).

It can directly cross the placenta causing fetal infection and adverse outcomes (Megli and Coyne, 2022). Furthermore,HCMV has been shown to alter the levels of certain human microRNAs in the placenta by interfering with their regulatory network affecting the vital functions of trophoblast cells, which are essential for invading the endometrium and nourishing the fetus (Carlino *et al.*, 2018).

2.3.History of Human Cytomegalovirus

The German physician Ribbert was the first to observe “protozoan-like” structures in the form of large intracellular inclusion bodies in the kidney of a dead child in 1881, these findings were not published until 1904 when Jesionek and Kiolemenoglou found similar inclusion bodies in various organs such as the lungs and liver of deceased children (Redheis and Lemermann, 2006).

In 1921, Goodpasture and Talbot suggested that the source of the inclusion bodies which they termed “cytomegaly” was similar to the varicella-zoster skin lesions discovered by Tyzzer in 1906 (Ho, 2008).

By the early 1930s, it was recognized as an infectious disease characterized by pathological cellular changes in the salivary glands, pancreas, and thyroid gland of postmortem infants (Knipe and Howley, 2007) .

In 1950, Wyatt and colleagues named it “generalized cytomegalia inclusion disease” or CID, later Enders, Robbins, and Weller isolated the virus in 1954, the name cytomegalovirus was proposed due to its cytopathic activity (large intracellular inclusion bodies), after an in vitro study by Rowe and colleagues, they named it “adenoid degeneration agent” in 1956 (Ho ,2008 ; Griffiths *et al.*, 2009).

In 1960, Weller, Scott, Hanshaw and Meadearis began serological tests that confirmed that human cytomegalovirus was pathogenic to humans (Halwachs-Baumann, 2011).

2.4. Classification of Human Cytomegalovirus

Herpes viruses are classified into major subfamilies (alpha, beta and gamma herpesviruses) including HCMV based on genetic makeup and growth characteristics HCMV is also known as human herpesvirus-5 (HHV-5) (Muller *et al.*,2021).

Group: Group I (dsDNA)

Order: Herpesvirales

Family : Herpesviridae

Subfamily: Betaherpesvirinae

Genus: Cytomegalovirus

Species: Human Cytomegalovirus (Al-Hakami *et al.*, 2016).

2.5. Human Cytomegalovirus structure

HCMV is the largest human herpesvirus, although structurally similar to other members of its family, it has the largest genome, a double-stranded DNA of 236 kilobases in length, containing 167 genes more than 40 of which are involved in the host immune response after initial infection and in maintaining HCMV's lifelong stability (Hama and Abdulrahman, 2013; Sijmons *et al.*, 2015; Njue *et al.*, 2020).

The viral genetic material is surrounded by an amorphous protein coat that surrounds the capsid, which is icosahedral and encased in a lipid bilayer derived from host cell membranes and coat contains numerous glycoproteins that help modify the coat and play important roles for the virus (Halwachs-Baumann, 2011; Vanarsdall and Johnson, 2012) .

The main function of the coat is to disassemble the virion upon entry and reassemble it after exit (Smith, 2017). Major glycoproteins and their receptors play a crucial role in facilitating viral entry and infection of various cell types such as epithelial and endothelial cells, a decrease in their content within viruses weakens their ability to infect cells (Wilkes and Kattoor, 2022) as shown in figure 2.1.

The capsid consists of 162 capsomeres and a very high content of guanine and cytosine in the DNA and two distinct regions with different segments one short (US) and one long (UL) as well as short and long internal and terminal sequence repeats (Griffiths *et al.*, 2009).

Gene products from the genome may interfere with or evade host immune responses to a significant extent, and may also use methods to interact with inflammatory molecules to allow the virus to survive and spread in the host (Luganini *et al.*, 2016).

MicroRNAs (miRNAs) are distributed throughout the genome including intergenic regions and introns and are not clustered in specific regions associated with latency this differs from other herpes viruses where miRNA genes are often clustered in regions associated with latency (Zhang *et al.* ,2020).

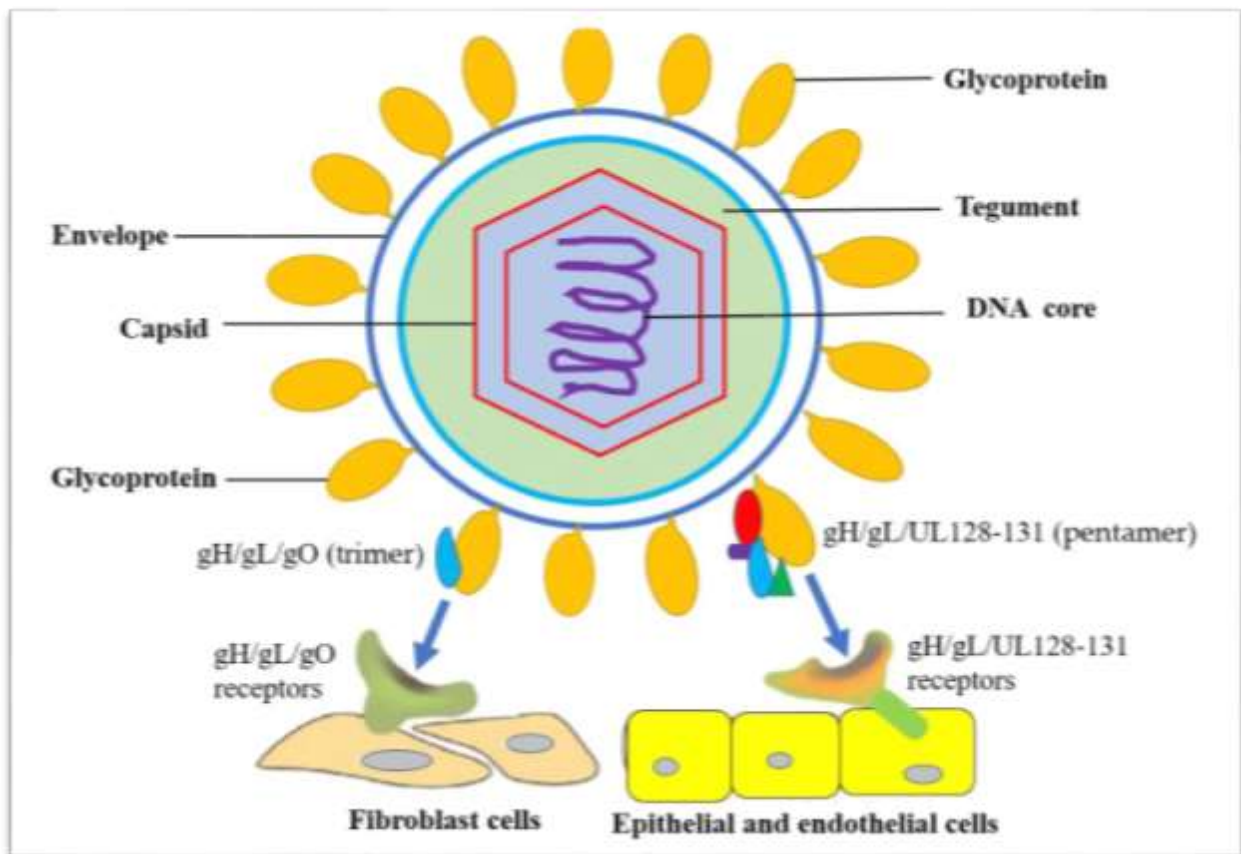


Figure (2-1) HCMV structure and entry into host cells

(Wilkes and Kattoor,2022)

2.6 .Human Cytomegalovirus Life Cycle

The virus replicates in various types of human cells including fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, myeloid cells ,macrophages and dendritic cells (Griffiths *et al.*,2009).The replication cycle takes 48 to 72 hours to initiate offspring release (Abd Alla, 2018).

Initially, HCMV binds primarily to proteoglycans present in the host cell and then binds to host cell specific receptors with the help of the major glycoprotein, subsequently the viral nuclear envelope enters the cell by one of two mechanisms, depending on the cell type in fibroblasts the envelope fuses with the cell membrane while in endothelial and epithelial cells phagocytosis occurs followed by endosome fusion (Bedford, 2018; Wilkes and Kattoor,2022).

This is facilitated by the fusion of the virion envelope and endosome with viral proteins, the cytoskeleton transports the viral nucleus to the nuclear pores where it is degraded and the linear genome is released through the nuclear pores into the nucleus, this process is believed to be mediated by envelope proteins (Tomtishen, 2013) figure (2-2).

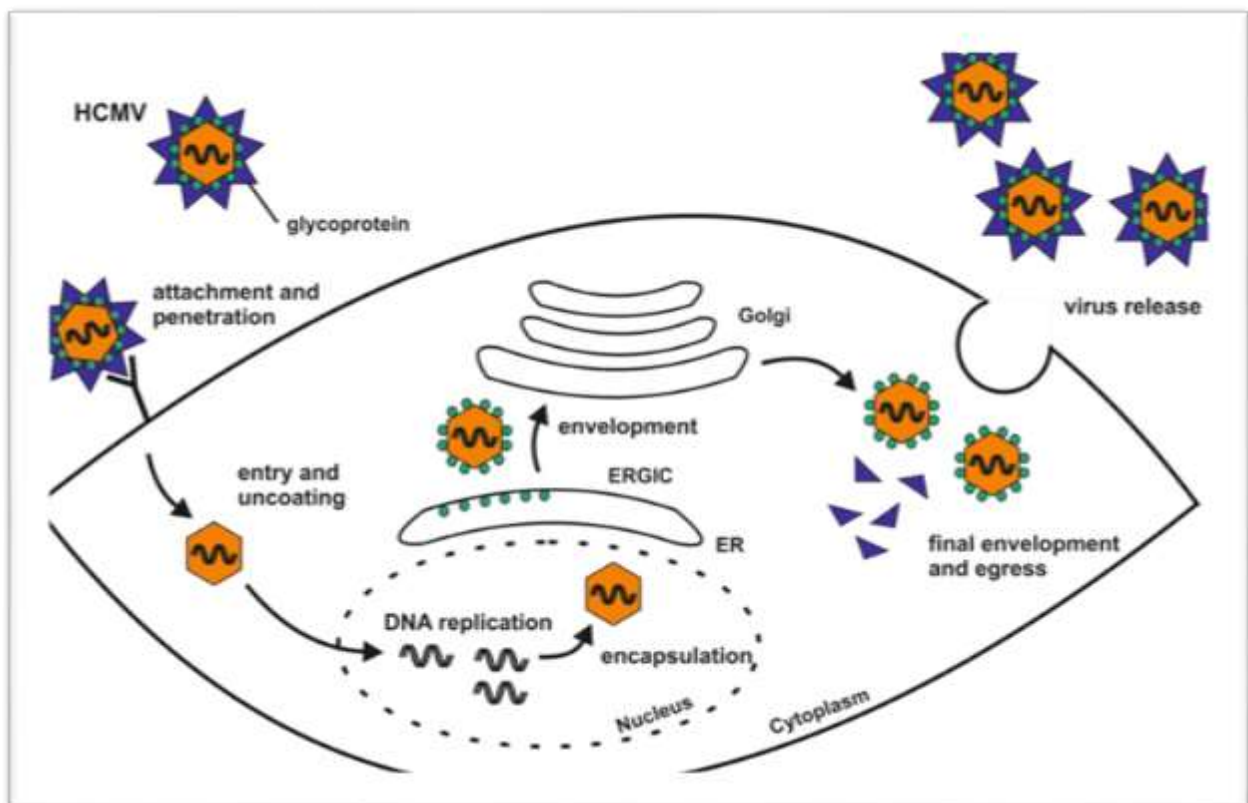


Figure (2-2): Illustration HCMV life cycle from viral entry to egress of new infectious virions, (Warnatsch,2013).

Once the viral genome enters the nucleus it takes on a circular shape by fusing its ends with rolling circular repeats, the envelope protein and cellular proteins then bind to the DNA to initiate replication (Warnatsch, 2013).

The viral genome is expressed by transcription, which produces: immediate early (IE) proteins which control the proteins responsible for initiating and terminating each of the subsequent steps in replication (such as IE1 and IE2 proteins); early (E) proteins which are responsible for replicating the viral DNA and late (L) proteins which are structural proteins responsible for virion assembly and maturation (Manandhar *et al.*, 2019).

During the late stages of replication the nuclear envelope assembles in the nucleus and its molecules accumulate in inclusions representing one of the cytopathological effects of cells infected with HCMV (intracellular inclusions) (Mocarski *et al.*, 2007).

Viral DNA maturation occurs when the viral envelope is first encapsidated into the virion, and then the genomes are inserted. The capsids then receive their primary envelope by budding across the nuclear membrane and then fuse with the endoplasmic reticulum (ER) membrane losing it in the process de-enveloping, HCMV then mature in the cytoplasm forming the envelope layer and then re-enveloping it by budding into Golgi vesicles (Gibson ,2008 ;Cowan and Smith, 2018).

The Golgi network then transports the mature viral particles to the cell surface, causing them to enlarge, thus being another typical cytopathic effect of HCMV (i.e., cytomegalovirus) (Griffiths *et al.*, 2009 ;Halwachs-Baumann ,2011).

2.7 .Epidemiology

Its importance and vital role particularly in the context of viral diseases that affect pregnancy and reproduction such as HCMV and herpesvirus is evident in determining the relationship between the timing of infection during pregnancy and the occurrence of miscarriage or congenital malformations, it also provides an assessment of incidence and prevalence rates and influencing social or environmental factors through the design of case control studies and the identification of vulnerable groups, this paves the way for the development of effective preventive strategies such as vaccinations or early screening during pregnancy (Bate *et al.*,2010; Celentano *et al.*,2023).

HCMV is distributed worldwide and varies greatly in distribution between countries,in sub-Saharan Africa, South Asia such as Bangladesh and India and Latin America such as Brazil prevalence rates exceed 90–95% among adults, due to poor health awareness, poverty and early transmission of the virus through bodily fluids.in Western Europe, the United States, Japan, Australia and England prevalence rates are lower ranging from 40% to 70% , infection is often transmitted during adulthood through sexual contact or from children in daycare (Manicklal *et al.*, 2013 ;Sharghi *et al.*, 2019).

Seroprevalence studies also confirmed the prevalence of HCMV (>90%) in Türkiye, Iran, India and Nigeria in addition to the Arab regions (Alghalibi *et al.*, 2016). Epidemiological studies around the world have documented a high seroprevalence of HCMV infection among people living with HIV ranging from 50% to 85% (Zuhair *et al.*, 2019).

HCMV DNA may be present in up to 20% of placenta and fetal tissue and it is possible that placental infection indirect effects and can lead to adverse pregnancy outcomes and may contribute to stillbirth (Al-dorri, 2018).

2.8. Pathogenesis

HCMV is a common opportunistic pathogen that infects humans and remains latent in the body for life, HCMV infection is usually mild or asymptomatic but it may progress to severe or life threatening disease in people with deficient adaptive host immune responses (Hughes *et al.*,2021).

The infection of HCMV can occur in four different ways include primary infection which occurs when an individual is first infected with the virus and lacks prior immunity,latent infection occurs when viral replication is very low and the individual remains asymptomatic during this period reactivation occurs when viral replication is stimulated after a latent period usually due to stress or other medical conditions superinfection occurs when a person is infected with a new strain despite natural immunity (Atabani *et al.*, 2012 ;Scaramuzzino *et al.*,2022;Paris *et al.*,2023) .

Congenital CMV often causes all of the above infections that can make pregnancy difficult, the virus remains one of the most common and serious opportunistic infections in hematopoietic stem cell transplantation (SCT) and solid organ transplantation (SOT) and remains a major opportunistic infection in people with acquired immunodeficiency syndrome caused by the human immunodeficiency virus (HIV) (Atabani *et al.*, 2012).

It poses a significant risk to elderly patients, neurological diseases and patients in intensive care units (Oiknine-Djian *et al.*, 2018). It has also been associated with several types of cancer, such as breast, prostate, and colon cancer, previously it was believed that HCMV played a tumor-modifying role by infecting cancer cells and modifying their malignant properties. However, some have hypothesized that cancer cells provide a genetic environment that allows HCMV to exert its tumor-modifying effects, there is also a strong link between HCMV and atherosclerosis (Saffert *et al.*, 2010).

The virus can infect glial cells, stromal cells, monocytes, endothelial cells, epithelial cells, smooth muscle cells, and fibroblasts in the nervous system. It can also be found in saliva, breast milk, serum, blood cells, urine, tears, feces, vaginal and cervical secretions and semen (Contreras *et al.*, 2014). Other important groups at risk are fetuses whose mothers were infected with HCMV during pregnancy and children or adults whose immune systems are weakened by illness or drug therapy (Yeroh *et al.*, 2015).

The mother acquires the primary infection and seroconversion occurs during pregnancy represented by reactivation of the latent virus or contamination with a new strain thus, all types of infection lead to vertical transmission (Zuhair *et al.*, 2019).

Fetal infections such as HCMV have been shown to cause pregnancy loss and proposed mechanisms include direct infection of the fetus or placenta, endometritis, endocervicitis, chorioamnionitis and altered immune response (Nigro *et al.*, 2011).

2.9. Clinical manifestations

HCMV is associated with diverse clinical manifestations in infected individuals, infection is usually acquired in childhood and patients may develop symptoms within 9 to 60 days of initial infection, human salivary glands are most commonly affected as the virus remains latent in myeloid cells after the initial infection resolves, cytotoxic T-cell immunity primarily mediates replication and reactivation, the virus is released into the body and then excreted and transmitted by patients through saliva and other body fluids, clinical manifestations may range from mild or asymptomatic to life-threatening medical conditions in immunocompromised patients (Nosotti *et al.*,2018; Acosta *et al.*,2020). It also often affects HIV patients and organ or bone marrow transplant recipients with organ failure (Ljungman *et al.*,2019 ; Sufiawati *et al.*,2021).

Clinical signs range from CMV syndrome to tissue-specific disease,CMV syndrome is characterized by flu-like symptoms, including fever, malaise, muscle aches, fatigue and headache as well as abnormal lymphocyte counts and liver function tests in addition, uncommon symptoms include pharyngitis, lymphadenopathy, rash and hepatomegaly the virus also invades tissues depending on the transplanted organ with incidence ranging from 35% to 75% in lung transplant patients and 9% to 35% in heart transplant patients (Kotton *et al.*,2018).

It may also show an elevated white blood cell count, as well as anemia, thrombocytopenia and sometimes abnormal immune antibodies (Ramchandar *et al.*, 2020).

HCMV infection also causes encephalitis, pneumonia, neuropathy, posterior eye damage, hepatitis, urinary tract infections and gastrointestinal disturbances (Gupta and Shorman ,2023).

The prevalence of HCMV infection in pregnant women increases with age, viruses, particularly CMV are important because they can cause chronic recurrent infections within the uterus and potential mechanisms proposed include the production of toxic metabolites, injury to the fetal or placental endometrium, or placental inflammation (Nigro and Adler, 2011).

If primary maternal HCMV infection occurs before pregnancy or during the first trimester it can affect fetal development and lead to severe malformations known as congenital CMV infection (cCMV) (Pass and Arav-Boger ,2018; Périllaud-Dubois *et al.*,2021).

It is considered the leading cause of congenital infection worldwide,if transmitted to the fetus it can lead to serious clinical manifestations, the most important of which are miscarriage or stillbirth, which may develop into serious problems such as birth defects, including intellectual disability, learning difficulties and developmental delay, it is also the most common cause of non-hereditary sensorineural hearing loss (SNHL) (Liu *et al.*, 2021b ; Hassan and Alzubaidy, 2024).

It also causes congenital facial and skull abnormalities, particularly microcephaly as well as ventricular enlargement (Boucoiran *et al.*, 2021).

2.10. Transmission

HCMV is widespread worldwide infecting more than 90% of the general population. The virus can be transmitted through various routes, including sexual contact, exposure to body fluids such as blood, saliva, or breast milk, or vertical transmission from mother to fetus during pregnancy or maternal vaginal secretions (Vora *et al.*,2018; Yu *et al.*,2021).

Direct contact with infected material is essential because the viral transmission period is relatively short the survival time of HCMV ranges from 5 minutes to 4 hours depending on the surface (Stowell *et al.*, 2012).

The infection is also transmitted through solid organ and stem cell transplantation (Parsons *et al.*,2021).

In mothers who do not have prior immunity HCMV can be transmitted ,when primary infection occurs during pregnancy for the first time, the risk of intrauterine transmission may be highest with a rate of vertical transmission in mothers of childbearing age,the risk of adverse effects on the fetus increases significantly if fetal infection occurs early in pregnancy especially during the first trimester when developmental defects are more severe (Barton *et al.*, 2020).

Several factors are likely to contribute to the increased vertical transmission of HCMV during advancing pregnancy. One contributing factor is the decrease in the number of uterine natural killer (uNK) cells, which account for approximately 70% of uterine leukocytes constitute the majority of leukocytes in early pregnancy (Bulmer *et al.* ,2010).

In addition, HCMV evades recognition by NK cells by downregulating MHC class I complexes making the placenta more susceptible to pathogen invasion (Narang *et al.*,2021).

Furthermore various factors have been associated with HCMV transmission including age, race, gender, and geographic and socioeconomic backgrounds (Fowler *et al.*,2022).

2.11. Cytokines

HCMV manipulates nuclear factor kappa-light-chain-enhancer of activated B cells signaling pathways (NF- κ B) in host cells using both proteins and microRNAs early in infection stimulating the production of pro-inflammatory antiviral cytokines which subsequently decline late in viral infection specifically, miR-US5-1 downregulates factors necessary for the propagation of NF- κ B signaling and the subsequent production of

IL-6 and TNF- α , regulation of these pro-inflammatory cytokines during lytic infection and during latency is critical for viral survival (Hancock *et al.*, 2017).

Cytokines are soluble proteins involved in the immune response and secreted by a variety of cells, such as natural killer (NK) cells, lymphocytes, macrophages and mast cells, they are characterized by a low molecular weight ($\approx 6-70$ kDa) (Kulbe *et al.*, 2012).

They are also considered important biomarkers of physical health disorders as any dysfunction in their secretion is responsible for the development of many of the most deadly diseases including cancer, infectious diseases and immune-mediated diseases, these diseases are associated with the massive release of pro-inflammatory cytokines known as a "cytokine storm," which triggers an inflammatory response to many pathogenic viruses leading to severe inflammatory syndromes multi-organ failure and potentially death (Kalayci *et al.*, 2019).

Essential roles are also evident in stem cell differentiation, embryonic development, wound healing and apoptosis, they regulate innate and adaptive immunity mediate communication between immune and non-immune cells and regulate inflammation due to their highly polymorphic activity cytokines may be present at very low or undetectable concentrations in body fluids and tissues under conditions of homeostasis, however in disease states they can rapidly increase up to 1000-fold, HCMV replication triggers a strong inflammatory response including the production of pro-inflammatory cytokines, it is unknown whether HCMV induced inflammatory cytokine production directly affects the risk of preterm birth through pathways similar to those caused by other reproductive infections (Biolatti *et al.*, 2018).

Interleukins are a type of cytokines secreted by white blood cell, they are small molecules that help each other by directing stimulating and sometimes inhibiting their activity and function is focused on transmitting signals between cells to regulate inflammatory responses whether systemic or local (Kalayci *et al.*, 2019).

2.11.1. Tumor necrosis factor alpha (TNF- α)

TNF- α is a cytokine with multiple effects on different cell types and is involved in the pathogenesis of some inflammatory and autoimmune diseases, it also plays a major role in immune modulation, fever ,inflammatory response, inhibition of tumorigenesis and inhibition of viral replication (You *et al.*,2021). It has many normal physiological functions that maintain homeostasis, health and combat microbial immunity and toxic to cancer cells (Lopatnikova *et al.*,2017).

In addition, TNF- α is a central mediator of immune processes to combat infection and autoimmune diseases and synergistic with interferons,it is also expressed by monocytes, macrophages and activated T cells, which are distinct from other cytotoxic cells , it is often classified as an inflammatory factor and has a number of critical roles in immunology linked to human diseases,members of the TNF family are targeted by viruses to evade or reduce immune responses (Gough and Myles, 2020).

Tumor necrosis factor receptors (TNFRs) are a family of structurally similar membrane proteins that act as communication pathways in many body systems most notably the immune system activating cell proliferation or death pathways and stimulating the expression of genes involved in cell differentiation and survival, these receptors bind to TNF-related cytokines regulating the development of the

immune system and initiating inflammatory reactions ensuring effective defenses against viral and bacterial pathogens (Varani and Landini, 2011).

HCMV infection in placental tissue contributes to elevated TNF- α levels, which promotes the death of trophoblasts adjacent to infected cells impairing the placental response and leading to local inflammation that reduces the effectiveness of trophoblast attack and blood flow necessary for fetal growth (Tsai *et al.*, 2022).

The incidence of miscarriage associated with HCMV infection is also increased in amniotic fluid confirming its association with a deterioration in the fetal environment (Smith *et al.*, 2024).

The activity of the inflammatory pathway is associated with an increase in TNF- α which leads to cell destruction especially in cells adjacent to the trophoblast which weakens the ability to remodel the uterine blood vessels and increases the resistance of the vessels to flexion may lead to spontaneous abortion or delayed fetal growth (Tsai *et al.*, 2022).

2.11.2 Interferon gamma (IFN- γ)

IFN- γ is a type of antiviral protein released in response to stimuli and has the ability to prevent viral replication, interferons are classified into three classes: type I, type II and type III, IFN- γ belongs to type II and its structure differs from that of type I and type III interferons (Heras *et al.*, 2020).

IFN- γ is a pro-inflammatory cytokine that stimulates activated immune cells and enhances antigen presentation, it is secreted by natural killer (NK) cells during the innate immune response and by Th1 CD4⁺ cells and (CTLs) cytotoxic CD8⁺ T lymphocytes after adaptive immunity is stimulated (Merli *et al.*, 2021).

It is also produced locally by antigen presenting cells to support the activation of self and neighboring cells for early control of the spread of pathogens (Matsushita *et al.*,2015).

It plays an important role in maintaining the hematopoietic stem cell environment and regulating the balance of immune cell functions and bone formation, furthermore IFN- γ -associated signals can exert immunosuppressive effects on autoimmune and antiviral responses, its cellular targets are of great importance for predicting patient responses especially in tumors because this cytokine can exert carcinogenic effects (Sosa *et al.*.,2015).

In pregnancy the maternal inflammatory response to viral infection stimulates the production of pro-inflammatory cytokines that can reach the developing fetal brain across the placenta and the fetal blood-brain barrier, these indirect effects of maternal infection add to the direct effects of vertical viral transmission that affect fetal neurodevelopment (Kalayci *et al.*, 2019).

During HCMV infection in early placental tissue IFN- γ production by CD8⁺ memory cells in the uterine wall increases reflecting an intense immune response aimed at limiting viral spread (Tabata *et al.*, 2019) .

Although IFN- γ enhances fetal immunity, it is a risk factor for spontaneous abortion when it is elevated sharply in the placental environment and has been linked to decreased trophoblast function and narrowing of the placental blood vessels leading to impaired implantation and fetal nutrition (Njue *et al.*, 2020).

2.11.3. Interleukin -8 (IL-8)

IL-8 is a pro-inflammatory chemokine that primarily recruits neutrophils, monocytes and cytotoxic T cells, although its expression is low or absent under normal conditions , it is induced by a wide range of extracellular stimuli such as TNF α , however excessive amounts of IL-8 lead to deleterious effects therefore its gene expression is tightly controlled at both the transcriptional and post-transcriptional levels, also activated in most cell types by the transcription factor NF- κ B (Hoffmann *et al.*, 2002).

The levels of IL-8 are carefully regulated in normal pregnancy to ensure a balance between the immune response and fetal survival, however infection with herpes viruses such as HCMV leads to a marked increase in IL-8 secretion in placental tissue and amniotic fluid contributing to chorioamnionitis and impairing trophoblast invasion, thereby disrupting the delicate immune balance required to maintain pregnancy and increasing the risk of miscarriage or preterm birth, in women who have miscarried following a viral infection, IL-8 levels are significantly higher in peripheral blood and uterine tissue than in normal pregnant women suggesting its role as a marker of infection-associated inflammation (Costa *et al.*, 2013 ;Romero *et al.*, 2014).

It is also a key component of many other viral infections regulating viral spread and replication (Medin and Rothman,2006). Cells infected with HCMV secrete increased levels of IL-8 because chemokines are effective mediators of immune cell migration across the endothelium this suggests that HCMV can significantly alter the inflammatory environment surrounding infected cells (Randolph-Habecker *et al.*, 2002).

During pregnancy IL-8 exhibits a dynamic balance in the immune responses necessary for the continuation of pregnancy in addition, it is considered a potent pro-inflammatory chemokine and a marker of inflammation which contributes significantly to recurrent pregnancy loss and plays an important role in attracting progenitor cells such as neutrophil, the levels were significantly lower in immune and cellular tissues of women who had spontaneous abortions in the first trimester of pregnancy compared to those in healthy pregnancies, which may hinder the remodeling of uterine blood vessels and contribute to embryo implantation failure (Pitman *et al.*, 2013).

On the other hand, higher levels of expression were shown in the serum of women suffering from recurrent miscarriage. compared to those of normal pregnant women reflecting a chronic inflammatory state that may lead to immune rejection of the fetus (Tyagi and Alharthi , 2020).

2.11.4.Interleukin -6 (IL-6)

IL-6 is a pleiotropic cytokine with diverse functions affecting acute phase reaction pathways, T and B lymphocytes, hematopoiesis and embryonic development ,it can switch between innate and adaptive immune responses and helps recruit macrophages and lymphocytes to sites of infection, also involved in immune homeostasis and the pathogenesis of several autoimmune diseases (Grebenciucova and VanHaerents ,2023).

It is produced by several major sources in the body including stimulated monocytes, macrophages, fibroblasts, B lymphocytes, granulocytes, endothelial cells, smooth muscle cells, eosinophils, osteocytes and mast cells, it is also considered an indicator of inflammation in the body and a mediator of regulating the acute phase response, replacing the immunoglobulin class (Maeda *et al.*, 2010;Tanaka *et al.*, 2014).

After stimulation IL-6 is found in glioma cells, cerebrospinal fluid and breast milk (Ting *et al.*, 2020).

It also plays a pivotal role as an acute indicator of inflammation in pathological conditions, especially during pregnancy, it is secreted by tissue specific immune cells and stromal cells in response to infection and tissue damage, women at risk of miscarriage due to HCMV infection have higher IL-6 levels than those not at risk of miscarriage, during infection high levels of IL-6 are transferred to circulating cells causing a pathological condition that negatively impacts fetal invasion and the balance necessary for the continuation of pregnancy, this can lead to decreased placental function, vasoconstriction and significant disturbances in maternal and fetal health depending on the severity of the miscarriage (Abu Gulel and Hasan, 2024).

2.12. Immunoglobulin G (IgG)

IgG is a type of antibody produced by the immune system after exposure to an infection, it is an indicator of previous infection and remains in the blood for life after recovery, it plays an important role in protecting against future infection with the same virus. (Hameed Haddad *et al.*, 2024).

It is important to determine whether the pregnant mother has immunity to the virus which reduces the risk of new infection during pregnancy (Liu *et al.*, 2021c).

2.13. Immunoglobulin M (IgM)

IgM is a type of antibody produced by the immune system as a primary response to infection, its high levels appear in the first weeks as an indicator of recent infection then gradually decline with the emergence of IgG antibodies (Hameed Haddad *et al.*, 2024).

The presence of IgM in the blood of pregnant mothers may be associated with an increased risk of miscarriage especially if HCMV infection is active during the first months of pregnancy and can cause serious complications such as inflammation of the placenta or fetal abnormalities and miscarriage (Ai Adi *et al.*,2022).

2.14. Immune Response to Human Cytomegalovirus

HCMV is characterized by its ability to remain latent in the body for long periods, it produces a set of microRNAs that play an important role in regulating the host immune response, these miRNAs are expressed in infected cells and contribute to inhibiting the production of pro-inflammatory cytokines which helps the virus evade the host immune response, thus promoting its long-term persistence ,on the other hand the host immune system responds to these miRNAs by producing its own miRNAs which can regulate the gene expression of immune cells and influence their ability to recognize and combat the virus (Zhang *et al.*, 2020).

The process of virus entry into the host cell stimulates an immune response through complex interactions with specific receptor molecules on the host cell (Safdar *et al.*,2023).

These interactions allow for viral uptake and establishment of infection and may activate cell signaling pathways to stimulate cellular miRNA production and create a favorable environment that is either supportive or antiviral (Trobaugh and Klimstra ,2017) .

HCMV infection occurs by infecting and lysing various cells and tissues as white blood cells and vascular endothelial cells after escaping T-cell immunity through various strategies to facilitate its survival, include

inhibiting major histocompatibility complex (MHC) regulation and modifying the efficiency of host cytokine production, in addition, HCMV carries the largest number of genes dedicated to evading the host's innate and adaptive immunity (Stack *et al.*,2012 ; Fruci *et al.*,2017).

HCMV persists for life after primary infection with a viral latent period in myeloid cells in healthy individuals ,the innate immune response detects viral elements through early protein receptors or specific peptides derived from the viral envelope protein,these are recognized by virus-specific CD8 T cells, which controls reactivation or prevents infection leading to the secretion of type I interferons and inflammatory cytokines and activation of many effector genes to create an antiviral state to protect neighboring cells from infection (Takeuchi and Akira, 2009).

The immune environment of the placenta is also modified when infected with HCMV and stimulating cytokines such as tumor necrosis factor alpha which may damage the placenta (Emery and Lazzarotto,2017).

HCMV can infect developing embryos and then the syncytial trophoblast layer covering the chorionic villi leading to incomplete development of the placenta and fetus, this can lead to early pregnancy loss associated with infection (Fisher *et al.*,2000).

The importance of natural defense mechanisms against reinfection with HCMV and congenital transmission is not well understood, therefore pre-existing immunity to this virus may protect an individual from new HCMV infection (Adler *et al.*,2019).

2.15. MiRNAs

HCMV expresses several viral microRNAs that contribute to immune evasion by inhibiting interferon pathways, enhancing its ability to persist in the body for long periods and also contribute to suppressing the anti-inflammatory immune response and reorganizing the cellular composition of infection (Zhang *et al.*, 2021).

The body also responds to infection using human miRNAs that regulate the expression of genes associated with susceptibility to infection and immune cell responses influence interferon pathways and reduce the risk of developing active infection (Fernández-Ruiz *et al.*, 2024).

MiRNAs are small single-stranded RNA molecules consisting of approximately 22 nucleotides, non-protein-coding molecules that are transcribed by RNA polymerases II and III, they regulate biological activities such as growth, immune response, metabolism and hematopoiesis, in addition they play an important role in cell proliferation, cell death, cancer development, and disease prognosis (Liu *et al.*, 2020).

The transcription process begins in the nucleus where the corresponding gene is transcribed into a primary miRNA (pri-miRNA), The resulting pre-miRNA is then modified and transported to the cytoplasm through nuclear pores (Rupaimoole and Slack, 2017).

The mature miRNA dimer binds to the RNA silencing complex (RISC), the Argonaute (AGO) protein family plays a role within RISC, along with several cofactors, where it unbinds the miRNA dimer and selects one of the strands (Correia de Sousa *et al.*, 2019). Virally encoded with over 250 v-miRNAs also exist and play a critical role in viral diseases (Mishra *et al.*, 2020).

HCMV encodes at least 26 small non-coding RNAs (miRNAs) (Meshesha *et al.*,2012). It targets both host and virus mRNA transcripts, regulates protein expression, mediates latency, virus replication, virus assembly, vesicle transport, cell cycle and immune evasion strategies (Ng *et al.*,2015) as shown in figure (2-3).

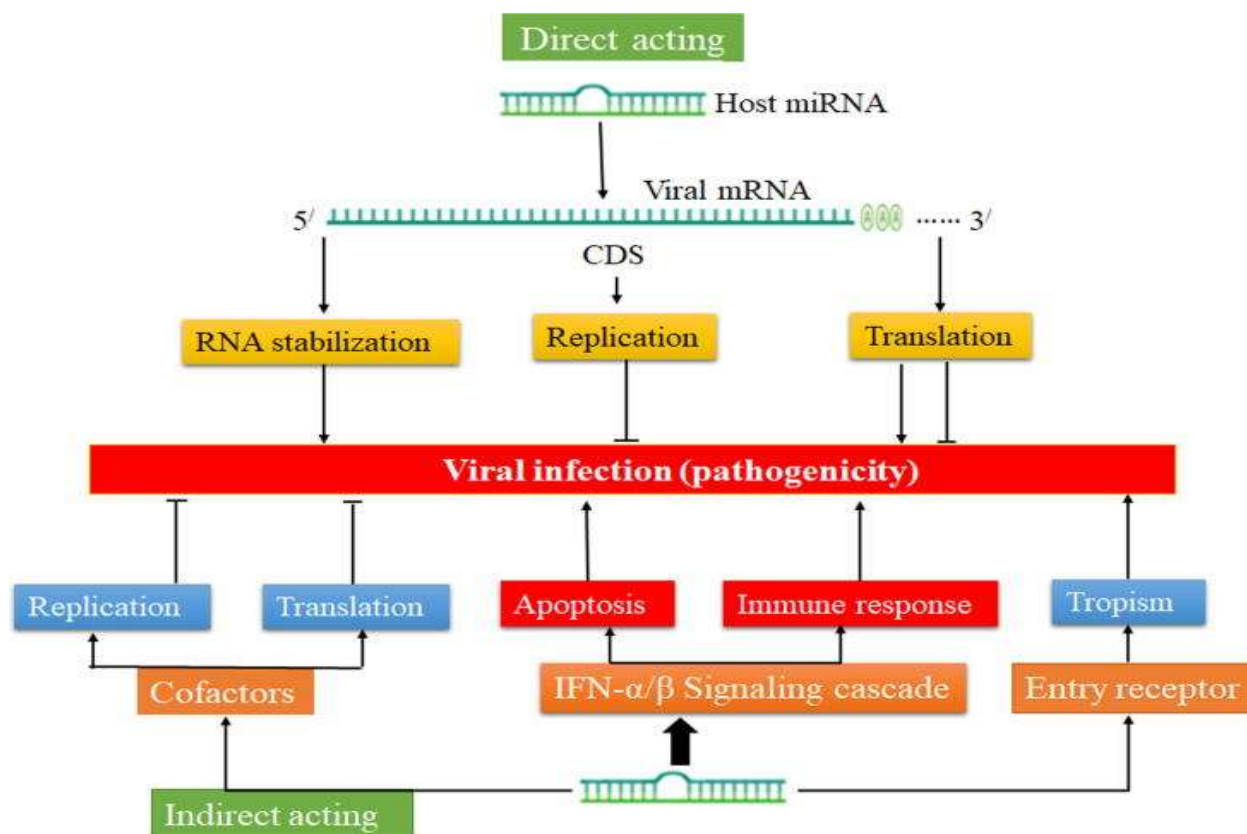


Figure (2-3) Mechanism of action of miRNAs during viral infection
(Girardi *et al.*,2018)

Mature miRNAs are located along HCMV genome and divided into unique long (UL) and unique short (US) regions, these regions are flanked by terminal and internal inverted repeats that present the sense and antisense microRNA precursors at the top and bottom of the viral genome, respectively (Melaiu *et al.*,2020) as shown in figure (2-4).

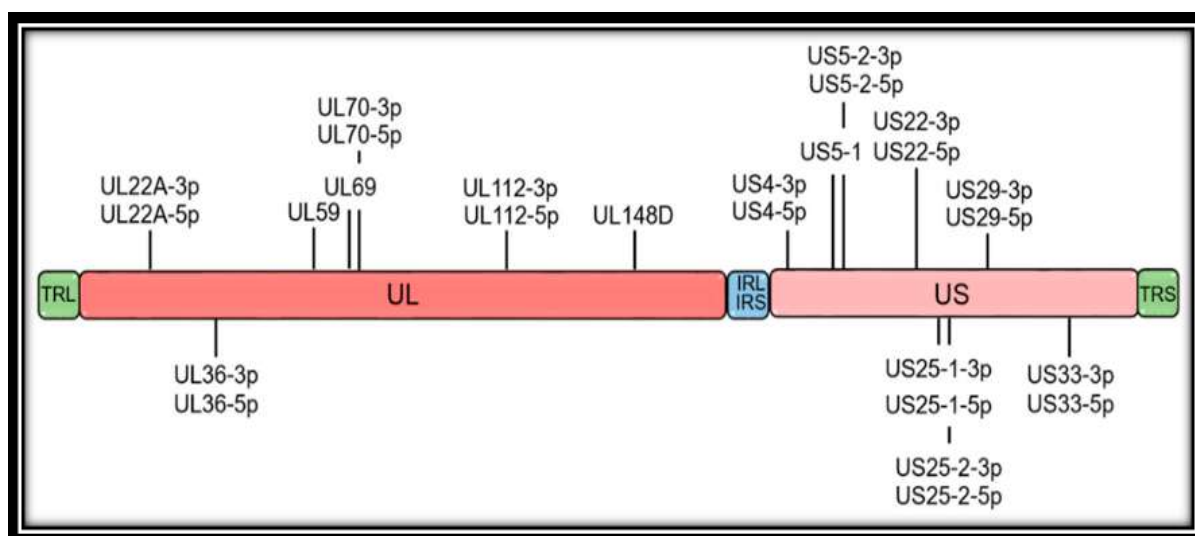


Figure (2.4) The genomic organization of (HCMV) miRNAs is illustrated

(Melaiu *et al.*,2020)

Most miRNAs produced during viral latency originate in the unique long (UL) region of the HCMV genome therefore, all known viral miRNAs even those absent during latency can be reexpressed upon viral reactivation from latency (Meshesha *et al.*,2016).The human genome contains approximately 500 distinct miRNA genes (Berezikov *et al.*,2005).

It plays essential roles in gene regulation in mammals directly or indirectly influence the ability of viruses to replicate and cause infection,cellular miRNAs bind to viral mRNA altering the stability of host mRNA by reducing translation of the viral genome and/or altering the levels of free miRNAs in the cell (Abu-Izneid *et al.*,2021).

During pregnancy many specialized human miRNAs are present in the placenta and play important roles in local immunity suppressing inflammatory responses and maintaining the growth of trophic cells,they also emerge as a risk factor for miscarriage due to their important role in maintaining the uterine environment and fetal growth (Bartel, 2018; Dong *et al.*, 2023).

Any disruption in the normal expression of these miRNAs may lead to impaired embryo implantation, abnormal placental growth, or excessive maternal immune responses, all of which may lead to spontaneous miscarriage (Fu *et al.*, 2023).

HCMV infection can alter host miRNA patterns reducing their levels exacerbating its pathological effects such as increased inflammation and apoptosis in placental tissue (Chen *et al.*, 2019). These changes may exacerbate immune activation or disrupt maternal and fetal tolerance, furthermore the co-expression of viral miRNAs with dysregulated human miRNAs in decidual tissues of women who miscarried after HCMV infection suggests a synergistic mechanism by which viral and host miRNAs interact to promote an unfavorable uterine environment (Zhou *et al.*, 2020). These types include Mir181a and Mir-US5-1

2.15.1.Mir-181a

Mir-181 family is one of the most abundant human miRNAs in lymphoid tissues and maintained an evolutionary trajectory across all vertebrates, it consists of four miRNAs that are considered to be nearly identical mature miRNAs (mir-181a, mir-181b, mir-181c and mir181d) (Neilson *et al.*, 2007).

Mir-181a is primarily expressed in immune cells such as T-cells, NK cells, the central nervous system, muscles and some cancerous tissues, it regulates T-cell sensitivity and acts as an immune sensitivity modulator by lowering the threshold for T-cell receptor (TCR) activation and increases the sensitivity of T-cells to antigens enhancing the immune response against viruses and cancer cells (Leung and Sharp, 2010).

It influences the immune system prenatally and has dynamic effects throughout life on immune cell processes and sharing several targets with them such as T cell development, activation , proliferation into natural killer (NK) cells, promoting the development of hematopoietic progenitor cells (HPCs), it also has a regulatory role in T cells during their development in the thymus, furthermore miR-181a contributes to impaired antiviral responses and its expression declines with age a related defect in adaptive immunity (Kim *et al.*,2021) .

It regulates the expression of genes associated with neuronal survival and synaptic morphology, it is involved in cognitive processes, learning and the response to oxidative stress (Mi *et al.*,2013).

The expression of miR181a is also described during B cell development in the bone marrow, in addition to regulating the functions of innate immune cells such as dendritic cells, macrophages and monocytes and the release of inflammatory cytokines (Lim *et al.*,2020).

It interacts with transcription factors to be selectively and/or highly expressed in immune cells and are important in the maturation, proliferation and differentiation of myeloid and lymphoid cells (Tsitsiou and Lindsay, 2009). Interactions that target mirRNA degradation or inhibit its translation Twenty-six mature microRNAs encoded by HCMV target both viral and host transcription contributing to immune evasion and cell cycle control (Tirabassi *et al.*,2011).

In normal pregnancy mir-181a is expressed in both the maternal and placental circulations particularly in placental trophoblast cells and elevated levels of mir-181a may be present in the plasma of women with

preeclampsia (PE) compared to women with normal pregnancies. Furthermore, mir-181a has been shown to play a role in trophoblast invasion and migration both of which are essential for normal placental development (Toljic *et al.*,2024) .

It also plays a pivotal role in reproductive immunity reducing levels of regulatory T cells by activating sphingosine-1-phosphate receptor 1 (S1PR1), which plays a vital role in regulating the transport of immune cells such as T cells across the lymph nodes. This leads to a stronger immune response against the fetus, which may contribute to immunological miscarriage, especially in cases of viral infections (Geng *et al.*,2022).

2.15. 2. Mir-US5-1

HCMV produces several miRNAs that are key tools in its invasive immune strategy, the most prominent is miR-US5-1 which contributes to complex modifications of gene expression for cellular antigens and immune signaling pathways, it targets the IKK α and IKK β kinase proteins responsible for activating the NF- κ B signaling pathway important in the inflammatory response and the activation of immune genes and cytokines, this inhibition of this pathway reduces the secretion of inflammatory cytokines such as IL-6 and TNF- α during the late stages of infection enabling the virus to survive without an acute immune response (Hancock *et al.*,2017).

Furthermore mir-US5-1 may interfere with cell cycle pathways by disrupting host DNA replication, slowing the growth of cells that detect infection (Jiang *et al.*,2017).

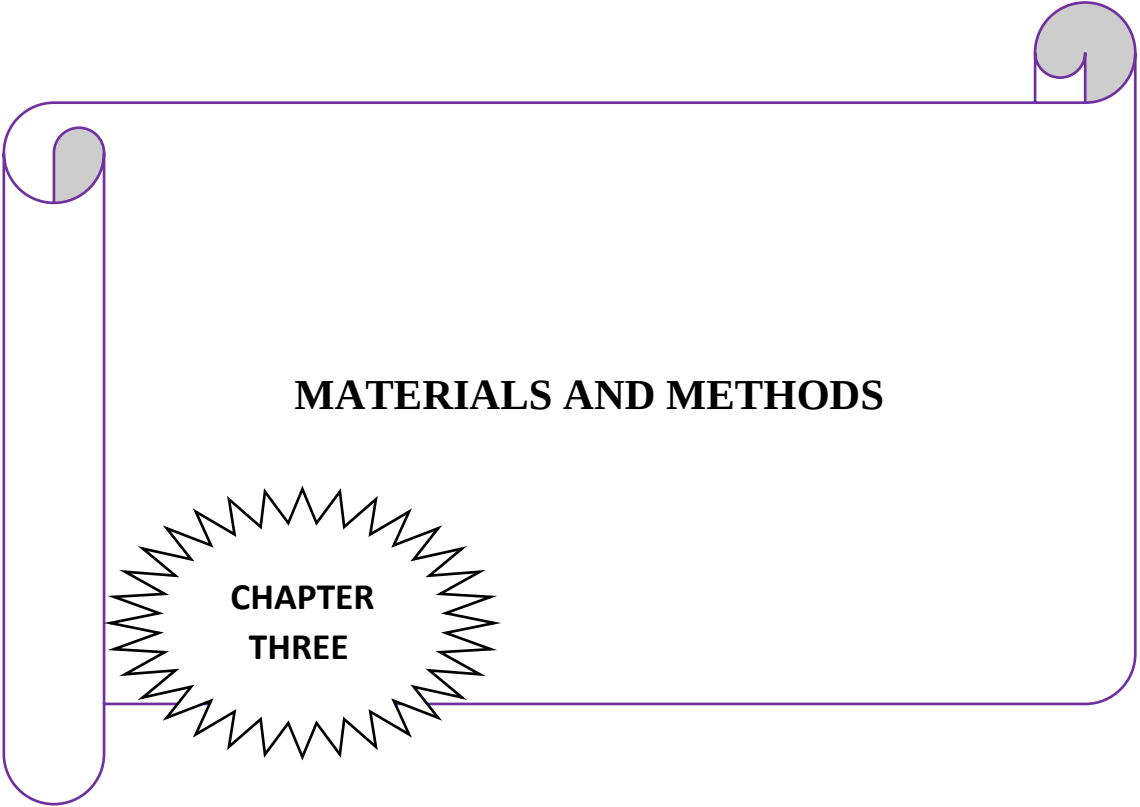
Mir-US5-1 targets endoplasmic reticulum aminopeptidase 1, contributing to immune evasion, ERAP1 is a key polymorphic component of antigen processing and HCMV has adopted multiple strategies to

manipulate host immune responses including the expression of viral miRNAs, ERAP1 variants with potent biological function are recruited to the 3'UTR contributing to modulation of miRNA-mRNA interactions and individual susceptibility to HCMV infection (Melaiu *et al.*,2020).

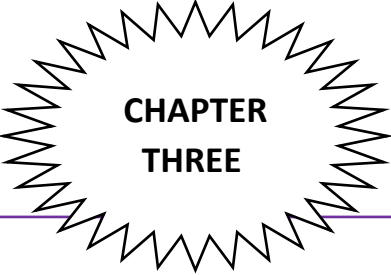
The role of miRNAs encoded by HCMV in the potential mechanism of infection is highlighted by targeting potential mediators of immune evasion and promoting viral replication, these SNPs are preferentially targeted by several different viral miRNAs, including mir-US5-1. Interestingly, this genetic variant is predicted to interact with mir-US5-1 suggesting its important role in HCMV immune evasion by inhibiting MHC class I antigen processing (Li *et al.*,2011).

mir-US5-1 levels are significantly elevated in the plasma of pregnant women with adverse pregnancy outcomes (APO) including miscarriage, this elevation has been verified in a similar independent cohort demonstrating the reliability of these findings and the importance of mir-US5-1 as a primary biomarker (Gao *et al.*,2021).

In addition mir-US5-1 has been shown to target Geminina key inhibitor of DNA replication which promotes the proliferation of host cells including placental cells creating a favorable environment for viral replication, this compromised barrier function is a contributing factor to tissue damage that can lead to miscarriage especially if it is associated with vertical transmission of the virus to the fetus (Zhang *et al.*.,2020).



MATERIALS AND METHODS



CHAPTER THREE

3. Materials and Methods**3.1. Materials:****1. Instruments and equipments:**

Table (3-1): laboratory equipment used in the current study with the name of the company and the country of origin.

Table (3-1): Instruments and equipments that used in current study:

No.	Instrument and equipment	Company	Country
1.	Centerifuge	Kokusan	Japan
2.	Deep freezer	Jeermaks	Germany
3.	Disposable syringe	Sterli Eo	China
4.	EDTA tubes (anticoagulant tubes)	Sun	Jordan
5.	ELISA apparatus	Bio metra	USA
6.	Eppendorf tubes	Sigma	England
7.	Exispin vortex centrifuge	Bioneer	Korea
8.	Gloves,Masks	Broche	China
9.	High speed Cold Centrifuge	Eppendorf	Germany
10.	Hot plat stirrer	Labtech	Korea
11.	Incubator	Memmert	Germany
12.	Microcentrifuge	Lab Tech	Korea
13.	Micropipettes (different volumes) and Tips	Eppendorf	Germany

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14.	Microtiter plate reader (450 nm filter)	Bio TeK	USA
15.	Micro-titer plate shaker	KAHN	Italy
16.	Miniopticon Real Time PCR	Bio-Rad	USA
17.	Multichannel micropipette	SIAMED	Germany
18.	Nanodrop	Thermo Scientific	UK
19.	PCR Tube	Sterilin Ltd.	UK
20.	PCR cabinet	PCR 1000	USA
21.	Rack	Sterellin Ltd.	UK
22.	Refrigerator	Concord	lebanon
23.	Sensitive balance	Sartorius	Germany
24.	T100 Thermal cycler PCR	BioRad	USA
25.	Vacuum blood collection tube	Jiangsu	China
26.	Vortex	CYAN	Belgium
27.	Water bath	Kottermann	Germany

2. Chemicals

The chemical and biological materials used in this work are listed below:

Table (3-2): Chemical materials with their remarks

No.	Chemicals	Company	Country
1.	Absolute Ethanol	Labort	India
2.	Chloroform	Labort	India
3.	DEPC water	Bioneer	Korea
4.	Isopropanol	Labort	India
5.	Nuclease free water	Bioneer	Korea

3. Kits

:The kits used in the current study:

A. Immunological kits :**1. Rapid diagnostic test of HCMV**

This test was performed to detect the presence of specific antibodies IgM, IgG in the serum against HCMV, the kit consists of the following components below:

Table (3 – 3) Components of rapid diagnostic test of HCMV

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

2. ELISA kits:

a.HCMV IgG and IgM antibodies

HCMV IgG and IgM ELISA test (kits) was used in the current study for the specific detection of HCMV IgG and IgM levels in human blood samples (serum) manufacturer's instructions (DRG Instruments GmbH, (Germany) in table (3-4).

Table (3-4) Components of ELISA IgG and IgM test

Reagent	Quantity(96T)
HCMV IgG and IgM Microwell plate	8 wells ×12 strips
Sample Diluent	1vial × 100ml
IgG-RF-Sorbent of HCMV IgM	1vial × 6.5 ml
Standard 1(colored green)	1×2.0 ml
Standard 2 (colored blue)	1×1.0 ml
Standard 3(colored red)	1×1.0 ml
Pos. control (colored black)	1vial × 1.0 ml
Neg. control (colored yellow)	1vial × 2.0 ml
Enzyme conjugate (colored red)	1vial × 20 ml
Substrate solution	1vial × 14 ml
Stop solution	1vial × 14 ml
Wash solution	1vial × 30 ml

b. Cytokines kits

Cytokines ELISA kits including: TNFa, INF- γ , IL-8 and IL-6 (BT Laboratory, Bio-Analytical Technology Laboratory, China) were used according to the manufacturer's instructions in Table (3-5).

Table (3-5) Components of cytokines kits

Reagent	Quantity(96T)
Standard Solution (960ng/L) of TNFa (480ng/L) of INF- γ (1280ng/L) of IL-8 (640ng/L) of IL-6	0.5ml x1
Pre-coated ELISA Plate	12 * 8 well strips x1
Standard Diluent	3ml x1
Streptavidin-HRP	6ml x1
Stop Solution	6ml x1
Substrate Solution A	6ml x1
Substrate Solution B	6ml x1
Wash Buffer Concentrate (25x)	20ml x1
Biotinylated Human IL-6Antibody	1ml x1
User instruction	1
Plate sealer	2pics
Zipper bag	1pic

B. Molecular kits

Table (3-6): the kits with their companies and countries of origin

No.	Kit	Company	Country
1	AccuZol Total RNA Extraction Kit	Bioneer	Korea
	Trizol reagent 100ml		
2	DNase I enzyme kit	Promega	USA
	DNase I enzyme		
	10x buffer		
	Free nuclease water		
	Stop reaction		
3	M-MLV Reverse Transcriptase kit	Bioneer	Korea
	M-MLV Reverse Transcriptase (10,000U)		
	5X M-MLV RTase reaction buffer		
	Dntp		
	100mM DTT		
	RNase Inhibitor		
4	GoTaq® qPCR Master Mix	Promega	USA
	qPCR Master Mix, 2X SYBER green dye, Taq DNA polymerase dNTPs (dATP, dCTP, dGTP, dTTP) and 10X buffer		

4. Primers

The qPCR Primers for human mir- l81a (MIMAT0000256) and HCMV mir-US5-1(MIMAT0001579) that used for direct detection of them by Real-Time PCR were design in this study by using (The Sanger Center miRNA database Registry) to selected miRNA sequence and using miRNA Primer Design Tool whereas, qPCR Housekeeping gene (GAPDH) was design in this study by using NCBI-Database and Primer3 plus design online, these primers were provided by Macrogen company (Korea) as following in table(3-7):

Table (3-7): Primers for human and HCMV:

Primer		Sequence (5'-3')
miRNA universal RT		GTCGTATCCAGTGCAGGGTCCGAGGTATTTCGCACTGGAT ACGACTTTTTTTTTTTVN
mir-US5-1 qPCR primer	F	AACAAGTGACAAGCCTGACG
	R	GTCGTATCCAGTGCAGGGT
Primer		Sequence (5'-3')
mir- l81a qPCR primer	F	AACAATAACATTCAACGCTGTCTG
	R	GTCGTATCCAGTGCAGGGT
GAPDH qPCR primer	F	AAAATCAAGTGGGGCGATGC
	R	TTCTCCATGGTGGTGAAGACG

3.2. Methods:

1. Study Samples

Complete samples were taken from aborted and pregnant women attending the Maternity and Children's Teaching Hospital, in addition to some medical laboratories and outpatient clinics in Diwaniyah Governorate during the period from 15 January 2024 to 15 December 2024, after obtaining informed consent from the participating women and using the samples for research purposes only, Approval was also obtained from the Research Ethics Committee at the Maternity and Children's Teaching Hospital in Diwaniyah.

The total number of study samples was 160 blood samples including 110 samples were taken from each women who had miscarried on the same day and 50 samples from healthy pregnant women, many questions were asked and recorded from the women; this information includes name, age, number of miscarriage , place of residence and whether they had previously infected with COVID-19 , blood samples were collected by venipuncture, with a volume of (5ml) for the purpose of diagnosing HCMV and performing ELISA tests for antibodies to the virus as well as cytokines and miRNA identified in this study.

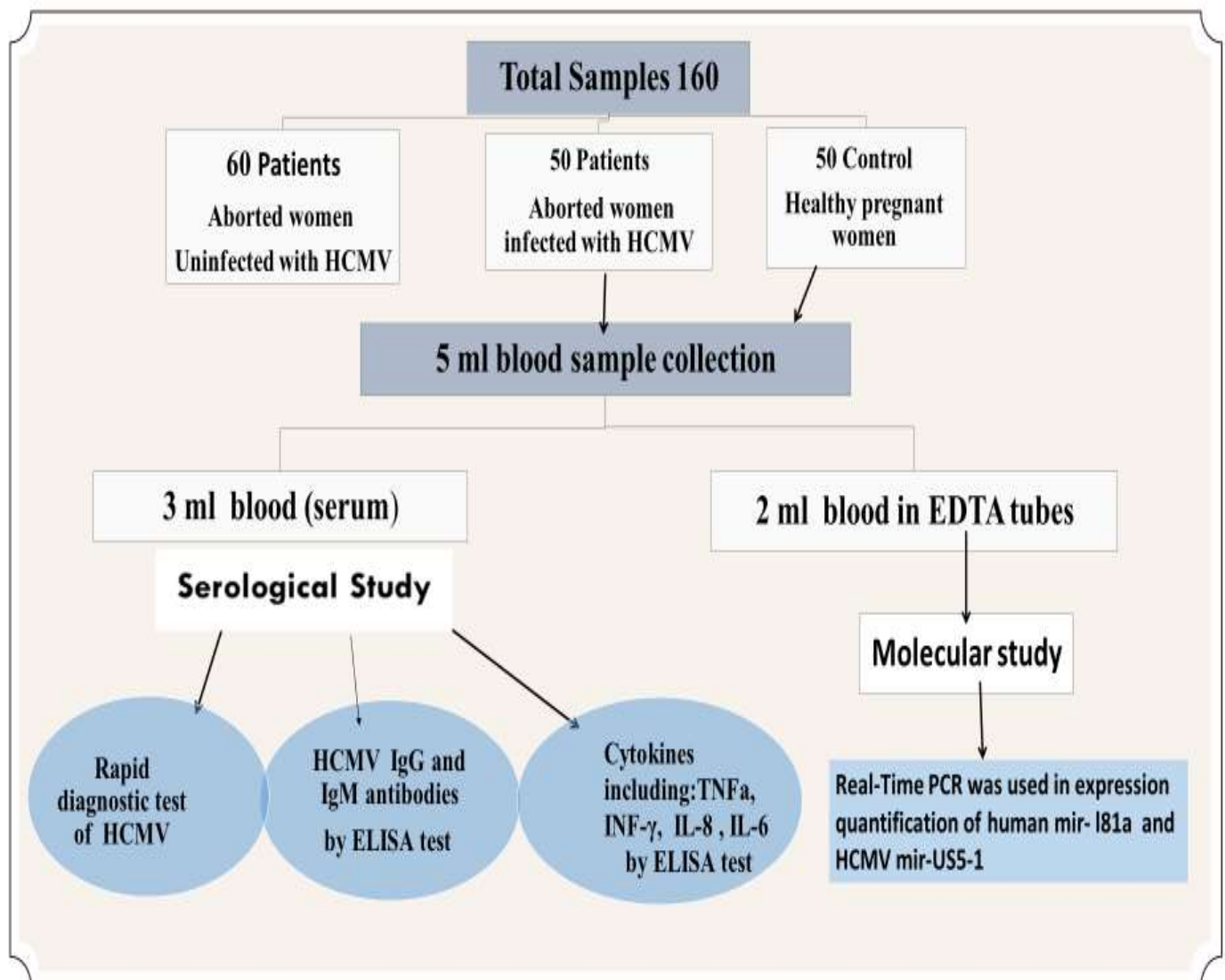


Figure (3-1): The Study Design

2. Rapid diagnostic test of HCMV assay

a. Principle

The rapid test was used for the primary detection of HCMV infection, as the principle of the test for the diagnosis of cytomegalovirus is as follows.

The serum mixing up anti-human IgG / IgM marker move alonge the membrane to the T line, and form the T line with recombinant HCMV antigen when the human serum contains HCMV IgM/IgG according to the principle of double antibody sandwich method and gold immunochromatography assay, which is a positive result,unreacted markers move forward continually to combine with anti-mouse antibody and form a control line. If the test line does not appear, it is a negative result.

b. Procedure

- The outer cover was removed and the test cassette was placed on a clean, flat surface with the sample opening well up.
- A Pasteur pipette was used to transfer (3) drops of serum sample to the cassette vertically with avoiding bubble formation.
- Test results were noted within 15-20 minutes and results were not valid for more than 20 minutes.

3. Detection of HCMV IgG and IgM antibodies by ELISA

The total samples in the current study were tested for HCMV antibodies (IgG and IgM) ,the test was done according to the manufacturing instructions of kit / DRG Instruments GmbH (Germany).

a.Assay procedure:

Before starting the analysis, mixed the diluted wash solution and prepared patient samples thoroughly before placing the pipette and carefully determined the distribution and identification plan included in the kit for all samples and controls.

1) The required number of microtiter strips or wells was selected and inserted into the holder, no less than

For the substrate blank, 1 well (e.g. A1)

For the Negative Control, 1 well (e.g. B1)

For the Standard 1-3 4 wells (e.g. from C1 on)

For the Positive Control ,1 well (e.g. G1) it is left to the user to determine standards, control and patient samples in duplicate.

2) Dispensed :

Neg. Control were added to well B1 in a volume of 100 μ L

Standard 1 100 μ L into well C1 + D1

Standard 2 100 μ L into well E1

Standard 3 100 μ L into well F1

Pos. Control 100 μ L into well G1 and 100 μ L of each diluted sample with new disposable tips into appropriate wells.,left well A1 for substrate blank.

3) The wells were covered with foil supplied with the kit,they were incubated for 60 minutes at 37°C.

4) The contents of the wells were shaken quickly and the wells were rinsed five times with diluted wash solution (300 μ L per well), the wells were then gently tapped on absorbent paper to remove the remaining droplets.

5) Enzyme conjugate was distributed into each well in a volume of 100 μ L except A1.

6) Incubated for 30 minutes at room temperature (20 $^{\circ}$ C to 25 $^{\circ}$ C).

7) The contents of the wells were shaken quickly. The wells were rinsed five times with diluted wash solution (300 μ L per well). The wells were then vigorously tapped on absorbent paper to remove residual droplets.

8) Substrate solution was added to all wells in a volume of 100 μ L. Incubated for exactly 10 minutes at room temperature (20 $^{\circ}$ C to 25 $^{\circ}$ C) in the dark.

9) Stopped the enzymatic reaction by adding 100 μ L of Stop Solution to each well. Any blue color developed during the incubation turns into yellow.

10) The optical density was read at 450 nm/620 nm using a microtiter plate reader within 30 minutes after adding the stop solution.

b. Interpretation of quantitative Results: The following values should be considered as a guideline of HCMV IgG and IgM antibodies below in table (3-8):

Table (3-8): quantitative values for HCMV IgG and IgM

Results	Quantitative of HCMV IgG	Quantitative of HCMV IgM
Negative:	< 9 DU/MI	< 45 DU/mL
Cut-off Value:	10 DU/mL	50 DU/mL
Grey zone (equivocal):	9 - 11 DU/mL	45 - 55 DU/mL
Positive:	> 11 DU/mL	> 55 DU/mL

4. Detection of cytokines

The Sandwich ELISA kit was used in this study for quantitative determination of human TNF α , IFN- γ , IL-8 and IL-6 from in HCMV patient and healthy control serum samples and done according to company instruction (BT-LAB) , The samples used were 100 serum samples including 50 women who had miscarried and were infected with HCMV and 50 healthy pregnant women as a control group.

A. Estimation of cytokines Level

Using ELISA technology TNF α , IFN- γ , IL-8 and IL-6 levels were estimated according to the manufacturer's instructions of ELISA kit provided by BT -LAB, Bioassay Technology lab (China).

B. Principle of Assay:

This kit is an enzyme-linked immunosorbent assay (ELISA). The plate was pre-coated with antibodies to TNF- α , IFN- γ , IL-8, and IL-6. Each cytokine present in the sample was added and bound to the antibodies coated on the wells, a biotinylated human antibody to the cytokines was then added and bound to the sample.

Streptavidin-HRP was then added and bound to the antibody. After incubation, unbound streptavidin-HRP was washed away during the wash step, substrate solution was then added, and color development was proportional to the amount of each cytokine, the reaction was terminated by adding an acidic stop buffer, and the absorbance was measured at 450 nm.

C. Reagents Preparation

- 1- All reagents were brought to room temperature before use.
- 2- Standards for TNF- α , IFN- γ , IL-8, and IL-6 respectively. :

The following standard solutions (960, 480, 1280, 640) ng/L were prepared in 120 μ L volumes respectively and 120 μ L of standard diluent was used to obtain standard solutions with concentrations of (480, 240, 640, 320) ng/L respectively, the standard solution was left for 15 minutes with gentle stirring before dilutions were made duplicate standard points were prepared by serially diluting the standard solution of each cytokine (480, 240, 640 - 320) ng/L in a ratio of 1:2 with standard diluent to obtain solutions with concentrations of (240, 120, 60 and 30) for TNF- α and produce solutions of (120, 60, 30 and 15) ng/L for IFN- γ and the standard diluent was used to produce solutions of (320, 160, 80 and 40) ng/L for IL-8, in addition (160, 80, 40 and 20) ng/L for IL-6

the standard diluent was used as the zero standard (0 ng/L) and any remaining solution should be frozen at -20°C and used within 1 month. dilution of standard solutions are as follows:

Table (3- 9) Dilution of the standard of TNF- α , IFN- γ

TNF-α (ng/L)	IFN-γ (ng/L)	Standard number	Dilution
480	240	Standard No.5	120ul Original standard + 120ul Standard diluent
240	120	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
120	60	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
60	30	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
30	15	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

Table (3- 10) Dilution of the standard of IL-8 and IL-6

IL-8 (ng/L)	IL-6 (ng/L)	Standard number	Dilution
640	320	Standard No.5	120ul Original standard + 120ul Standard diluent
320	160	Standard No.4	120ul Standard No.5 + 120ul Standard diluent
160	80	Standard No.3	120ul Standard No.4 + 120ul Standard diluent
80	40	Standard No.2	120ul Standard No.3 + 120ul Standard diluent
40	20	Standard No.1	120ul Standard No.2 + 120ul Standard diluent

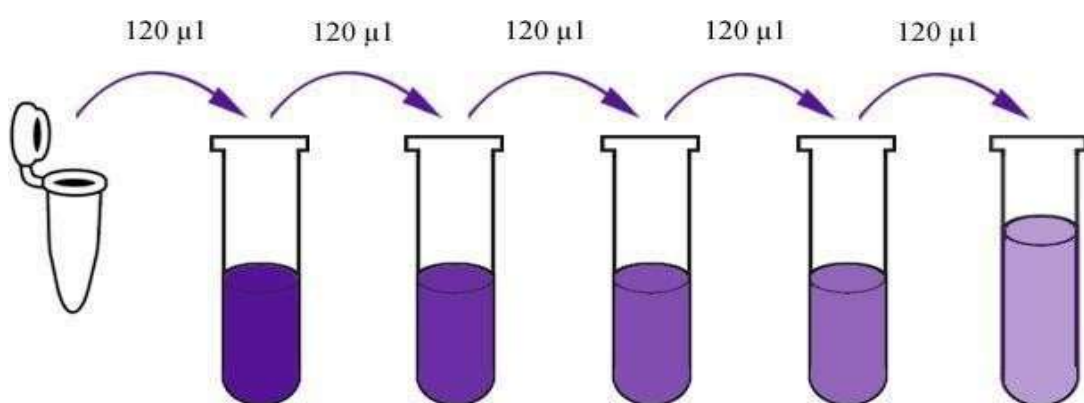


Fig (3-2) Serial dilution of cytokines

3- The wash Buffer concentrate was taken in a volume of 20 ml and diluted 25x with deionized or distilled water to obtain 500 ml of 1x Wash Buffer ,when crystals formed in the concentrate, it was gently mixed until the crystals completely dissolved

Table (3- 11) Standard concentration of cytokines

Cytokine	Standard concentration (ng/L)	Standard No.5 (ng/L)	Standard No.4 (ng/L)	Standard No.3 (ng/L)	Standard No.2 (ng/L)	Standard No.1 (ng/L)
TNF-α	960	480	240	120	60	30
IFN-γ	480	240	120	60	30	15
IL-6	640	320	160	80	40	20
IL-8	1280	640	320	160	80	40

D. Assay Procedure

1. All reagents standard solutions and samples were prepared according to the instructions,all reagents were prepared to room temperature before use,the test was performed at room temperature.
2. The number of strips required for analysis was determined and the strips were inserted into the designated trays,unused strips were stored at a temperature between 2 and 8°C.

3. Standard solution was added at a volume of 50 μL to the standard well.

Note: Don't added antibody to standard well because the standard solution contains biotinylated antibody.

4. Approximately 40 μL of sample was added to the sample wells, then 10 μL of human antibodies to the cytokines identified in this study were added to the sample wells and 50 μL of streptavidin-HRP was added to the sample wells and standard wells (not the blank control well),mixed well then cover the plate with a sealant and incubated for 60 minutes at 37°C.

5. The sealer was removed and the plate was washed 5 times with wash buffer,the wells were soaked in 300 μL of wash buffer for 30 seconds to 1 minute per wash,for automated washing, each well was withdrawn or decanted and washed 5 times with wash buffer ,the plate was dried on paper towels or other absorbent material.

6. Substrate solution A was added at a volume of 50 μL to each well, followed by 50 μL of substrate solution B, the plate was incubated, covered with new sealer for 10 minutes at 37°C in the dark.

7. Stop solution was added at a volume of 50 μL to each well and the color change from blue to yellow was observed immediately.

8. The optical density (OD value) of each well was determined immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

E. Calculate the Results

The standard curve were plotted by the mean OD of each parameter on the vertical (Y) axis versus the focus on the horizontal (X) axis and drawing the most appropriate curve through the points on the Graph. The sample result was calculated using a special program in the computer, and the best fit line could be determined by regression analysis.

Table (3-12) Calibration Sheet for cytokines

Standard	Mean O.D	Concentration (pg/ml)			
		TNF- α	IFN- γ	IL-8	IL-6
ST.1	2.74	480	240	640	320
ST.2	1.411	240	120	320	160
ST.3	0.742	120	60	160	80
ST.4	0.419	60	30	80	40
ST.5	0.111	30	15	40	20

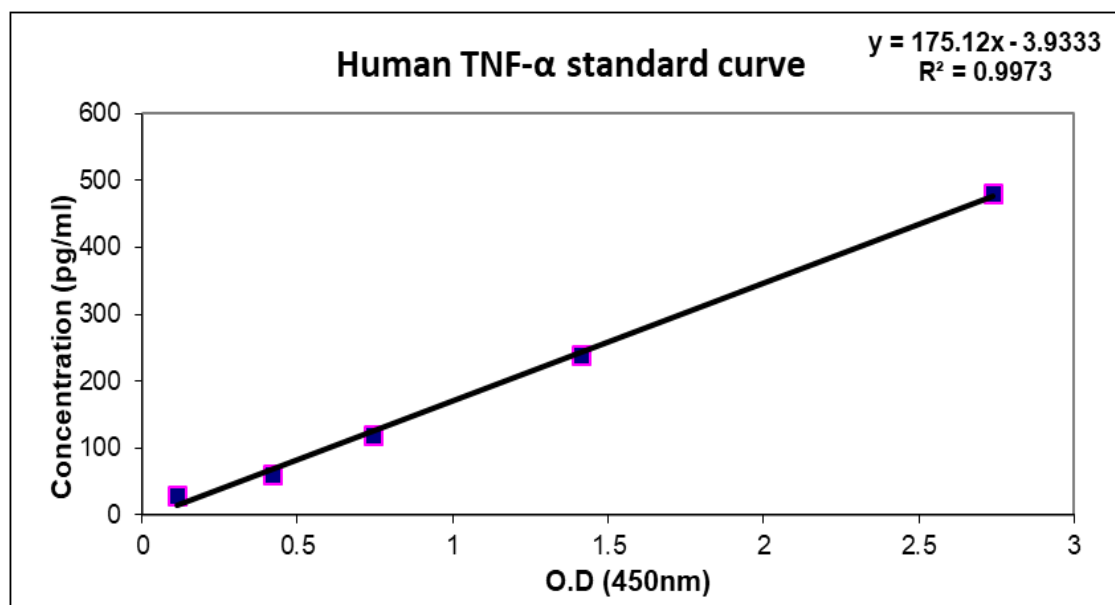


Fig. (3-3) Standard curve of TNF α concentration

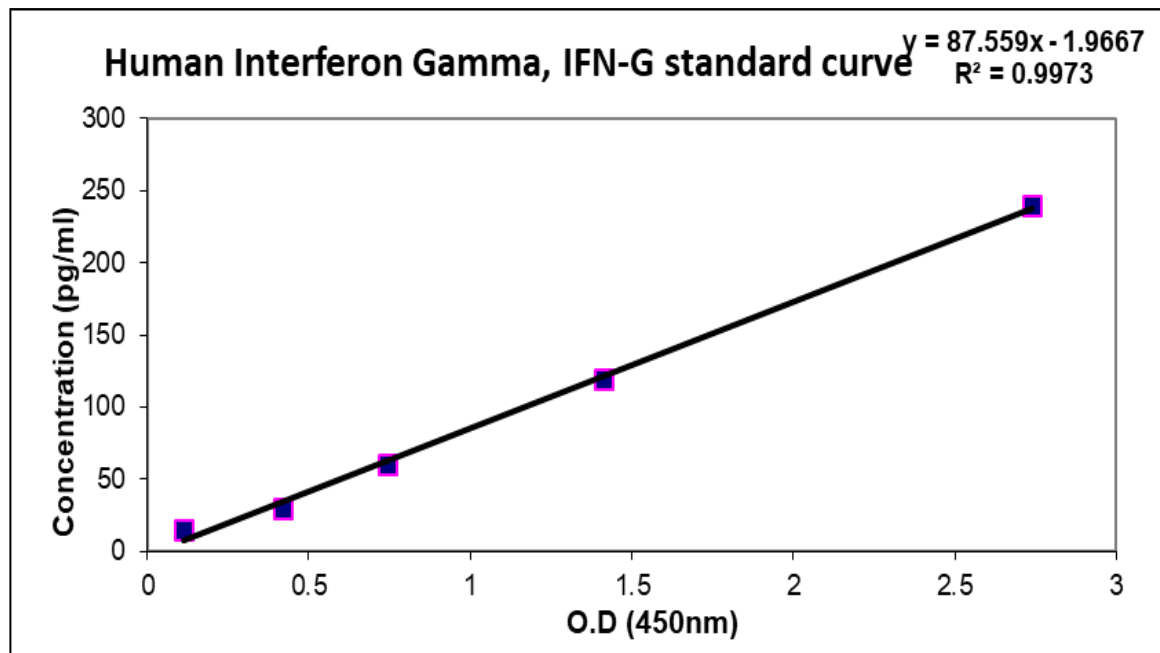


Fig. (3-4) Standard curve of IFN- γ concentration

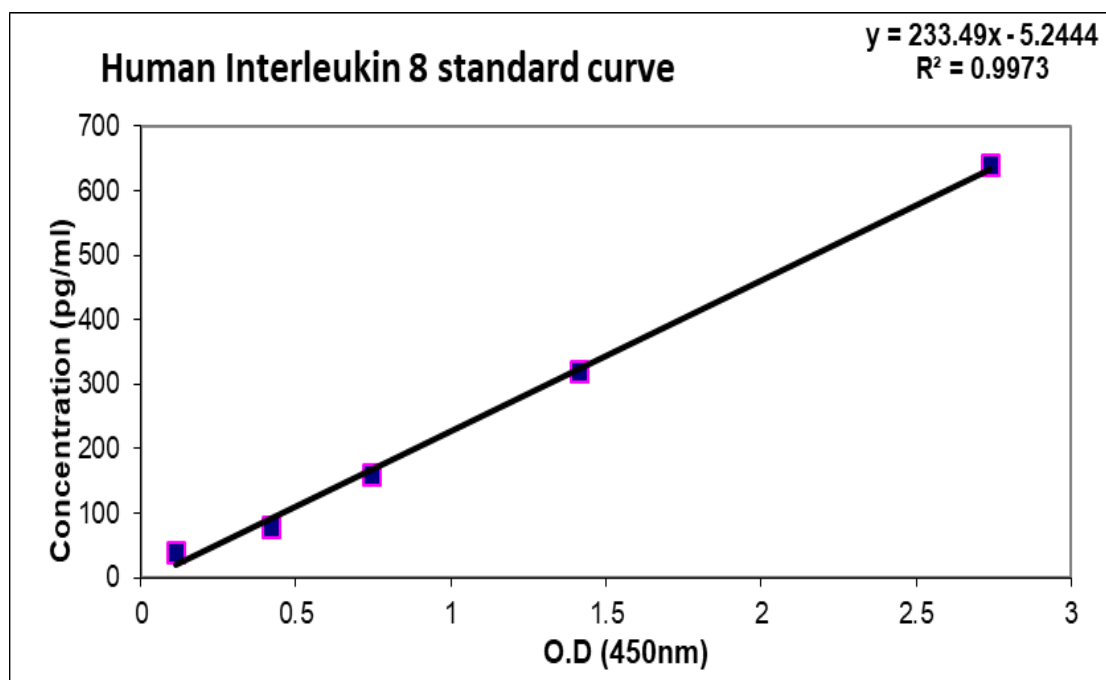


Fig. (3-5) Standard curve of IL-8 concentration

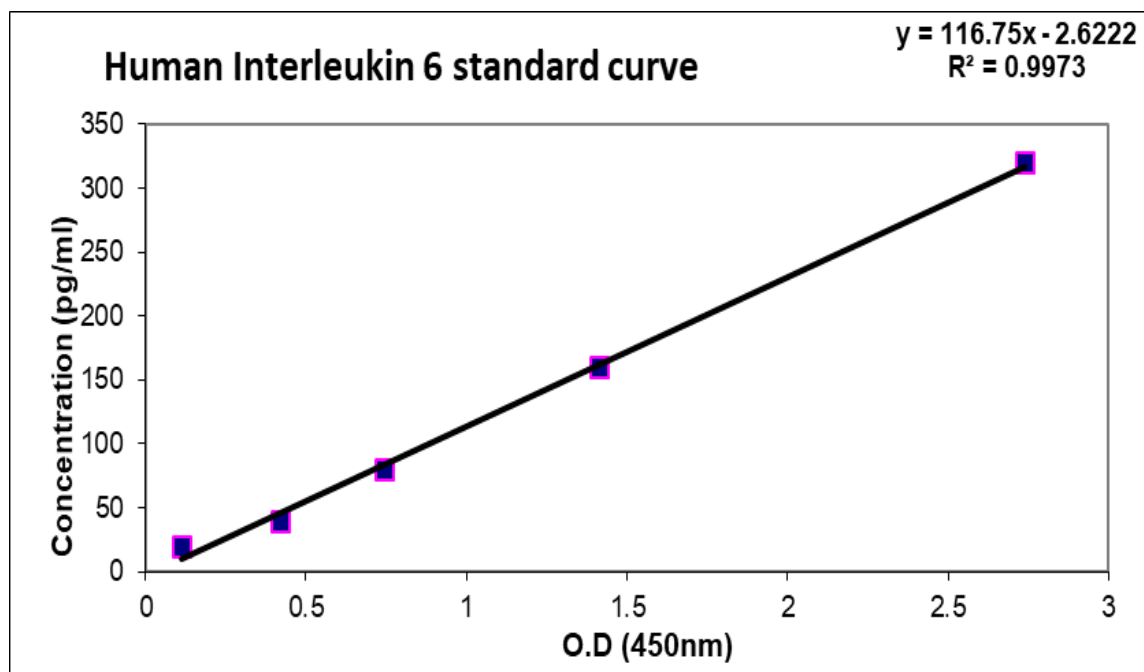


Fig. (3-6) Standard curve of IL-6 concentration

5. Molecular assay:

A. STEM-LOOP RT-qPCR

The stem loop RT-qPCR was used in expression analysis quantification of miR- l81a in human and HCMV mir-US5-1 as well as housekeeping gene (GAPDH) in blood patients and normal samples by using Real-Time PCR technique and this method was carried out according to method described by Qattan *et al.*, (2017) and included the following:

1. Total RNA extraction

Total RNA were extracted from blood samples by using (TRIzol® reagent kit) and done according to company instructions as following:

- a) Blood samples were placed in 1.5 microcentrifuge tube a volume of 250µl then 750µl TRIzol® reagent was added to each tubes.
- b) Chloroform was added to each tube a volume of 200µl and shaken vigorously for 60 seconds.

- c) The mixture was incubated on ice for 5 minutes, then centrifuged at 12000 rpm, 4C°, for 15 minutes.
- d) Supernatant was transferred into a new eppendorf tube and 500µl isopropanol was added, then, mixture mixed by inverting the tube 4-5 times and incubated at 4C° for 10 minutes then centrifuged at 12,000 rpm, 4C° for 10 minutes.
- e) Supernatant was discarded and 1ml 80% ethanol was added and mixed by vortex again. Then, centrifuge at 12000 rpm, 4C° for 5 minutes.
- f) The supernatant was discarded and the RNA pellet was left to air to dry.
- g) DEPC water was added to each sample a volume of 50µl to dissolve the RNA pellet, Then, the extracted RNA sample was kept at -20.

2. Estimation RNA yield and quality

The extracted genomic RNA was checked by using nanodrop spectrophotometer (THERMO. USA) that check RNA concentration and estimation of RNA purity through reading the absorbance in at (260 /280 nm) as following :

- a) After opening up the Nanodrop software, chosen the appropriate application (Nucleic acid, RNA).
- b) A dry wipe was taken and cleaned the measurement pedestals several times. Then carefully pipet 2µl of ddH₂O onto the surface of the lower measurement pedestal.

- c) The sampling arm was lowered and clicking OK to blank the Nanodrop, then cleaning off the pedestals.
- d) The pedestals were cleaned and pipet 1 μ l of RNA sample for measurement.

3. DNase I Treatment

The extracted RNA were treated with DNase I enzyme to remove the trace amounts of genomic DNA from the eluted total RNA by using samples (DNase I enzyme kit) and done according to method described by Promega company, USA instructions as follow:

Mix	Volume (μL)
Total RNA 100ng/ μ l	10
DNase I enzyme	1
10X buffer	4
DEPC water	5
Total	20

After that, The mixture was incubated at 37C° for 30 minutes. also, 1 μ l stop reaction was added and incubated at 65C° for 10 minutes for inactivation of DNase enzyme action.

B. cDNA synthesis

1. cDNA synthesis for miRNA

DNase-I treated RNA samples were used in miRNA cDNA synthesis step for miRNA by using **M-MLV Reverse Transcriptase kit** and done according to company instructions as following tables:

Step 1

RT master mix	Volume (μL)
Total RNA 100ng/ μ L	8
miR RT primer	1
DEPC water	1
Total	10

RNA and primer was denatured for 10 min at 65 °C, after that immediately cool on ice.

Step 2

RT master mix	Volume (μL)
Step 1 RT master mix	10
M-MLV RTase (200u)	1
5X M-MLV RTase reaction buffer	4
100mM DTT	2
Dntp	2
RNase inhibitor	1
Total	20

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions:

Step	Temperature (°C)	Time
cDNA synthesis (RT step)	42	1 hour
Heat inactivation	95	5 minutes

2. cDNA synthesis for GAPDH gene

DNase-I treated RNA samples were also used in cDNA synthesis step for GAPDH gene by using **M-MLV Reverse Transcriptase kit** and done according to company instructions as following tables:

Step 1

RT master mix	Volume (µL)
Total RNA 100ng/ul	8
Random Hexamer primer	1
DEPC water	1
Total	10

RNA and primer was denatured for 10 minutes at 65 °C, after that immediately cool on ice.

Step 2

RT master mix	Volume (μL)
Step 1 RT master mix	10
M-MLV RTase (200u)	1
5X M-MLV RTase reaction buffer	4
100mM DTT	2
Dntp	2
RNase inhibitor	1
Total	20

Then the tubes were placed in vortex and briefly spinning down. The RNA converted into cDNA in thermocycler under the following thermocycler conditions:

Step	Temperature ($^{\circ}$C)	Time
cDNA synthesis (RT step)	42	1 hour
Heat inactivation	95	5 minutes

a. qPCR master mix preparation

qPCR master mix was prepared by using **GoTaq® qPCR Master Mix kit** that dependent on SYBER Green dye detection of gene amplification in Real-Time PCR system and include the follow:

Standard protocol:

qPCR master mix	Volume (μL)
miRNA cDNA template (20ng)	5
Forward primer(10 pmol)	1
Reverse primer (10 pmol)	1
qPCR master mix	10
Nucelaes free water	3
Total	20

Then, these qPCR master mix component that mentioned above placed in qPCR strip standard plate tubes and mixed by Exispin vortex centrifuge for 3 minutes, then placed in MiniOpticon Real-Time PCR system.

b. MiRNA qPCR Thermocycler conditions

After that, the qPCR plate was loaded and the following thermocycler protocol in the following table:

qPCR step	Temperature (°C)	Time	Repeat cycle
Initial Denaturation	95	5 minutes	1
Denaturation	95	20 seconds	45
Annealing\Extention Detection(scan)	60	30 seconds	

C. Quantitative Real-Time PCR (qPCR)

The quantitative Real-Time PCR used in quantification **GAPDH** gene expression analysis as following:

1. qPCR master mix preparation

qPCR master mix was prepared by using **GoTaq® qPCR Master Mix kit** and include the follow:

qPCR master mix	Volume (µL)
cDNA template (20ng)	5
Forward primer(10pmol)	1
Reverse primer (10pmol)	1
qPCR master mix	10
DEPC water	3
Total	20

After that, these qPCR master mix component that mentioned above placed in qPCR strip plate tubes and mixed by Exispin vortex centrifuge for 3 minutes, then placed in Miniopticon Real-Time PCR system.

2. qPCR Thermocycler conditions

The qPCR plate was loaded and the following thermocycler protocol in the following table:

qPCR step	Temperature (°C)	Time	Repeat cycle
Initial Denaturation	95	5 minutes	1
Denaturation	95	20 seconds	45
Annealing\Extention Detection(scan)	60	30 seconds	

D. Data analysis of qPCR

The data results of q RT-PCR for target and housekeeping gene were analyzed by the relative quantification gene expression levels (fold change) (The Δ CT Method Using a reference gene) that described by (Livak and Schmittgen, 2001). as following equation:

$$\Delta\text{CT (Test)} = \text{CT (target gene, test)} - \text{CT (HKG gene, test)}$$

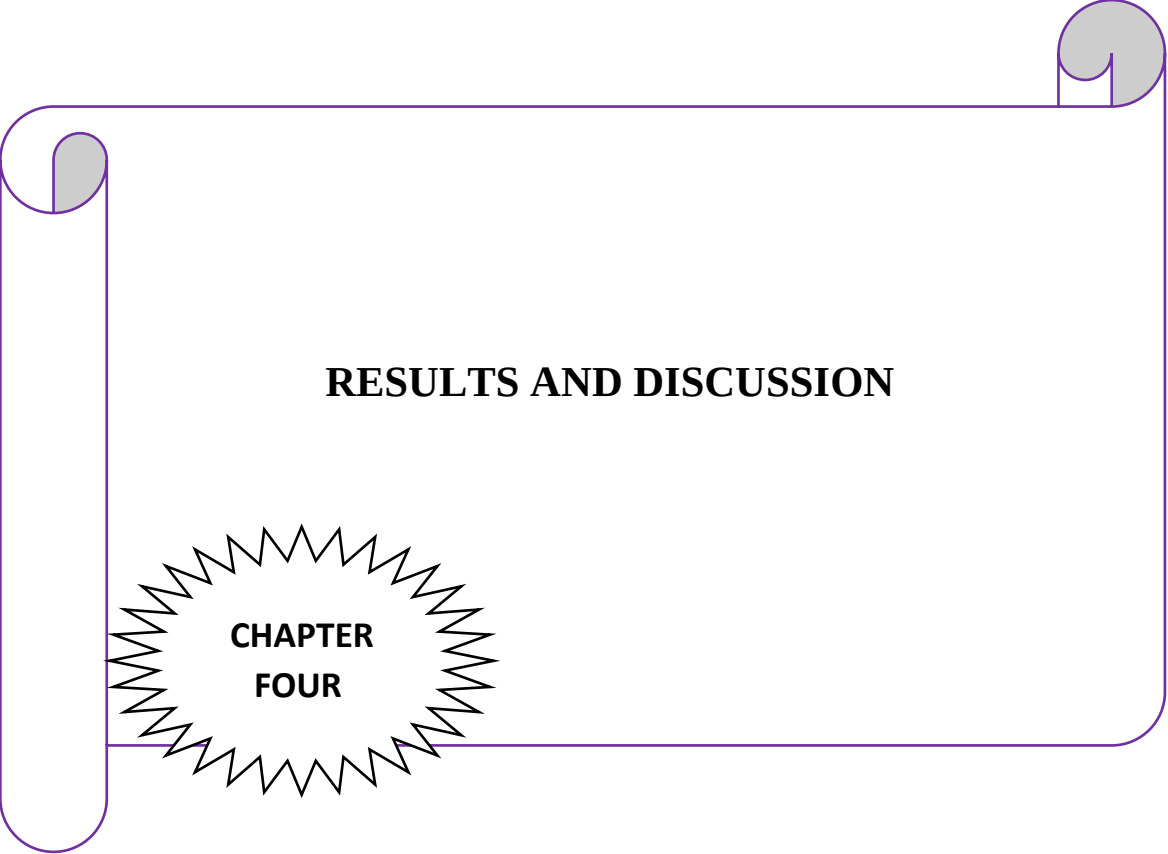
$$\Delta\text{CT (Control)} = \text{CT (target gene, control)} - \text{CT (HKG gene, control)}$$

$$\Delta\Delta\text{CT} = \Delta\text{CT (Test)} - \Delta\text{CT (Control)}$$

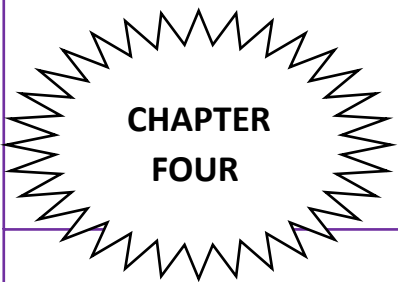
$$\text{Fold change (target / HKG)} = 2^{-\text{CT}} \Delta\Delta\text{CT}$$

E. Statistical analysis :

Data were collected and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26 and Microsoft Office Excel 2010. The independent samples t-test was used to examine the difference in means between any two groups and the one-way analysis of variance (ANOVA) and chi-square test were used to examine the association between any two categorical variables, the Pearson correlation was used, and the results were expressed in terms of the correlation coefficient (r) and the significance level (P), where P is less than 0.05, and a high significance level is 0.01 or less (Daniel and Cross, 2018).



RESULTS AND DISCUSSION



CHAPTER FOUR

4. Results and discussion

4.1. Detection of Human Cytomegalovirus

4.1.1. Rapid diagnosis of HCMV

The current study enrolled 160 blood samples from (110 miscarried women and 50 healthy pregnant women as control) to investigated HCMV by using rapid diagnostic test (cassette) and the results in table (4-1) showed as 50 (45.5%) of women with miscarriage had positive results of rapid diagnostic test and all healthy pregnant women had negative results, also, 60 (54.5%) women who had miscarried were negative for the rapid test and not infected with HCMV.

Table (4-1): Rapid diagnosis of HCMV in miscarried women and healthy pregnant women

Rapid diagnostic test	Patients with miscarriage n = 110	Healthy pregnant women n = 50
HCMV infected women n (%)	50 (45.5%)	0
HCMV uninfected women n (%)	60 (54.5%)	50 (100.0%)

The results of the current study were supported by the results of the study by Jassim *et al.*, (2024) which used the rapid HCMV test for women who had abortions recording a positive rate of 50% (15 out of 30 cases) while the remaining cases were negative for the test which enhances the reliability of this type of test as an effective initial screening tool for detecting cases of HCMV infection. It is also consistent with a study by Bander and Mohammad, (2024) that indicated that 42.6% of women with

spontaneous miscarriage were positive for HCMV compared to only 13.6% in the group of uninfected pregnant women, which supports the relationship between HCMV infection and an increased risk of miscarriage.

Another study conducted by Jaber *et al.*,(2023) on 719 miscarried women showed the test was used as a primary screening method for detecting HCMV 53 cases (7.37%) were positive for the rapid test.

Unlike other infections HCMV often became active during the reproductive years and can be transmitted to the fetus despite maternal immunity. Infection during pregnancy poses a significant challenge, leading to miscarriage in most cases. Up to 40% of HCMV infections during pregnancy cross the placenta and infect the fetus, potentially leading to HCMV syndrome (Rufati *et al.*,2018). HCMV is endemic in most parts of the world, with a geographic seroprevalence ranging from 30% to 100%. Early and accurate diagnosis of infection before its effects become apparent, with minimal pathogens, is essential (Saravi *et al.*,2023) .

4.1.2. Detection of HCMV by ELISA technique

To confirm the HCMV infection, the suspected patients and healthy control subjects submitted to IgG and IgM detected by ELISA technique and the results demonstrated in table (4-2) showed all of miscarried women 50 (100.0%) for IgG had active or previous HCMV infection ,whereas 20 (40.0%) of miscarried women had active HCMV infection by positive results for IgM, while all healthy pregnant women 50 (100.0 %) had negative results of both IgM and IgG .

Table (4-2): Prevalence HCMV infection according to ELISA technique in studied groups

ELISA	Women with miscarriage n =50	Healthy pregnant women n = 50
IgG		
Positive, n (%)	50 (100.0%)	0
Negative, n (%)	0	50 (100.0%)
IgM		
Positive, n (%)	20 (40.0%)	0
Negative, n (%)	30 (60.0%)	50 (100.0%)

The results of the current study were similar to the studies by Bander and Mohammed., (2023) and Jaber *et al.*, (2023) in Diwaniyah Governorate whom showed the percentage of IgG was higher than IgM , another study in Babylon Governorate by Khikani *et al.*, (2024) and Saleh *et al.*, (2018) was similar to the results of the current study and showed positive serological results for IgG higher than IgM.

HCMV seropositivity is widespread worldwide the current study is similar to study of Gubran, (2023) in Yemen, Sharmin *et al.*,(2023) in Bangladesh and Choi *et al.*, (2021) in Korea whom showed the prevalence of IgG of HCMV infection was higher than IgM.

While the results of current study differed from the results of a study presented by Anwar and Al-Bayati, (2018) in Samarra city and study by

Al-Moussawi and Al-Hajjar,(2021) in Babylon Governorate whom showed IgM rate was higher in women infected with HCMV compared to IgG using the ELISA method.

Elevated levels of (HCMV) IgG in women who have miscarried are attributed to a previous infection or a latent virus in the body, during pregnancy the infection may reactivate or be reinfected, stimulating a strong immune response with the production of large amounts of IgG, the virus can cause indirect damage to the placenta by activating immune cells, secreting inflammatory cytokines and reducing the efficiency of nutrient and oxygen exchange between the mother and fetus, increasing the risk of miscarriage. Furthermore, the presence of IgG does not necessarily indicate an active infection, but it indicates potential risks if the mother's immunity is unable to suppress the latent infection or if it reactivates. (Pontes *et al.*, 2024).

IgM antibodies may indicated a new infection with human HCMV or reinfection with a new strain, because the virus was the most common source of congenital infectious disease more than 70% of infections during the first trimester of pregnancy can result in miscarriage and the seroprevalence rates of IgM and IgG to HCMV and association with recurrent miscarriage may represent a real risk based on pregnancy complications (Vasani and Kumar, 2019).

4.2. Demographic study of miscarried women with HCMV and healthy pregnant women subjects

The demographic characteristics of 50 miscarried women with HCMV and 50 healthy pregnant women subjects (after excluded the aborted women without positive HCMV infection) were shown in table (4-3), the mean age of patients was 28.82 ± 6.27 and that of control was 26.76 ± 5.49 years and there was no significant in mean age ($P = 0.118$), again, there was no significant difference in the frequency distribution of patients and control subjects according to age group ($P = 0.146$). on the other hand, most of patients with HCMV enrolled in the present study were between 21-25 years of age, 16 (32.0%).

Table (4-3): Comparison between patients and control groups in Age

Characteristic	Miscarried women with HCMV $n = 50$	Healthy pregnant women $n = 50$	<i>P</i>
Age (years)			
Mean $\pm$ SD	28.82 ± 6.27	26.76 ± 5.49	0.118 †
Age groups (years)	15– 44	18 – 38	NS
15-20 , n (%)	7 (14.0%)	10 (20.0%)	0.146 ¥ NS
21-25 , n (%)	16 (32.0%)	16 (32.0%)	
26-30 , n (%)	5 (10.0%)	10 (20.0%)	
31-35 , n (%)	12 (24.0%)	12 (24.0%)	
36-40 , n (%)	6 (12.0%)	2 (4.0%)	
41-45 , n (%)	4 (8.0%)	0	

n: number of cases; *SD*: standard deviation; †: independent samples t-test; ¥: Chi-square test; NS: not significant at $P > 0.05$

In a study supporting the results of the current study by Jaber *et al.*, (2023) whom found the frequency distribution of HCMV positive patients by age group included 16-26 years, which had the highest number of cases. Another study, continuing with the current study, conducted by Sharmin *et al.*, (2023) in Bangladesh, whom showed that the target age group was the 18-22 year group, with the highest prevalence rate of 37.7%, while the lowest prevalence was recorded among the 38 year group and above, with 4.3%. In another study supporting the current study by Mackenzie *et al.*, (2019), whom found the frequency distribution of HCMV positive patients by age group included 21-25 years.

Some studies differed from the current study , such as the study by Al-Zubaidi *et al.*, (2025) in Diwaniyah whom found that the highest rate of IgG positivity of HCMV was in the age group 26-35 years (43.33%), while the lowest rate (13.0%) was in the age group 36-45 years. Also study conducted by Ghailan and Mohammed, (2020) in Kirkuk Governorate reported that the majority of participants in their study were aged 26 to 37, representing the highest percentage (59%), the lowest percentage of infected women was aged 37 to 47, representing 19% .

Also study by Sahu *et al.*, (2019) whom showed that the age group above 31 years had the highest prevalence of HCMV infection.

HCMV infection during pregnancy poses a significant challenge, the virus often reactivates during the reproductive years and can be transmitted to the fetus despite the mother's immune system. In most cases, it led to miscarriage (Pinar *et al.*, 2018; Saravi *et al.*, 2023).

The reason for the 21-25 age group having the highest incidence of abortions compared to other age groups may be that this group is the most married, and that pregnancy rates in this group will be very high, and studies have shown that the highest rate of miscarriage was among women in their twenties, because this is the period during which pregnancy occurs and it is more common in miscarried women with a TORCH infection (Coskun *et al.*, 2020).

4.3. Distribution of miscarried women with HCMV infection and healthy pregnant women by residency

Results of the current study included 75 woman (75%) from urban areas and 25 woman (25%) from rural areas, the group of patients with miscarriages included 35 patients (70%) from urban areas and 15 patients (30%) from rural areas, while the control group included 40 healthy pregnant women (80%) from urban areas and 10 healthy pregnant women (20%) from rural areas as shown in the table (4-4),

Table(4-4): Distribution of patients with HCMV and control groups in residency

		Residency		Total
		Urban	Rural	
Groups	Patients with HCMV	35 (70.0%)	15 (30.0%)	50.0
	Healthy pregnant women	40 (80.0 %)	10 (20.0.0%)	50.0
Total		75 (75.0%)	25 (25.0%)	100

Studies consistent with the current study included a study by Al-Kinani and Al-Kaabi ,(2022) whom showed that the incidence HCMV infection in aborted women was higher in urban areas than in rural areas. Also study conducted by Ghailan and Mohammed ,(2020) in Kirkuk Governorate reported that the majority of participants in their study lived in urban areas (64%) and in rural areas (30%).

While studies that differed with the current study included a study by Al-Zubaidi *et al.*, (2025) in Diwaniyah whom found that the incidence rate of HCMV infection in aborted women in rural and urban areas was (96.48%) and (95.26%), respectively. another study conducted by Jaber *et al.*, (2023) and Al-Jbouri *et al.*,(2020) whom found that the prevalence of HCMV infection in aborted women was higher in rural areas than in urban areas.

The prevalence of miscarriages resulting from HCMV infection in urban areas is attributed to several environmental and social factors, most notably high population density, increased social interaction and mobility, and frequent exposure to contaminated body fluids (such as saliva and urine), especially in crowded settings such as health centers and nurseries, this increases the likelihood of transmission of the virus between individuals, particularly among pregnant women ,low levels of health awareness about latent infection and the absence of early screening during pregnancy contribute to delayed detection of infection and increased rates of primary HCMV infection, which leads to miscarriage (Kenneson and Cannon, 2007; Pesch and Schleiss ,2022).

4.3. Some clinical features associated miscarriage

The results of the current study showed the frequency distribution of aborted women with HCMV, as well as healthy pregnant women, according to some clinical features, as shown in table (4-5), the total number of pregnancies was shown as follows: 24 (48.0%) of patients had fewer than 4 pregnancies and 26 (52.0%) of patients had 4 or more pregnancies, the current results showed that most of the healthy pregnant women had fewer than 4 pregnancies, 38 (76.0%), and the difference was significant ($p=0.004$).

In addition to the number of miscarriage, the frequency distribution of patients was as follows: 29 (58.0%) of patients with HCMV had one miscarriage, 17 (34.0%) of patients with HCMV have two miscarriage, while only 4 (8.0%) of patients have three or more miscarriage , but all healthy women did not have miscarriages 50 (100%) and the difference was highly significant ($p=0.001$).

Also the current results showed non-significant difference miscarried women patients with HCMV and healthy control according to Covid-19 infection.

Table (4-5): The frequency distribution patients with HCMV and healthy control according to number of pregnancies , miscarriages and COVID-19 infection

Characteristic	Patients with HCMV n = 50	Healthy pregnant women n = 50	<i>P</i>
Number of total pregnancies			
< 4, <i>n</i> (%)	24 (48.0%)	38 (76.0%)	0.004 ¥ S
≥ 4, <i>n</i> (%)	26 (52.0%)	12 (24.0%)	
Number of miscarriages			
One, <i>n</i> (%)	29 (58.0%)	0	0.001 ¥ S
Two, <i>n</i> (%)	17 (34.0%)	0	
≥ three, <i>n</i> (%)	4 (8.0%)	0	
Without miscarriages, <i>n</i> (%)	0	50 (100.0%)	
Covid-19 infection			
Positive, <i>n</i> (%)	29 (58.0%)	26 (52.0%)	0.546 ¥ NS
Negative, <i>n</i> (%)	21 (42.0%)	24 (48.0%)	

n: number of cases; ¥: Chi-square test; NS: not significant at P > 0.05; S: significant at P < 0.05

In a study that was similar to the results of the current study, conducted by Mocanu *et al.*, (2024), included 465 women who had previously been pregnant twice or more, despite the presence of miscarriages among them, were included, as 84 women had a history of miscarriage at least once, moreover, there are not enough studies that address the number of pregnancies in aborted women with HCMV, it is believed that a previous healthy pregnancy is possible in aborted women due to HCMV because the virus was in a latent state and did not reactivate or affect the pregnancy (Rahav *et al.*,2007).

Many studies supporting the findings of the current study by Hameed Haddad *et al.*, (2024) who showed that the highest percentage of women with a history of miscarriage was 33.53% (57) of women with a history of miscarriage more than once, out of 150 women who were examined and had a history of miscarriage.

Another study converged with the current study by Ghailan and Mohammed ,(2020) in Kirkuk Governorate reported that the majority of participants in their study had a miscarriage rate of 71%, with known causes, including HCMV, the highest percentage (38%),the remainder were distributed among other viral, parasitic, and physiological causes. The highest percentage of miscarriages was recorded among those who had had a single miscarriage (67%), while those who had two miscarriages (26%), three miscarriages (4%) and the lowest percentage was recorded among those who had had four or more miscarriages (3%).

There may be a close association between HCMV infection and increased miscarriage rates, especially in women who have previously had successful pregnancies by affecting the immune response in the placenta, the virus is believed to disrupt the delicate immune balance that contributes to impaired trophoblast development and adhesion, leading to implantation failure or stunted fetal growth (Abdulla ,2024).

The results of the current study were consistent with a prior study by Jaber *et al.*,(2023) whom found that aborted women with HCMV had higher rates of COVID-19 infection, as well as previous convergent studies Le Balc'h *et al.*, (2020) and Schuierer *et al.*,(2020) indicated that herpes virus reactivation at higher rates is common in patients with severe acute respiratory distress syndrome associated with COVID-19.

COVID-19 infection can temporarily weaken immunity during or after pregnancy leading to reactivation of latent or primary HCMV infection in women with a previously latent infection, also COVID-19 infection before or during pregnancy has been associated with a significantly increased rate of miscarriage (Sandoval *et al.*, 2025). The presence of latent HCMV infection in women is also associated with a higher rate of previous miscarriage, suggesting that reactivation of latent infection or vertical transmission is more likely when immunocompromised, such as that caused by COVID-19 occurs (Mocanu *et al.*, 2024).

4.4. Immunological Analysis Results

4.4.1. Serum Tumor Necrosis Factor- α (TNF- α) level

The results of the current study showed that the level of serum TNF- α in the serum of miscarried women infected with HCMV and the healthy pregnant women reached 172.72 ± 8.99 pg/ml and 61.74 ± 8.93 pg/ml, respectively, as shown in table (4-6) and figure (4-1), the level was significantly higher in patients compared to the healthy control group ($P < 0.001$).

Table (4-6): TNF- α level in miscarried women with HCMV and healthy control subject

Tumor Necrosis Factor- α level (pg/ml)	Miscarried women with HCMV <i>n</i> = 50	Healthy pregnant control <i>n</i> = 50	<i>P</i>
Mean $\pm$ SD	172.72 $\pm$ 8.99	61.74 $\pm$ 8.93	< 0.001
Range	128.46 – 165.41	43.17- 78.55	† HS

n: number of cases; **SD:** standard deviation; **†:** independent samples t-test; **HS:** Highly significant at $P \leq 0.001$

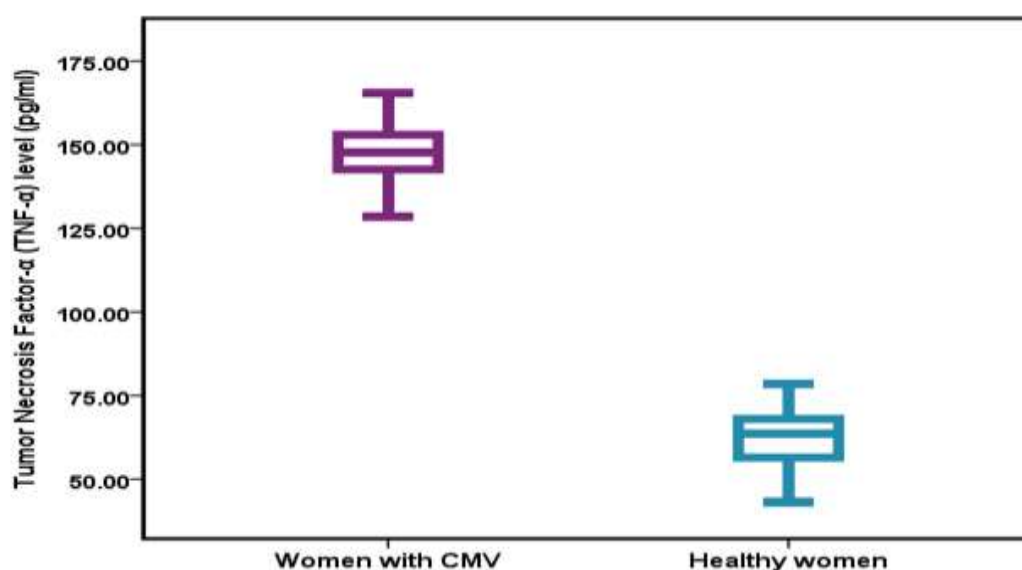


Figure (4.1): Mean Serum TNF- α level of patients and healthy controls

The results of the current study were similar to a study by Ahmed and Raad, (2024) on pregnant women who had previously had a miscarriage showed that TNF- α levels were higher in the miscarried group than in the control group (915.0 pg/ml vs. 784.6 pg/ml) with a p-value of 0.001.

The results of the current study were also consistent with previous studies by Al-Kinani and Al-Kaabi ,(2022) ; Vesce *et al.*, (2022) and Sherif *et al.*, (2014) as the results showed a significant increase in the concentration of TNF- α in miscarried women .

While in a study that differed from the current study by Chmaj-Wierzchowska *et al.*,(2018) whom results focused on cytokine levels in women participating in the study, which included a group at risk of miscarriage and a group with normal pregnancies, no significant changes in cytokine concentrations were observed in all groups.

The virus disrupts the regulation of cytokines during pregnancy, leading to changes in the placental immune environment. Increased concentrations of TNF- α may be associated with an increased risk of obstetric complications particularly fetal loss and recurrent growth restriction, the human immune system cannot eliminate HCMV infection and its replication period, but rather acts by promoting active immune defense, including TNF- α , which targets several genes important for immune evasion and encoded by HCMV, the intensity of the HCMV-specific cellular immune response may also cause early immune exhaustion and, consequently, a rise in T-cell cytotoxicity (Haider and Knofler, 2009; Chaudhuri *et al.*, 2009) .

4.4.1.1 Distribution of TNF- α levels according to some characteristics

The comparison of TNF- α levels according to some characteristics ,the results were demonstrated in table (4-7). The current results show non-significant difference of TNF- α levels according to all studied characteristic including IgG and IgM levels as well as the number of miscarriages in miscarried women with HCMV and COVID-19 infection ($P > 0.05$).

Table (4-7): Distribution of serum TNF- α level according to some characteristic

Characteristics		N	TNF- α Mean $\pm$ SD	P
IgG	Positive	50	172.72 $\pm$ 8.99	
	Negative	0		
IgM	Positive	20	147.26 $\pm$ 8.19	0.811 † NS
	Negative	30	148.03 $\pm$ 9.67	
Number of miscarriages	One	29	148.34 $\pm$ 7.25	0.111 K NS
	Two	17	148.24 $\pm$ 10.84	
	$\geq$ three	4	141.02 $\pm$ 11.77	
Covid-19 infection	Positive	29	147.74 $\pm$ 8.92	0.804 † NS
	Negative	21	147.69 $\pm$ 9.31	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

In a study related to the current study by Al-Kinani and Al-Kaabi ,(2022) conducted in Najaf province, the serological profile of 65 miscarried women infected with HCMV was evaluated using ELISA , the results showed a predominant elevation of IgG compared to IgM with a significant immuno-inflammatory elevation of TNF- α in HCMV-associated miscarriages.

A study by Muhammad Faizal *et al.*, (2022) continued with the current study, whom showed that miscarried women had significantly higher levels of IgG compared to IgM, indicating a latent or previous infection,also, higher levels of TNF- α suggested an immune-inflammatory role in miscarriage, even in cases of non-primary infection.

The results of the current study also support a Canadian study by Balegamire *et al.*, (2024), whom showed that the presence of HCMV IgG in the first trimester of pregnancy was significantly associated with an increased risk of preterm birth and preeclampsia, the study demonstrated that 97% of the effect of HCMV on preterm birth was direct, attributed to inflammation in the placenta associated with latent infection, including elevated TNF- α in placental tissue, which may negatively impact trophoblast function and pregnancy stability.

Elevated IgG levels compared to IgM levels in miscarried women with HCMV infection may indicate recurrent or chronic infection, rather than primary infection, this reflects prior immune exposure, as IgG persists and accumulates, whereas IgM rapidly declines after acute infection, in early pregnancy, immunosuppression increases the risk of HCMV reactivation, even in the absence of elevated IgM levels, notably, significantly elevated serum TNF- α concentrations have been observed in miscarried women (Stuart *et al.*, 2012).

TNF- α is a proinflammatory cytokine that contributes to the disruption of cellular motility and the immune environment within the placenta, potentially inducing apoptosis in trophoblast cells and impairing placental growth. These mechanisms have been directly linked to spontaneous abortion and poor pregnancy outcomes (Haider and Knofler, 2009).

The current study differed from previous studies by Piosik *et al.*, (2013) and Lee *et al.*, (2013) whom reported that elevated TNF- α levels in women with secondary recurrent pregnancy loss are characteristic of women with higher TNF- α levels at the beginning of pregnancy compared to women with primary recurrent pregnancy loss.

A similar study to the current study by Garcia-Flores *et al.* (2024) found elevated levels of TNF- α in the placenta of women who miscarried after contracting COVID-19, this reflects a strong inflammatory response that may contribute to pregnancy complications such as preterm birth or miscarriage. While the results of the current study differ from those of Chen *et al.* (2020), whom examined results from mothers who tested negative for COVID-19, although there is no reported evidence of vertical transmission of the virus, the maternal immune response to viral infection tends to be enhanced by an inflammatory response, characterized by elevated levels of inflammatory cytokines, such as TNF- α .

Inflammation leading to miscarriage may arise from impaired control of fetal cytokines therefore, maintaining Th1 immunity is associated with other late complications, such as fetal growth restriction, preterm birth, preeclampsia, and even miscarriage, under fetal growth restriction, stimulation of maternal peripheral mononuclear cells with trophoblast antigens results in elevated levels of the pro-inflammatory cytokines such as ,TNF α compared to normal fetal growth. In such cases, trophoblasts are enhanced as a result of the maternal inflammatory response, rather than suppressed (Raghupathy *et al.*, 2012 ;Al-Azemi *et al.*, 2017). Pregnant women undergo immunological changes that can increase maternal susceptibility to infectious diseases (WHO ,2020).

The severe inflammatory response caused by COVID-19—a “cytokine storm” that damages multiple organs, including the placenta—is believed to foster an environment conducive to the transmission of CMV to the fetus and may lead to miscarriage (Rad, 2021).

4.4.2. Serum Interferon gamma (IFN- γ) level

The results were shown in table (4-8) and figure (4-2), the mean levels of IFN- γ in serum were 53.87 ± 4.81 pg/ml and 36.65 ± 6.80 pg/ml in miscarried women infected with HCMV and the healthy control group, respectively; the level was significantly higher in patients compared to healthy women ($P < 0.001$).

Table (4-8): IFN- γ level in miscarried women with HCMV and healthy control subject

Interferon gamma (IFN- γ) level (pg/ml)	Miscarried women with HCMV $n = 50$	Healthy pregnant women $n = 50$	P
Mean $\pm$ SD	53.87 ± 4.81	36.65 ± 6.80	< 0.001
Range	43.65 – 68.43	26.31- 61.78	† HS

n : number of cases; SD: standard deviation; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

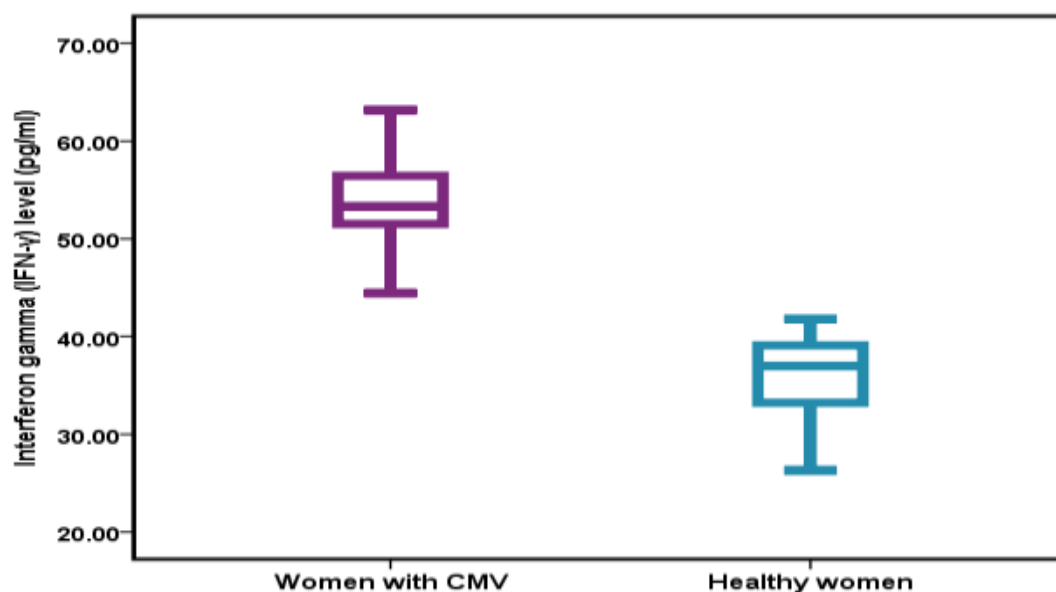


Figure (4.2): Mean serum IFN- γ level of patients and healthy controls

Similar studies to the current study's findings were presented by Ahmed and Raad , (2024) on pregnant women who had previously had a miscarriage also showed that interferon gamma levels were higher in miscarried group than in the control group. Also a similar study by Jaber *et al.*, (2023) was conducted on a number of 45 women who had miscarried and were infected with HCMV showed that INF- γ levels were higher in the women who had miscarried 49.41 ± 2.94 compared to the control group, which was 28.37 ± 1.61 .

Another convergent study by Sharif *et al.*, (2014) whom showed a significant increase in mean concentrations of Th1 cytokines, including IFN- γ , in women with recurrent miscarriage, compared to pregnant and non-pregnant women.

While the results of the study by Chmaj-Wierzchowska, *et al.*, (2018)) differed regarding cytokine levels in women at risk of miscarriage and a control group with normal pregnancies, as interferon-gamma levels were similar in all groups, no significant changes in cytokine concentrations were observed

The reason for the excessive increase in IFN- γ levels may be due to the immune response, which is a defensive reaction to eliminate the pathogen, such as human cytomegalovirus infection (Kak *et al.*, 2018).

4.4.2.1 Distribution of IFN- γ levels according to some characteristics

The results of the current study showed that IFN- γ levels according to some characteristics as shown in table (4-9), it showed insignificant differences in IFN- γ levels according to all the studied characteristics

including IgG and IgM levels as well as the number of miscarriages in miscarried women with HCMV and COVID-19 infection ($P < 0.05$).

Table (4-9): Distribution of serum IFN- γ level according to some characteristic

Characteristics		N	IFN- γ Mean $\pm$ SD	P
IgG	Positive	50	53.87 $\pm$ 4.81	
	Negative	0		
IgM	Positive	20	52.92 $\pm$ 3.95	0.416 † NS
	Negative	30	54.43 $\pm$ 4.43	
Number of miscarriages	One	29	54.49 $\pm$ 3.83	0.366 K NS
	Two	17	53.69 $\pm$ 4.49	
	$\geq$ three	4	49.60 $\pm$ 4.16	
Covid-19 infection	Positive	29	53.78 $\pm$ 4.22	0.683 † NS
	Negative	21	53.90 $\pm$ 4.31	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

Results of the current study did not show a significant difference compared to the study conducted by Jaber *et al.*, (2023). However, the mean levels of INF- γ in the serum of patients with HCMV Igm positive were higher than those in patients with HCMV Igm negative and IgG postive , which was higher than in this study, but the difference was not significant, but the difference was not statistically significant at ($P = 0.114$).

Also in a study by Muhammad Faizal *et al.*, (2022), interferon- γ levels were significantly higher in primary HCMV infection and significantly higher in pregnant women with HCMV infection.

IFN- γ stimulates B cell activation, increasing the production of IgG antibodies, particularly those associated with inflammatory responses, the presence of these antibodies disrupts the function of endothelial cells in the placenta and increases microscopic blood clots, leading to early or late pregnancy failure (Erlebacher, 2013).

The results of study showed an increase in (IFN- γ) levels in women who had miscarried, but this increase was not statistically significant. This result is partially consistent with that reported in a study by Daher *et al.*, (2004), which included women with recurrent miscarriage (three or more), where the study recorded a significant increase in the mean serum IFN- γ concentration in the women compared to the control group (355.8 ± 32.5 pg/ml vs. 98.0 ± 28.3 pg/ml; $P < 0.01$).

Recurrent miscarriages are associated with impaired regulatory T cell function, which plays a key role in suppressing effective immune cells, such as Th1 cells responsible for producing interferon- γ , imbalanced immune systems at the maternal-fetal interface may lead to increased Th1 cell activity, creating an inflammatory environment that is intolerant to the fetus and contributing to pregnancy failure (Alijotas-Reig *et al.*, 2014).

A similar study to the current study by Garcia-Flores *et al.*, (2024) found elevated levels of, IFN- γ in the placenta of women who miscarried after contracting COVID-19. This reflects a strong inflammatory response that may contribute to pregnancy complications such as preterm birth or miscarriage. Also a study by Joneborg and Marions, (2014) showed that women diagnosed with COVID-19, compared to uninfected pregnant women, had a higher risk of developing serious pregnancy complications, including preeclampsia and preterm birth.

While studies that differed from the current study showed by Valdespino-Vázquez *et al.*, (2021) that the risk of spontaneous abortion and preterm birth was not increased in women who became infected with COVID-19 in the third trimester of pregnancy.

The results of the study by Elshafeey *et al.*, (2020) showed no complications of miscarriage during pregnancy due to COVID-19 infection.

Pro-inflammatory cytokines were significantly elevated in pregnant women with primary HCMV infection, suggesting a predominant Th1 response (Muhammad Faizal *et al.*,2022). Elevated levels of pro-inflammatory cytokines in pregnant women with COVID-19 can cause serious consequences, including miscarriage or fetal developmental abnormalities., changes in the maternal immune system during pregnancy may also affect the response to infections, especially viruses, in general, the immune system adapts during pregnancy to the developing fetus, resulting in altered immune responses to infections during pregnancy, in placental trophoblast cells from women with a history of miscarriage, this response includes increased production of pro-inflammatory cytokines, such as IFN- γ (Han *et al.*, 2020) .

4.4.3. Serum Interleukin-8 (IL-8) level

The results of the current study showed that the mean serum IL-8 level among miscarried women infected with HCMV and the healthy control group was 127.74 ± 10.80 pg/ml and 66.17 ± 8.26 pg/ml, respectively, as shown in table (4-10) and figure (4-3). The level was significantly higher in miscarried women infected with HCMV compared to the healthy control group ($P < 0.001$).

Table (4-10): IL-8 level in miscarried women with HCMV and healthy control subject

Interleukin-8 (IL-8) level (pg/ml)	Miscarried women with HCMV <i>n</i> = 50	Healthy pregnant women <i>n</i> = 50	<i>P</i>
Mean± SD	127.74 ± 10.80	66.17 ± 8.26	< 0.001 † HS
Range	99.36 – 163.10	50.09- 77.88	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

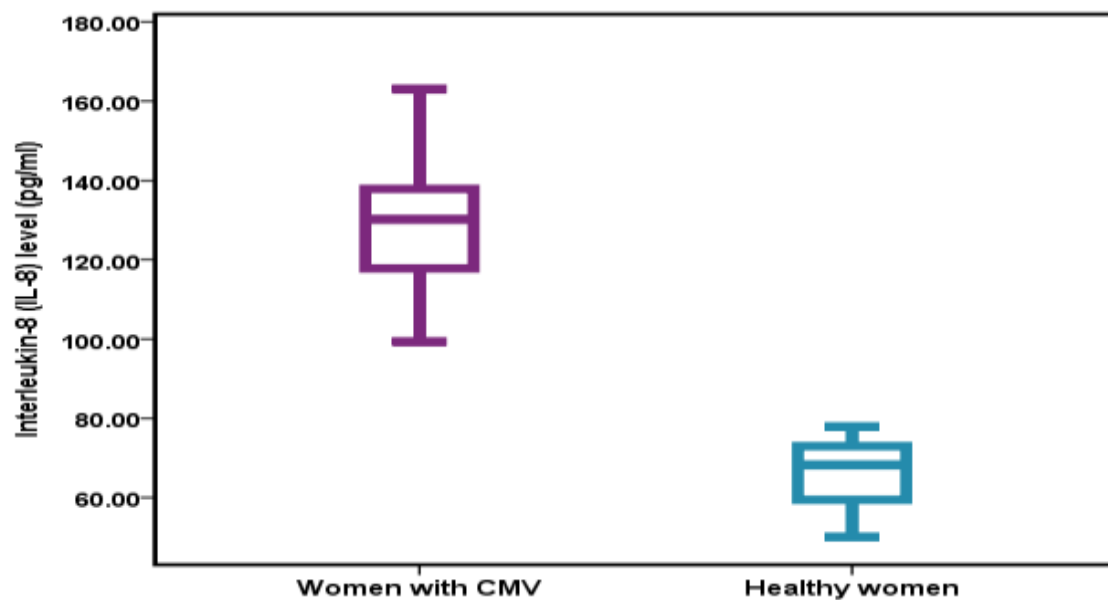


Figure (4.3): Mean Serum IL-8 level of patients and healthy controls

Recent studies continuing with the results of the current study, such as Zhao *et al.*, (2022), Chen *et al.*, (2021) , Wang *et al.*, (2021), and Aboud, (2010) whom indicated increase in IL-8 expression in Recurrent Pregnancy Loss (RPL) patients compared to normal pregnancies.

In a study different from the current study by Pitman *et al.* (2013), had reported decreased expression/secretion of IL-6 and IL-8 at the fetal-maternal junction was reported in cases of unexplained early pregnancy loss and in isolated Sporadic Pregnancy Loss (SPL), expression IL-8 was found to be lower compared to normal pregnancies.

Elevated IL-8 levels indicated an exacerbate inflammatory state at the point of contact between the fetus and the mother, which may harm the implanted embryo and affect the pregnancy, both insufficient and excessive levels of IL-8 disrupt the inflammatory network, which may affect the pregnancy. Furthermore, differences in the expression pattern of IL-8 in reproductive tissues in cases of recurrent pregnancy loss support the hypothesis that these complications may have a significantly different etiological background (Galazios *et al.*, 2011; Laisk *et al.*, 2020).

4.4.3.1 Distribution of IL-8 levels according to some characteristics

The results were shown in table (4-11), which included non-significant differences in IL-8 levels according to all the characteristics studied including IgG and IgM levels as well as the number of miscarriages in aborted with HCMV and COVID-19 infection ($P < 0.05$).

Table (4-11): Distribution of serum IL-8 level according to some characteristic

Characteristics		N	IL-8 Mean $\pm$ SD	P
IgG	Positive	50	127.74 $\pm$ 10.80	
	Negative	0		
IgM	Positive	20	126.38 $\pm$ 13.39	0.877 † NS
	Negative	30	128.64 $\pm$ 13.85	
Number of miscarriages	One	29	128.10 $\pm$ 13.67	0.298 K NS
	Two	17	128.55 $\pm$ 12.66	
	$\geq$ three	4	121.65 $\pm$ 11.46	
Covid-19 infection	Positive	29	126.57 $\pm$ 12.39	0.815 † NS
	Negative	21	129.34 $\pm$ 11.56	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

In studies supportive to the current study by Karachrysafi *et al.*, (2023) there was indicated that chronic IgG-positive immune conditions are associated with elevated IL-8, also there was a significant increase in the expression of IL-8 in the decidual tissue and trophoblastic tissue of patients with recurrent pregnancy loss.

While a different study by Leños-Miranda *et al.*, (2021), Romero *et al.*, (2015) and Kamity *et al.*, (2014) observed increased expression of IL-8 in acute placental inflammation (API) with consistent release of other acute-phase mediators in trophoblasts in response to inflammatory stimulation.

The presence of positive IgG reflects previous or ongoing exposure to an antigen such as HCMV, leading to long-term immune activation. This chronic activation stimulates the secretion of IL-8, a cytokine that attracts

neutrophils and activates the vascular endothelium, disrupting the intrauterine immune environment and increasing the risk of miscarriage (Karachrysafi *et al.*, 2023).

A study similar to the current study by Hattori *et al.*, (2007) who indicated a clear role for immune inflammation in cases of recurrent pregnancy loss (RPL), as elevated levels of (IL-8) are one of the most prominent inflammatory markers associated with these conditions. Their study showed that women with a history of two or more miscarriages showed elevated levels of IL-8 in their cervical mucus compared to women who successfully completed their pregnancies, suggesting the role of IL-8 as an early indicator of immune dysfunction in the uterine environment in recurrent miscarriages.

While a different study presented by Chen *et al.*, (2020) whom showed that in mothers who tested negative for COVID-19, despite no evidence of vertical transmission of the virus, the mothers' immune response to viral infection tended to be enhanced by an inflammatory response, characterized by elevated levels of inflammatory cytokines, such as IL-8.

However, there are no further published studies that directly link the number of miscarriages as a numerical variable (e.g., 2, 3, 4 cases), as well as COVID-19 infection and its relationship with women who have had miscarriages with HCMV and its association with IL-8 levels.

Jovanovic-Krivokuca *et al.*, (2016) whom found elevated levels of IL-8 expression in placental inflammation as a result of an inflammatory immune response . IL-8 may be involved in regulating trophoblast invasion

and spiral artery remodeling through IL-8 signaling, suboptimal levels of stromal IL-6 and IL-8 may lead to insufficient trophoblast invasion and spiral artery remodeling and ultimately to early PL (De Oliveira *et al.*, 2010; Pitman *et al.*, 2013).

4.4.4. Serum Interleukin-6 (IL-6) level

The results were shown in table (4-12) and figure (4-4). The mean serum IL-6 level among miscarried women infected with (HCMV) and the healthy control group was 113.36 ± 6.22 pg/ml and 35.24 ± 6.08 pg/ml, respectively, the level was significantly higher in miscarried women infected with HCMV compared to the healthy control group ($P < 0.001$).

Table (4-12): Serum IL-6 level in miscarried women with HCMV and healthy control subject

Interleukin-6 (IL-6) level (pg/ml)	Miscarried women with HCMV <i>n</i> = 50	Healthy pregnant women <i>n</i> = 50	<i>P</i>
Mean± SD	113.36 ± 6.22	35.24 ± 6.08	< 0.001 † HS
Range	101.05 – 129.31	26.57- 56.92	

n: number of cases; **SD**: standard deviation; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$

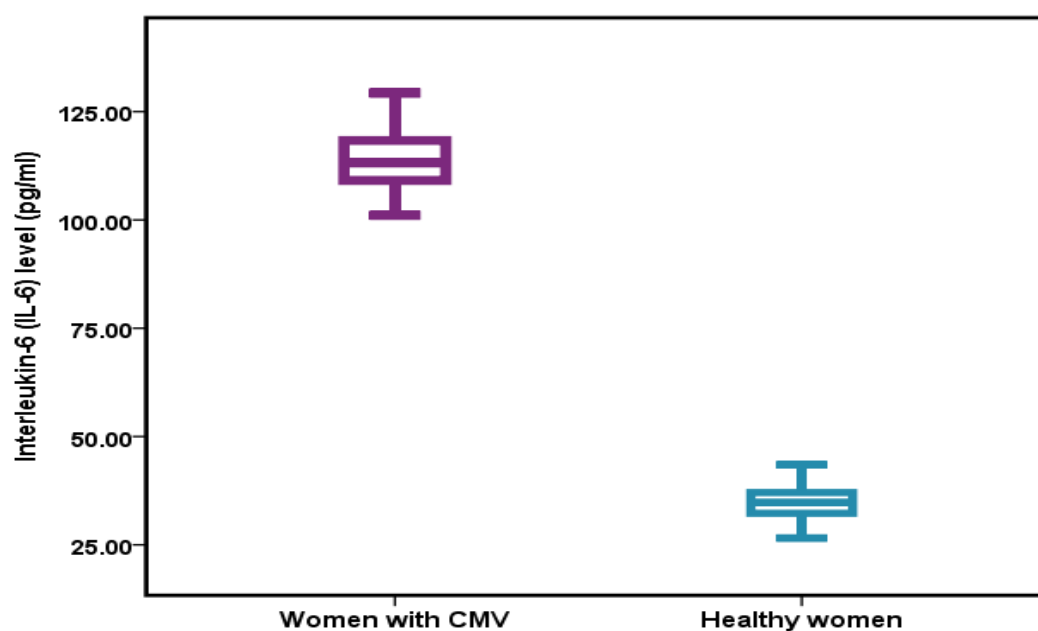


Figure (4.4): Mean Serum IL-6 level of patients and healthy controls

Previous studies have been presented that were consistent with the current study by Amin *et al.*, (2024), the mean IL-6 level in females was 38.98, compared to the control group, which was less than 7 pg/ml. The mean highest IL-6 level was 80.6 in females, and was recorded in patients aged 51 to 60 years. Also study by Jaber *et al.*, (2023) conducted on 45 women who had aborted their pregnancy with HCMV found that levels of IL- 6 were higher in the miscarried women 32.67 ± 2.47 compared to the control group, which was 19.62 ± 2.4 .

In addition, a study conducted by Vesce *et al.*, (2022) whom showed that elevated levels of IL- 6 during inflammation are the main cause of miscarriage, pregnancy loss, and premature birth and study by Leños-Miranda *et al.*, (2021) whom found that high IL-6 concentrations in Amniotic Fluid (AF) were significantly associated with an increased risk of perinatal morbidity and mortality.

In a study different from the current study by Ahmed, (2017) and Pitman *et al.*, (2013), had reported decreased expression/secretion of IL-6 at the fetal-maternal junction was reported in cases of unexplained early pregnancy loss and in isolated Sporadic Pregnancy Loss (SPL) expression of IL-6 was found to be lower compared to normal pregnancies and a study conducted by Aboud, (2010) also showed a decrease in IL-6 levels in Austrian women with recurrent miscarriages in the first trimester compared to pregnant and non-pregnant women.

While the results of a study by Chmaj-Wierzchowska, *et al.*, (2018) whom that IL-6 levels were evaluated in women at risk of miscarriage compared to a healthy control group, no significant differences in IL-6 levels were recorded between women with normal pregnancies and women at risk of miscarriage and the results were similar in all groups.

Increased IL-6 levels following viral infection are associated with an increased number of CD8 T cells, this elevation may be due to infection of the placenta and the membranes surrounding the fetus, as infection is associated with increased expression of IL-6. In the placental trophoblast, excessive secretion of IL-6 following viral infection suppresses the secretion of certain hormones, including estrogen and estradiol, two hormones that play an important role in creating a balance between Th1 and Th2, leading to miscarriage (Gupta and Chia, 2013).

4.4.4.1 Distribution of IL-6 levels according to some characteristics

The comparison of IL-6 levels according to some characteristics including IgG and IgM levels as well as the number of miscarriages in aborted with HCMV and COVID-19 infection that has been carried out and the results were demonstrated in table (4-13), show non-significant difference of IL-6 levels according to all studied characteristic ($P > 0.05$).

Table (4-13): Distribution of serum IL-6 level according to some characteristic

Characteristics		N	IL-6 Mean $\pm$ SD	P
IgG	Positive	50	113.36 $\pm$ 6.22	
	Negative	0		
IgM	Positive	20	113.10 $\pm$ 4.82	0.844 † NS
	Negative	30	113.54 $\pm$ 7.08	
Number of miscarriages	One	29	113.52 $\pm$ 5.78	0.190 K NS
	Two	17	114.44 $\pm$ 6.46	
	$\geq$ three	4	107.65 $\pm$ 6.93	
Covid-19 infection	Positive	29	112.94 $\pm$ 5.91	0.691 † NS
	Negative	21	113.95 $\pm$ 6.73	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

The results of the current study also supported a Canadian study by Balegamire *et al.*, (2024) whom showed that the presence of HCMV IgG in the first trimester of pregnancy was significantly associated with an increased risk of preterm birth and preeclampsia, this is attributed to inflammation in the placenta associated with latent infection, including elevated IL-6 in placental tissue, which may negatively affect trophoblast function and pregnancy stability.

Another study, conducted by Jaber *et al.*, (2023), confirmed that the difference was not significant, as the mean IL-6 levels in the serum of patients with HCMV IgG levels were found to be higher than IgM levels and Igm-positive patients were lower than those in patients with HCMV Igm-negative patients. However, the difference was not statistically significant ($P = 0.580$).

The current study also continued with study by Pourroostaei Ardakani *et al.*, (2022) which demonstrated that chronic HCMV infection and IgG seropositivity were significantly more prevalent among Iranian women with recurrent pregnancy loss compared to the control group (44% vs. 25.45%, $P = 0.005$), with increased IL-6 expression in the presence of chronic viral antigenic stimulation.

While the current study differed from study by Muhammad Faizal, *et al.*, (2022), whom showed that the expression of cytokines in pregnant women with primary and non-primary HCMV infection was determined to determine their concentrations IL-6 were significantly elevated in pregnant women with primary HCMV infection a positive correlation was observed between IL-6 and HCMV IgM only. This is consistent with a study conducted by Hussain *et al.*, (2018) on cytokine expression in women with primary cytomegalovirus compared to healthy controls showed elevated levels of IL-6 indicating a Th1 predominance.

IgG-positive chronic HCMV infection contributes to a pro-inflammatory environment and the body may be in a persistent inflammatory state, leading to the secretion of IL-6 as a primary inflammatory signal, which may impair maternal and fetal tolerance and promote immune-mediated pregnancy loss (Pourroostaei Ardakani *et al.*, 2022).

In another study that continued with the current study, it was conducted by Han *et al.*, (2020) whom showed elevate levels of pro-inflammatory cytokines, such as IL-6, in pregnant women with COVID-19. and study by Cavalcante *et al.*, (2021) whom showed a comparison of the miscarriage rate among 113 asymptomatic women with COVID-19 in the first trimester of pregnancy with 172 uninfected pregnant women found that the overall number of miscarriages (22.1% vs. 16.9%, $P = 0.32$) did not differ significantly, suggesting that the risk of miscarriage appears to increase during the acute phase of COVID-19, relative to disease severity and obstetric risk factors.

Also study by Afrooz *et al.*, (2022) whom reported elevate levels of pro-inflammatory cytokines in pregnant women with COVID-19, including IL-6, these changes ultimately lead to serious pregnancy risks, including miscarriage and weakened immune systems.

While the results of the current study differ from those of Chen *et al.*, (2020), that examined mothers who tested negative for COVID-19, despite no reported evidence of vertical transmission of the virus, the maternal immune response to viral infection tends to be enhanced by an inflammatory response, characterized by elevated levels of inflammatory cytokines, such as IL-6.

In pregnant women with COVID-19, pro-inflammatory cytokines such as IL-6 increase this factor has serious consequences, including miscarriage or fetal developmental abnormalities modulation of the maternal immune system during pregnancy may affect the response to infections, especially viruses (Han *et al.*, 2020).

The immune system maintains the body's balance disturbances can trigger systemic immune responses, COVID-19 infection can lead to the spread of viral agents and inflammation causing tissue damage which then triggers the release of cytokines from damaged cells, due to the activated signaling cascade, these cytokines may also affect gene expression, high levels of cytokines associated with a variety of infectious conditions are often called cytokine storms referring to an immune response characterized by the release of interferons, interleukins, tumor necrosis factors, chemokines, and many other mediators (Wong *et al.*, 2021).

4.5. Correlation between cytokine parameters in miscarried women with HCMV

The correlations between cytokine parameters in miscarried women with HCMV were shown in tables (4-14), the current results showed significant positive correlation between TNF- α and IFN- γ ($r=0.610$, $p=0.001$), TNF- α and IL-6 ($r=0.549$, $p=0.001$), TNF- α and IL-8 ($r=0.633$, $p=0.001$), IFN- γ and IL-6 ($r=0.561$, $p=0.001$), IFN- γ and IL-8 ($r=0.510$, $p=0.001$), and IL-6 and IL-8 ($r=0.681$, $p=0.001$).

Table (4-14): Correlation between cytokine parameters in miscarried women with HCMV

Characteristic	Cytokine parameters							
	TNF- α		IFN- γ		IL-6		IL-8	
	<i>R</i>	<i>P</i>	<i>R</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
TNF- α	1							
IFN- γ	0.610	0.001*	1					
IL-6	0.549	0.001*	0.561	0.001*	1			
IL-8	0.633	0.001*	0.510	0.001*	0.681	0.001*	1	

r: correlation coefficient.

When HCMV infects the placenta, it stimulates the release of $\text{TNF-}\alpha$, creating a local inflammatory environment that negatively impacts fetal perfusion and nutrition, $\text{TNF-}\alpha$ is associated with the activation of trophoblast apoptosis, impeding implantation the fetus (Hamilton *et al.*, 2012). $\text{IFN-}\gamma$ is a part of the Th1 pattern and enhances MHC production, increasing the innate immune response , also increasing immune stress in the uterine environment (Giannubilo *et al.*, 2012).

This environment plays a role in increased production of IL-6 and IL-8, particularly in the second trimester of pregnancy, their elevation is associated with the prediction of miscarriage, as IL-6 enhances inflammation and stimulates immune cell pathways, IL-8 acts as a factor attracting neutrophils and other cells to the endometrium (decidua), causing trophoblast damage (Hannan *et al.*, 2014).

The positive relationship between $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, IL-6, and IL-8 suggests a positive feedback loop called a local cytokine storm leads to a Th1/Th2 imbalance, an excessive increase in Th1 with a possible insufficient decrease in Th2, which reduces the tolerance to pregnancy (Pocock *et al.*, 2017). Consequently, chronic inflammation, destruction of placental tissue, failure of implantation and poor supply, leading to spontaneous abortion or preterm birth, this may increase the possibility that these cytokines involved in Th1 inflammatory responses, particularly in the context of HCMV, constitute a major risk factor for pregnancy failure.

4.6. Logistic regression correlations between cytokine parameters

The Logistic regression model between studied cytokine parameters show that the $\text{TNF-}\alpha$ directly correlate with each of $\text{IFN-}\gamma$, IL-6 and IL-8 among patients as in figures (4-5) to (4-7) this result might be refer to

HCMV infection condition enhances production of TNF- α in relation the expression of IFN- γ , IL-6 and IL-8, also this result show there was directly correlation between all IFN- γ , IL-6 and IL-8 among patients as in figures (4-8), (4-9) and (4-10).

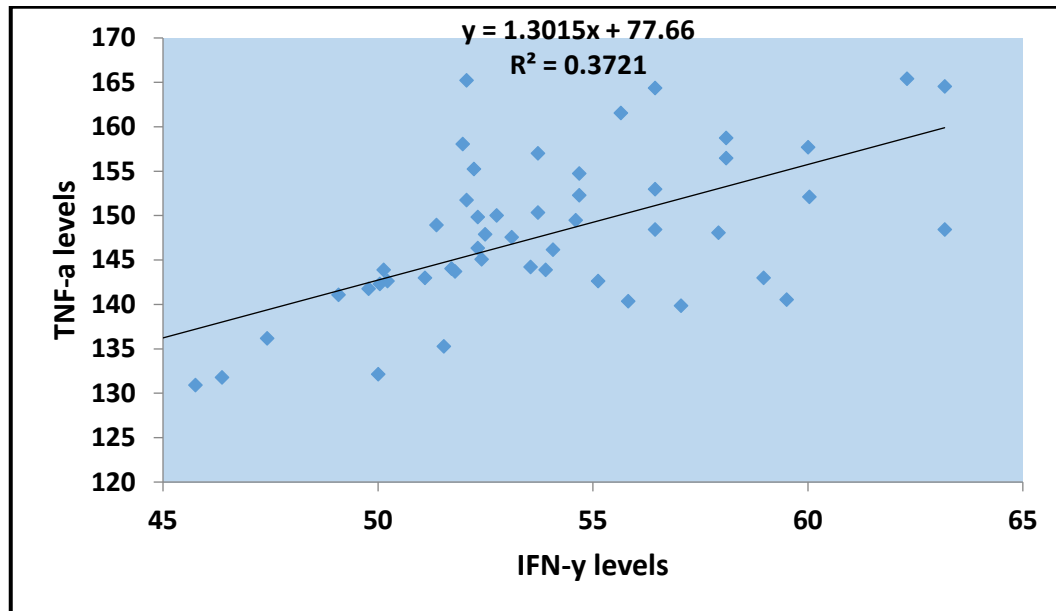


Figure (4-5): The Logistic scatter plot of TNF- α and IFN- γ among patients

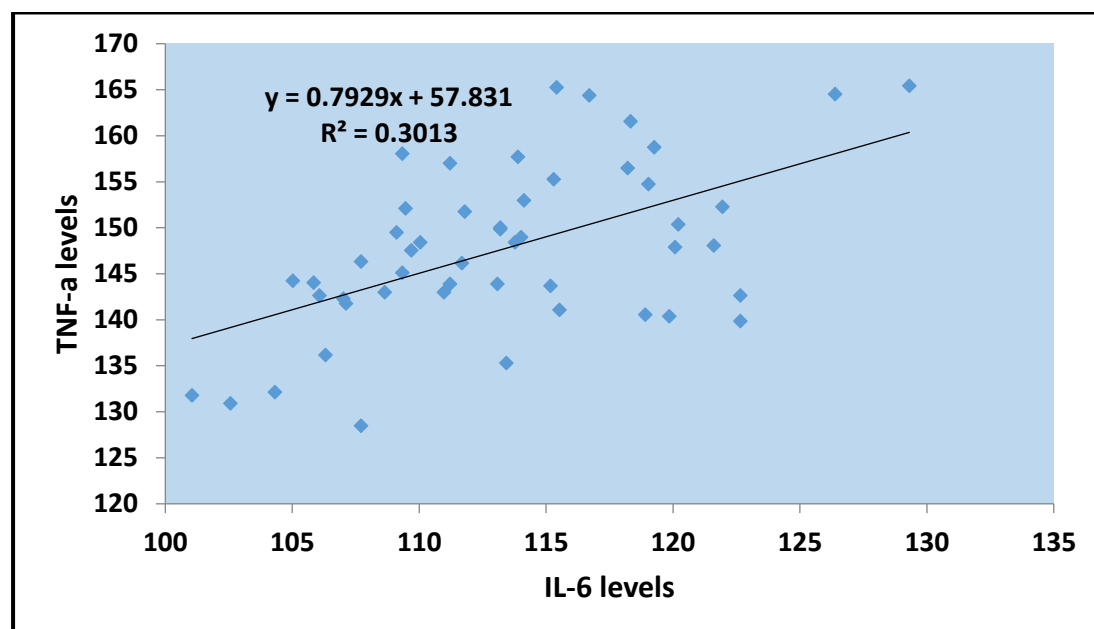


Figure (4-6): The Logistic scatter plot of TNF- α and IL-6 among patients

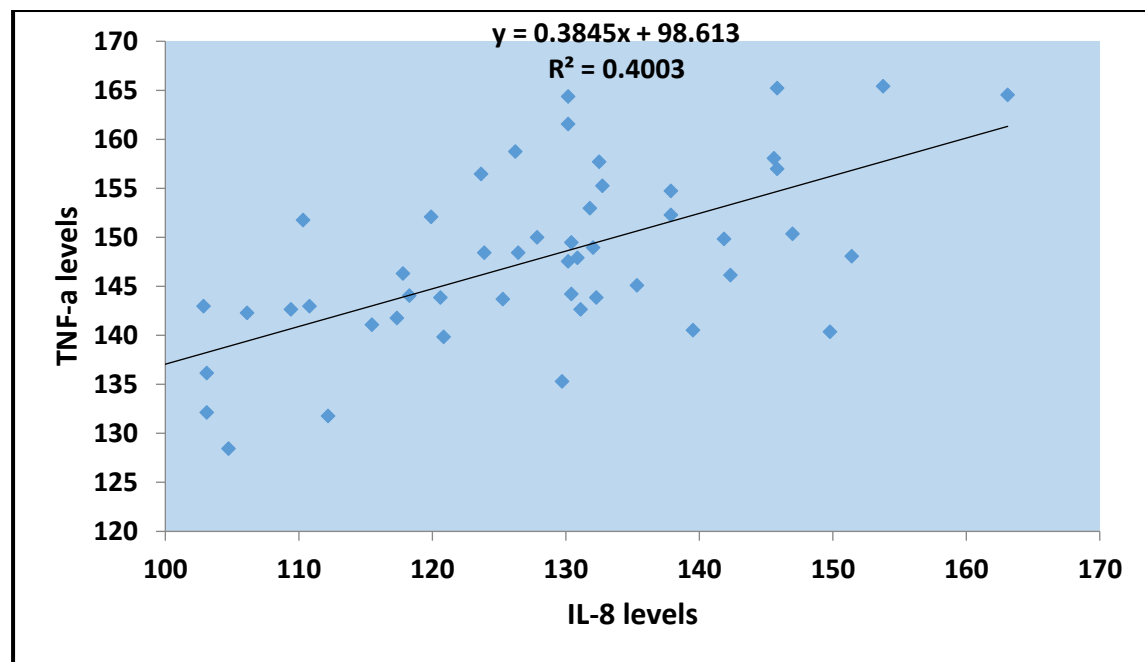


Figure (4-7): The Logistic scatter plot of TNF- α and IL-8 among patients

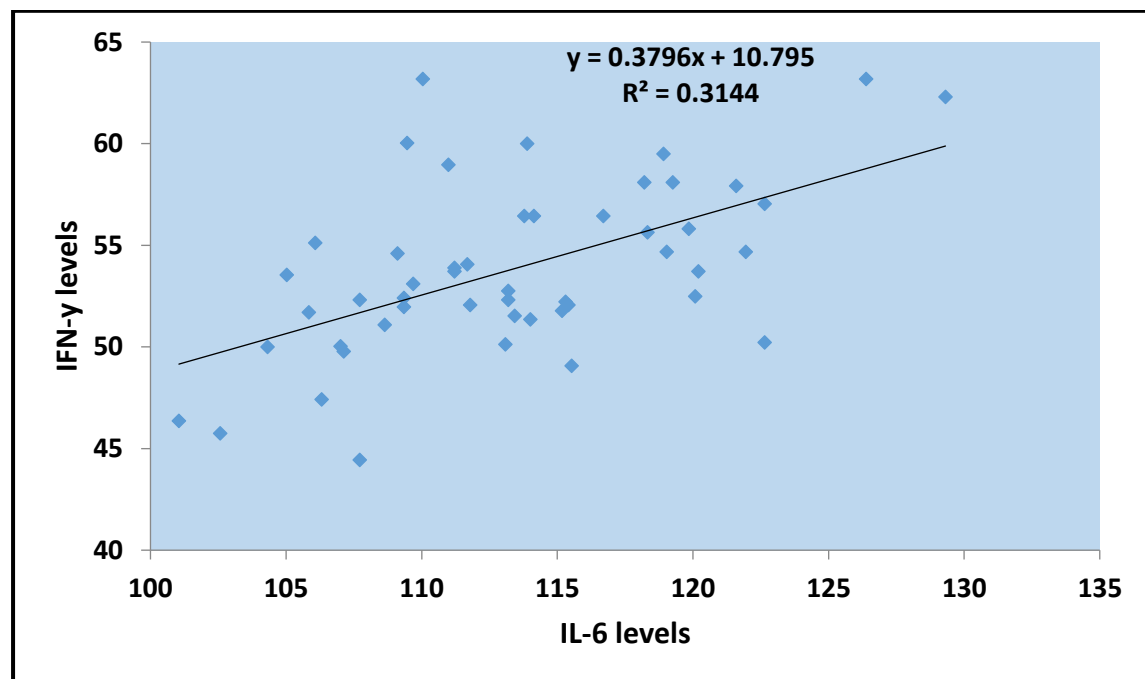


Figure (4-8): The Logistic scatter plot of IFN- γ and IL-6 among patients

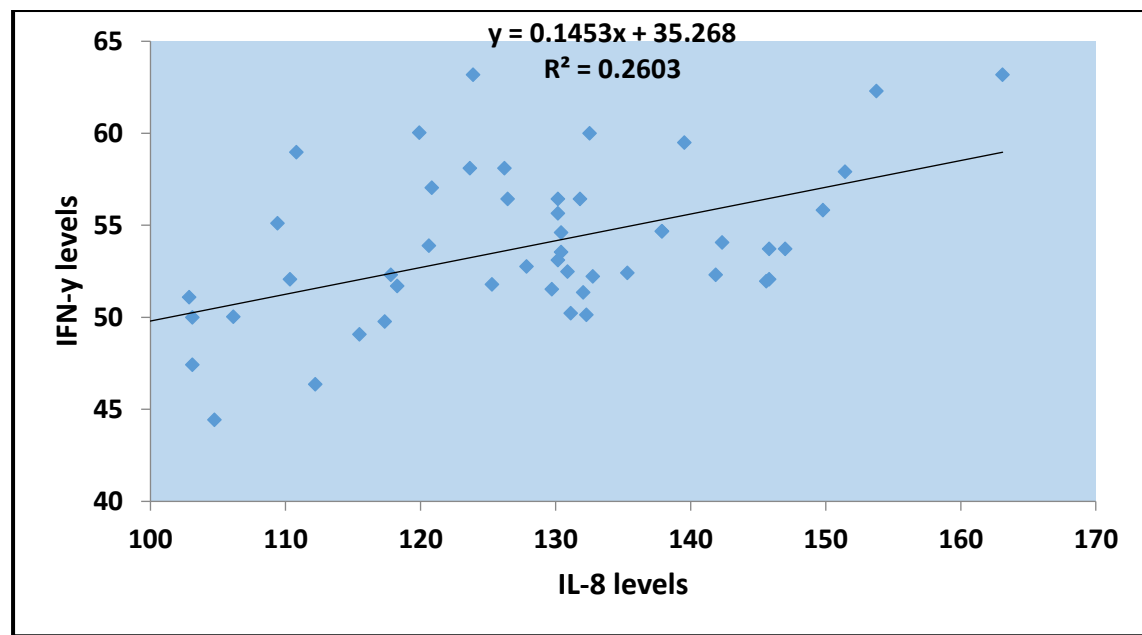


Figure (4-9): The Logistic scatter plot of IFN-γ and IL-8 among patients

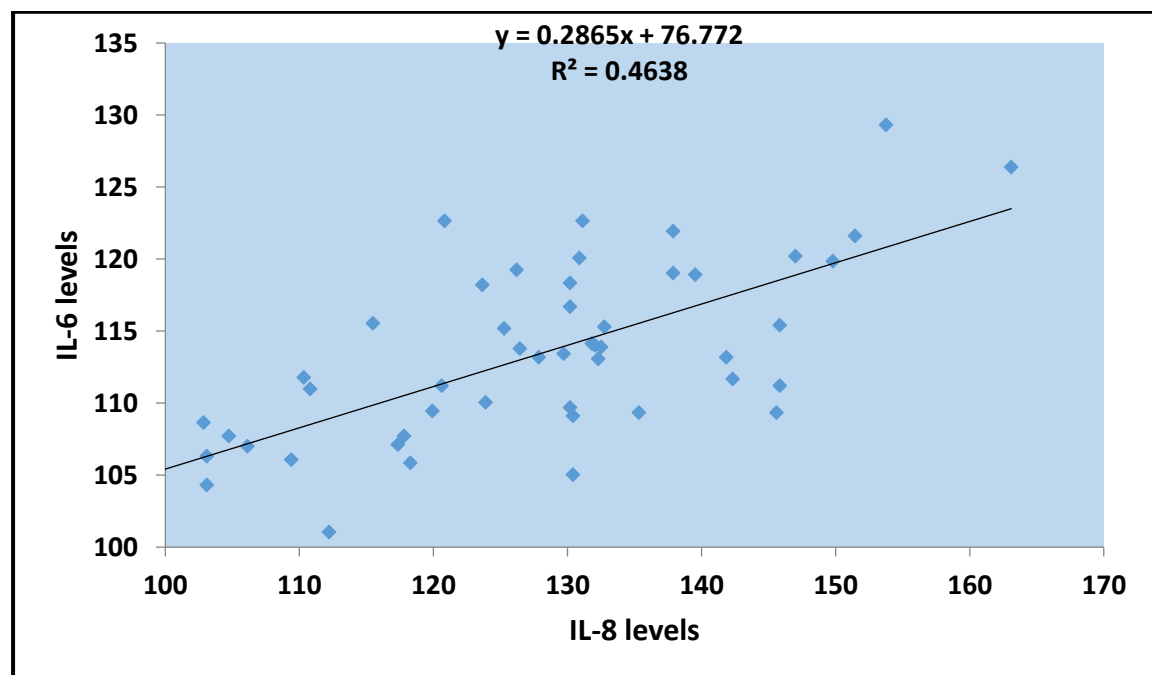


Figure (4-10): The Logistic scatter plot of IL-6 and IL-8 among patients

4.7. Gene expression profile study by Real Time PCR

4.7.1. The purity of total RNA

The purity of total RNA samples ranged from 1.9 to 2 ng/μl in the study groups, the RNA purity of the study groups as well a good yield with a high concentration of total RNA depends on the extraction conditions where by strict aseptic techniques must be used, the utilization of TRIzol in the Total RNA extraction from blood is well documented, the use of nuclease free water and RNase free equipment like tips and microfuge tubes and decontamination of the working field by the use of UV light and suprase, all these could be considered working aids in reaching the best result, a high concentration of RNA was associated with nill DNA.

4.7.2. Results of quantitative Real Time PCR

Real time PCR quantification” applied in the present experiment utilized the SYBR green, a fluorescent dye that recognizes any double stranded DNA including cDNA, the amplification was recorded as a Ct value (cycle threshold). “The lower Ct value indicated the presence of higher copies of the target and vice versa,in terms of gene expression, high Ct values indicated low gene expression and low Ct value indicated a high gene expression (Livak and Schmittgen, 2008).

4.7.3. Real time PCR quantification of GAPDH expression

The Ct value of GAPDH, the housekeeping gene used in the present study is the mean of Ct value for GAPDH CMV patients group was (23.23), for the in control group was (22.84).

Basic Assumption of Household Molecular Genes Used Studies showed that their expression remains constant in the cells or tissues underneath achievement (Reboucas *et al.*, 2013). One of the most common household genes associated with gene expression data is GAPDH (Barber *et al.*, 2005). Also studied the expression of 1718 genes using qRT-PCR, which used GAPDH as a reference gene in 72 types of normal human tissue, figure (4-11) showed GAPDH amplification diagrams and dissociation curves.

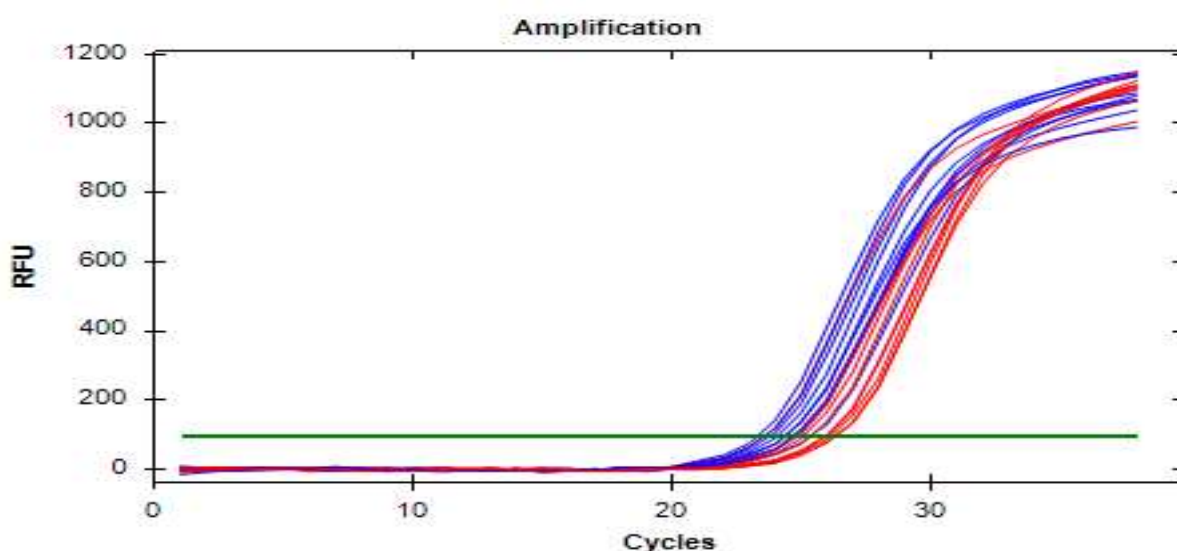


Figure (4-11) The Real Time PCR amplification plots of housekeeping (GAPDH) gene expression patient and healthy control samples. The blue plots patients and the red plots (control)

4.7.4. Real time PCR quantification of mir-181a expression

The mean Ct value of miRNA-181a amplification was (24.25) in the HCMV patients. While Ct values in control were mean (26.49), the mean Ct values in control group were higher than those of HCMV group.

Each quantitative PCR reactions was run in duplicate for each sample.,in each run, samples from HCMV and control were run in addition to non-template and non-primer controls.” This was important to make the statistical calculation of each group and in order to specify the calibrator.

Plots of each run were recorded including the amplification plots and dissociation curves. figure (4-12) showed the amplification plots and dissociation curves for miRNA-181a.

In this study, a quantitative analysis of RT-PCR analyzed expression of miRNA-181a and comparison of its expression between, apparently healthy group and the group HCMV, the change in gene expression was calculated using a relative quantitative measurement (Livak and Schmittgen, 2008).

This is based on the normalization of the Ct values for calculating ΔC_t and represents the difference between the average Ct values of the miRNA-181a amplification replica for each case and case of GAPDH.

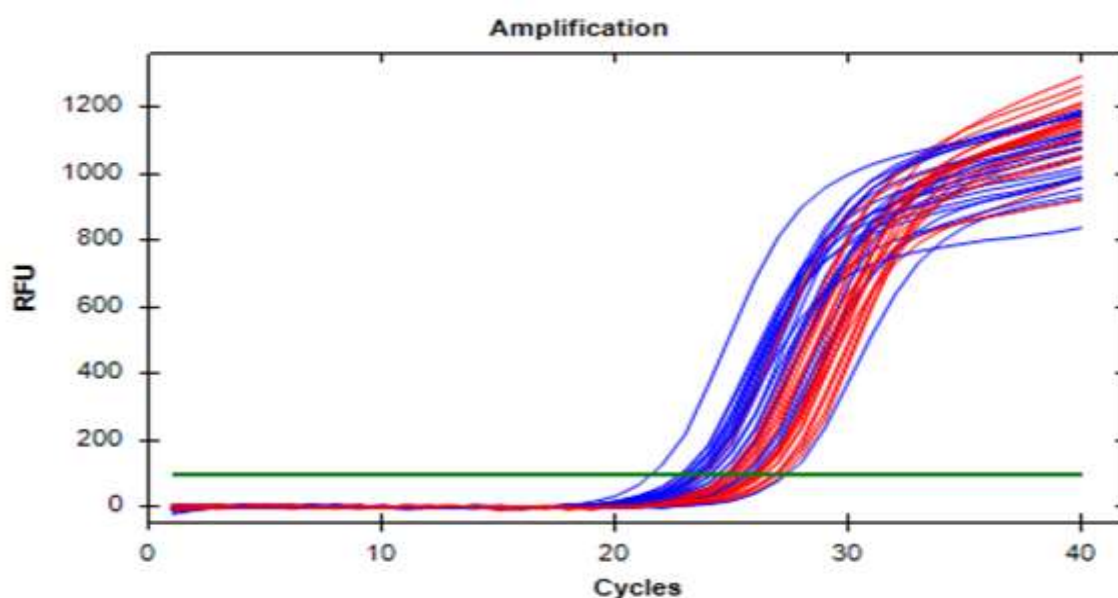


Figure (4-12): The Real Time PCR amplification plots of miRNA 181a gene expression patient and healthy control samples. The blue plots patients and the red plots (control)

In calculation of the relative expression of miRNA-181a gene in both study groups the $2^{-\Delta Ct}$ results was applied.

The results of the current study showed the gene expression of miRNA-181a in miscarried women infected with HCMV and healthy controls, the mean gene expression of miRNA-181a was 9.07 ± 1.40 and 1.0, respectively, the results were shown in table (4-15) and figure (4-13). the level was significantly higher in miscarried women infected with HCMV compared to healthy controls ($P < 0.001$).

Table (4-15): Comparison of mean of mir-181a gene expression between patients and healthy controls

mir-181a gene expression	Miscarried women with HCMV <i>n</i> = 50	Healthy pregnant control <i>n</i> = 50	<i>P</i>
Mean± SE	9.07 ± 1.40	1.0	< 0.001
Range	0.12 – 31.39	0.09-6.76	† HS

n: number of cases; SE: standard error; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

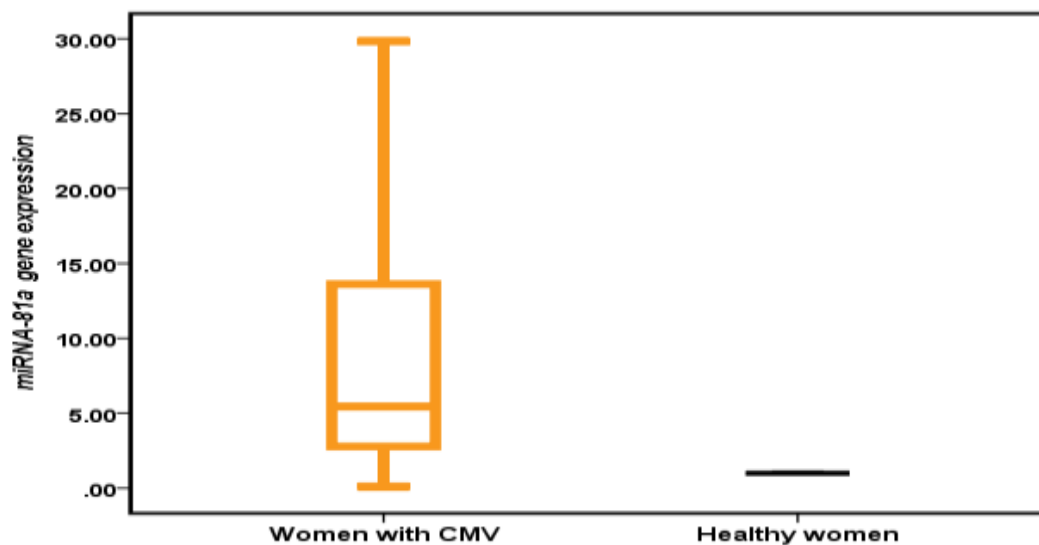


Figure (4.13): miRNA-181a gene expression in patients with HCMV and healthy controls.

The current study agreed with a study by An *et al.*,(2022) that miR-181a was associated with an increased risk of recurrent pregnancy loss (RPL) and Imbar and Eisenberg ,(2014) whom indicated that miRNAs play critical roles in the pathophysiology of various reproductive disorders,elevated miR-181a expression was associated with a higher risk of RPL in a cohort of Korean women, this occurred more frequently in patients with RPL than in controls, indicating a significant association with an increased risk of RPL (all $p < 0.05$).Furthermore, Ardakani, *et al.*,(2023) showed that miR-181a expression increased as the date of vaginal delivery approached ($p < 0.001$).

A study different from the results and case of the current study, An *et al.*, (2025) whom indicated that microRNA was associated with an increased risk of primary ovarian insufficiency, they noted a significant decrease in miR-181a expression in women with primary ovarian insufficiency ($P < 0.05$). Also the current study contradicts by Geng *et al.*, (2022) there was a decrease in gene expression levels in a study conducted on 36 patients with recurrent miscarriage and 40 as a control group.

Also study by Goharitaban *et al.* ,(2022) also indicated that miR-181a was decreased in cases of recurrent implantation failure (RIF), which weakens the uterus's readiness for fertilization and increases the likelihood of miscarriage.

Dysregulation of certain miRNAs is associated with an increased incidence of recurrent miscarriage, they also play a role in the growth and differentiation of trophic cells, embryo activation and implantation, immune tolerance and endometrial reception (Patronia *et al.*,2024) .An imbalance of miRNAs that promote healthy immunity in cases of recurrent

miscarriage, for example, elevated miR-181a is accompanied by a deficiency in other miRNAs within Treg cells, which weakens immune tolerance and thus creates an uncontrolled inflammatory environment that leads to miscarriage (Guan, 2024).miR-181a plays a role in modulating the sensitivity of immune cells, especially T cells,HCMV infection stimulates the release of inflammatory cytokines such as TNF- α and IL-6.as a secondary effector, miR-181a expression in the blood is elevated as part of post-transcriptional regulation (Zhang, 2015).

4.7.4.1 Frequency distribution of mir-181a gene expression according to some characteristics

The results are presented in table (4-16) which showed the gene expression of miRNA-181a gene according to some characteristics including IgG and IgM levels as well as the number of miscarriages in miscarried women with HCMV and COVID-19 infection, the current results show a statistically insignificant difference for miRNA-181a gene.

Table (4-16): Distribution of mir-181a expression according to some characteristic

Characteristics		N	mir-181a gene Mean $\pm$ SE	P
IgG	Positive	50	9.07 $\pm$ 1.40	
	Negative	0		
IgM	Positive	20	6.42 $\pm$ 1.36	0.157 † NS
	Negative	30	10.84 $\pm$ 1.81	
Number of miscarriages	One	29	7.33 $\pm$ 1.01	0.129 K NS
	Two	17	10.89 $\pm$ 1.48	
	$\geq$ three	4	14.00 $\pm$ 2.19	
Covid-19 infection	Positive	29	9.54 $\pm$ 1.52	0.635 † NS
	Negative	21	8.43 $\pm$ 1.76	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

Due to the lack of studies linking IgG and IgM levels with CMV infection and elevated microRNA mir-181a, there are studies that have recorded the number of previous pregnancy losses for Korean women by An *et al.*, (2022) at 3.01 $\pm$ 1.50 and there is evidence in their study that miRNAs play critical roles in the pathophysiology of various reproductive disorders.

Its elevation in the plasma of pregnant women during the early months of pregnancy is used as a predictive marker associated with miscarriage, the elevation may indicate an abnormal cellular response, affecting the immune balance and regulatory flows in the endometrium, this leads to impaired implantation, trophoblast development, and local immune regulation, which increases the likelihood of recurrent miscarriage (Zhang *et al.*, 2015).

In previous studies that continued with the current study by Khatami *et al.*, (2023) indicated that differences in miRNA expression patterns between the studied groups indicated that miR-181a may be useful as effective biomarkers for diagnosing COVID-19 and regulating several inflammatory parameters. In addition to the previous study by Grand *et al.*, (2022), which confirmed that asymptomatic HCMV infection occurs in a high proportion of patients diagnosed with COVID-19 as a result of viral reactivation due to the use of high doses of corticosteroids, which may have a direct effect on viral reactivation.

miRNA-181a has functional purposes, having been shown to play important roles in pregnancy and infertility, in recent years, the study of circulating miRNAs has been proposed as potential novel biomarkers of infectious diseases and as useful biomarkers for clinical use, potentially

improving diagnostic accuracy, the study of selected miRNAs, which may be directly or indirectly involved in the inflammatory pathway, may interact with SARS-CoV-2 receptors additionally, potential prognostic factors are associated with high miscarriage rates in hospitals (Lippi *et al.*,2020) .

Both virally encoded and host-encoded miRNAs may be effective biomarkers for COVID-19 disease, in recent years, patients have demonstrated immune dysfunction in severe cases of SARS-CoV-2 infection, characterized by hyperinflammation, COVID-19 infection can lead to reactivation of HCMV in pregnant women, contributing to pregnancy loss in some cases, reactivation of latent HCMV in patients may be due to immunosuppression or the use of corticosteroids as a treatment for COVID-19 infection (Paul *et al.*, 2022).

4.7.5. Real time PCR quantification of mir-US5-1 expression

The mean Ct value of mir-US5-1 amplification was (7.57) in the CMV patients. While Ct values in control were mean (zero), each quantitative PCR reactions was run in duplicate for each sample. in each run, samples from HCMV and control were run in addition to non-template and non-primer controls.” This was important to make the statistical calculation of each group and in order to specify the calibrator. Plots of each run were recorded including the amplification plots and dissociation curves.figure (4-14) show amplification plots and dissociation curves for mir-US5-1.

In this study, a quantitative analysis of RT-PCR analyzed Expression of mir-US5-1 and comparison of its expression between, apparently healthy group and the group HCMV, the change in gene expression was calculated using a relative quantitative measurement (Livak and Schmittgen, 2008).

This is based on the normalization of the Ct values for calculating ΔCt and represents the difference between the average Ct values of the mir-US5-1 amplification replica for each case and case of GAPDH.

In calculation of the relative expression of mir-US5-1 gene in both study groups the $2^{-\Delta Ct}$ results was applied.

The current study showed that of mir-US5-1 gene expression between miscarried women with HCMV and healthy control subject has been carried out and the results were demonstrated in table (4-17). Mean of mir-US5-1 gene expression were in miscarried women with HCMV was 5.49 ± 1.20 but the this gene not expressed in healthy control.

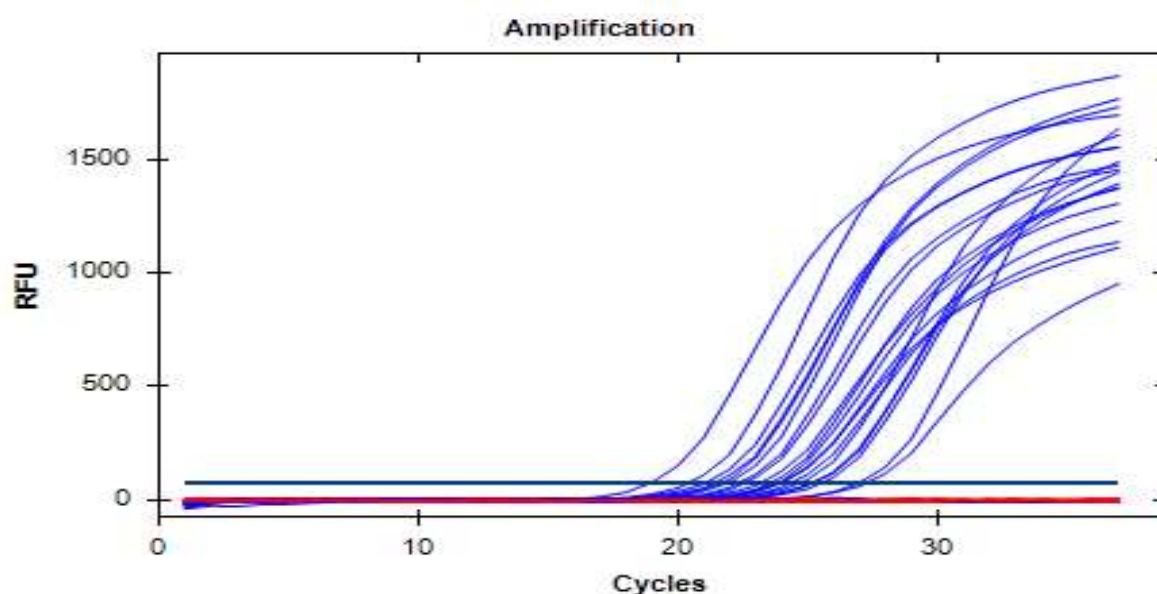


Figure (4-14): The Real Time PCR amplification plots of mir-US5-1 gene expression patient and healthy control samples. The blue plots patients and the red plots (control)

Table (4-17): Comparison of mean of mir-US5-1 gene expression between patients and healthy controls

mir-US5-1 gene expression	Women with HCMV <i>n</i> = 50	Healthy pregnant control <i>n</i> = 50	<i>P</i>
Mean± SE	5.49 ± 1.20	Zero	< 0.001 † HS
Range	0 – 84.07		

n: number of cases; *SE*: standard error; †: independent samples t-test; HS: Highly significant at $P \leq 0.001$.

In studies that agreed with the results of the current study, it was presented by Zhou, *et al.*, (2020), Lisboa *et al.*,(2015) and Shen *et al.*,(2014) revealed that the level of hcmv-mir-US25-1 in tested serum HCMV particles was significantly elevated in HCMV patients compared to controls ($P < 0.01$) .

Elevated levels of mir-US5-1 in the tissues of women infected with HCMV who have miscarried create a favorable cellular environment for viral replication, with potentially negative effects on trophoblast cells (Zhang *et al.*, 2020).This suggests that HCMV uses mir-US5-1 as a means of evading the immune response and infecting embryonic cells, miR-US5-1 is highly expressed during the active (lytic) inflammatory cycle, while its expression decreases during the latent period (Nappi *et al.*, 2023).

4.7.5.1 Frequency distribution of mir-US5-1 gene expression according to some characteristics

The comparison of mir-US5-1 gene expression according to some characteristics including IgG and IgM levels as well as the number of miscarriages in aborted with HCMV and COVID-19 infection that has been carried out and the results were demonstrated in table (4-18).

Mean of mir-US5-1 gene expression were 9.75 ± 2.21 and 2.65 ± 1.0 , in patients with positive IgM and patients with negative IgM respectively; the level was highly significant higher than in patients with positive IgM in comparison with patients with negative IgM ($P < 0.001$). But the present results show non-significant difference of mir-US5-1 gene expression according to other studied characteristic ($P > 0.05$).

Table (4-18): Distribution of mir-US5-1 expression according to some characteristic

Characteristics		N	mir-US5-1 gene Mean $\pm$ SE	P
IgG	Positive	50	5.49 ± 1.20	
	Negative	0		
IgM	Positive	20	9.75 ± 2.21	0.016 † S
	Negative	30	2.65 ± 1.0	
Number of miscarriages	One	29	7.0 ± 1.58	0.180 K NS
	Two	17	4.22 ± 1.11	
	$\geq$ three	4	Zero	
Covid-19 infection	Positive	29	5.06 ± 1.04	0.823 † NS
	Negative	21	6.08 ± 1.05	

n: number of cases; SD: standard deviation; †: independent samples t-test; K: Kruskal–Wallis test; NS: not significant at $P > 0.05$.

In a study contrasting with the current study by Zhou, *et al.*, (2020) HCMV infection and serology were evaluated in 5,664 peripheral blood leukocyte samples, which were characterized using real-time quantitative PCR from 59 patients who were seronegative for HCMV IgM.

Another precedent study conducted by Mikell, *et al.*, (2019) ,Meshesha *et al.*,(2016) revealed that this hcmv-miR-US25-1 was greatly expressed when it was revitalized after the period of inactivity. In addition to study by Fu *et al.*, (2014) whom found that miR-US25-1 was expressed by human cytomegalovirus (HCMV) during latent infection, host microRNA expression was also affected by latent HCMV infection.

Supporting the findings of the current study, Gao *et al.*,(2021) whom showed that hcmv-miR-US5-1 was expressed at higher levels in the plasma of pregnant women with APO (adverse pregnancy outcomes) such as miscarriage compared to the plasma of healthy women ($P < 0.05$).

During primary viral infection, HCMV enters the production phase (lytic cycle), during this phase, the virus abundantly expresses certain microRNA molecules, such as mir-US5-1, in infected cells (e.g., fibroblasts),this expression is observed to be strongly induced during active infection, and IgM antibodies are produced as a rapid initial response, the period coincides with the active HCMV cycle, when mir-US5-1 is significantly elevated as a mechanism to mitigate the initial immune response and make the environment more hospitable to viral success (Zeng *et al.*,2023).

4.8. Logistic regression correlations between genes expression

The Logistic regression model showed that the correlation of gene expression such as miRNA-181a gene expression in which had directly correlate with mir-US5-1 gene among miscarried women with HCMV as in figure (4-15) this result might be refer to that HCMV condition enhance production of miRNA-181a gene in relation the expression of mir-US5-1 gene.

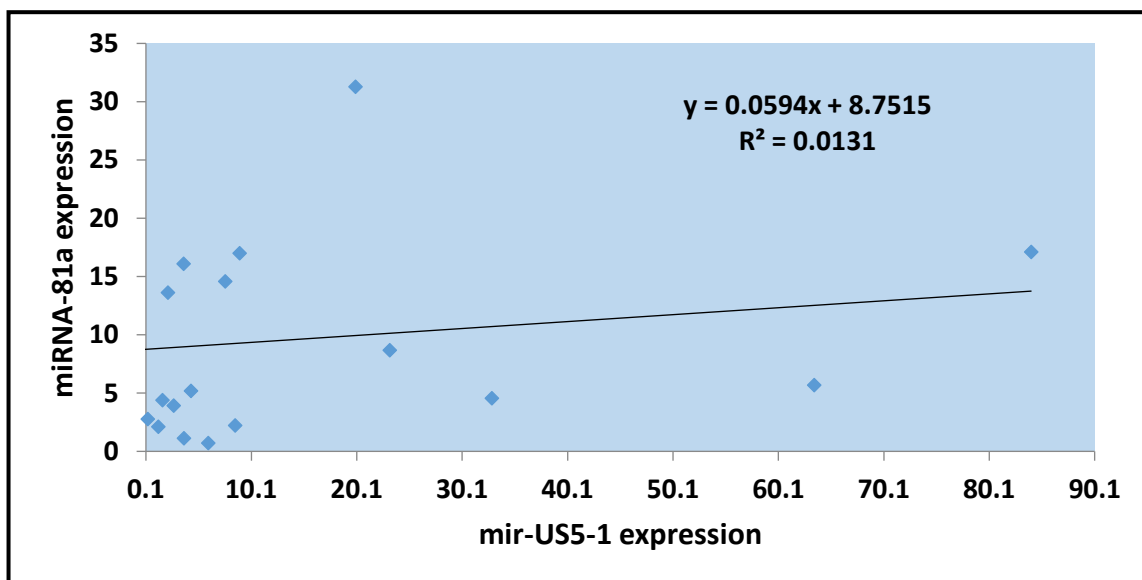


Figure (4-15): The Logistic scatter blot mir-181a and mir-US5-1 gene expression among patients

4.9. Logistic regression correlations between mir-181a expression and other cytokine parameters

The Logistic regression model show that the correlation miRNAs expression such as miRNA-181a in which have directly correlate with other parameters (TNF- α , IFN- γ , IL-6 and IL-8) among miscarried women with

HCMV as in figure (4-16), (4-17), (4-18) and (4-19) this result might be refer to that HCMV condition enhances production of TNF- α , IFN- γ , IL-6 and IL-8 in relation the expression of miRNA-181a.

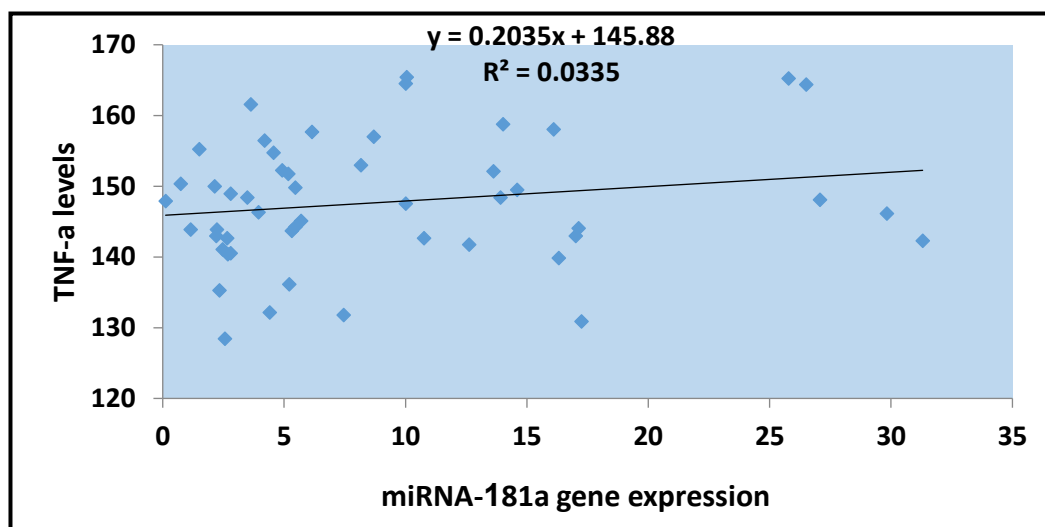


Figure (4-16): The Logistic scatter mir-181a and TNF- α level among HCMV patients.

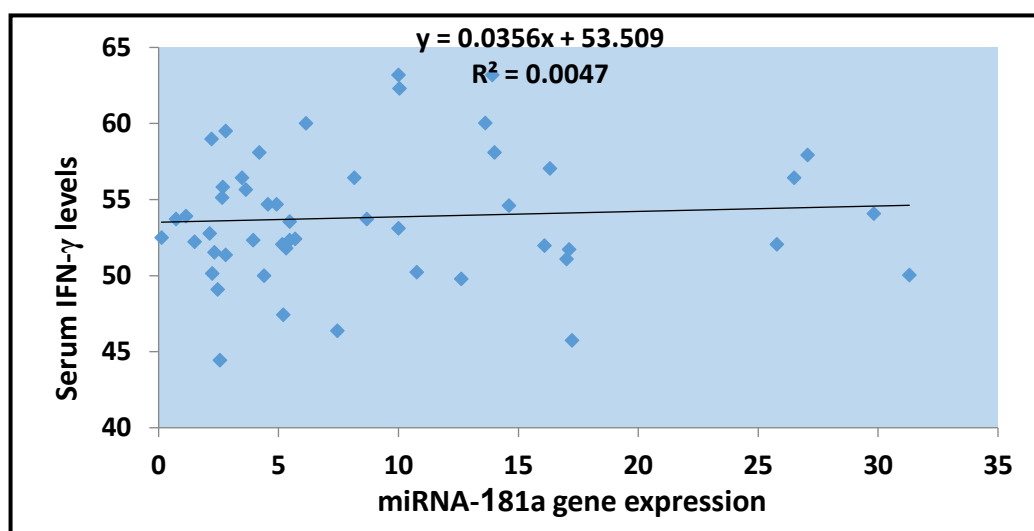


Figure (4-17): The Logistic scatter mir-181a and IFN- γ level among HCMV patients.

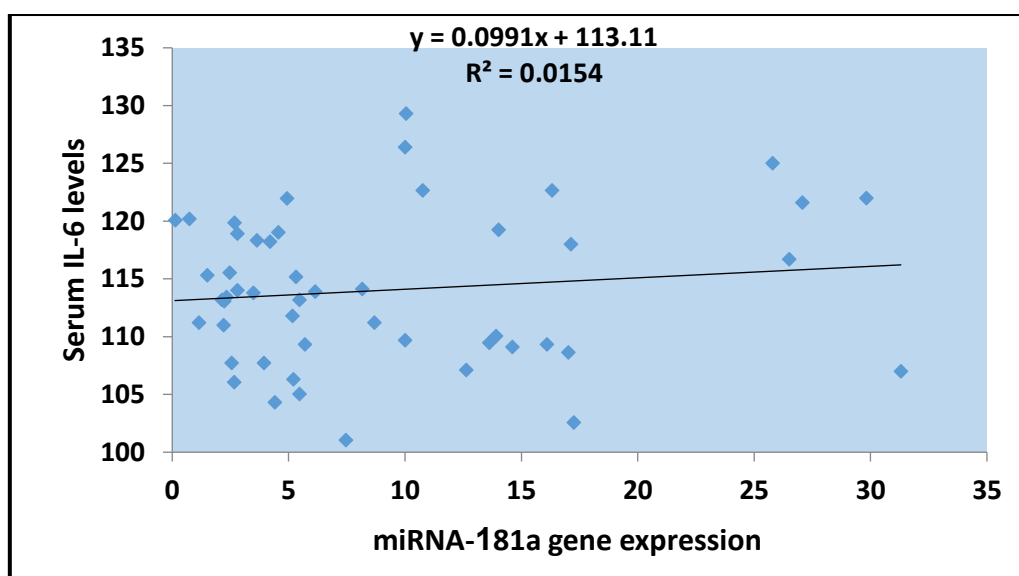


Figure (4-18): The Logistic scatter mir-181a and IL-6 level among HCMV patients.

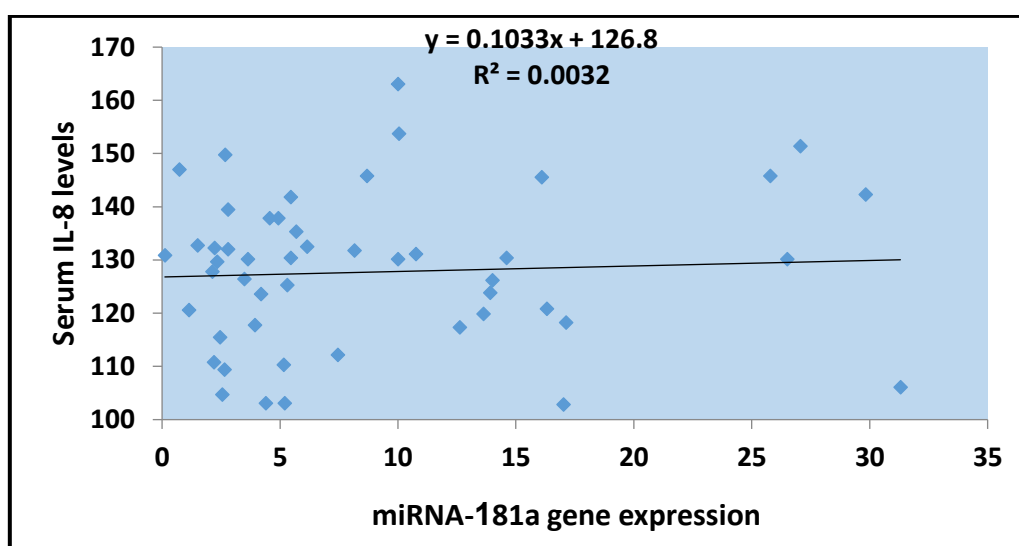


Figure (4-19): The Logistic scatter mir-181a and IL-8 level among HCMV patients.

Scientific studies have shown that human mir-181a plays an important role in regulating the balance between inflammatory and tolerogenic immune responses during pregnancy, elevated mir-181a is associated with increased production of Th1 inflammatory cytokines such as $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, IL-6 and IL-8 which play a crucial role in causing excessive inflammation in the placenta during HCMV infection, miR-181a promotes activation of the NF- κ B pathway, which increases the expression

of inflammatory cytokine genes, leading to an imbalance in the gestational immune environment and increasing the risk of miscarriage, this delicate immune balance can be altered by elevated mir-181a, contributing to the exacerbation of the Th1 hyperresponsiveness associated with HCMV (Li *et al.*, 2019). A recent study also indicated that mir-181a plays a central role in gestational pathogenesis by influencing the production of inflammatory cytokines (Sun *et al.*, 2021).

4.10. Logistic regression correlations between mir-US5-1 expression and other cytokine parameters

The Logistic regression model show that the correlation miRNAs expression such as mir-US5-1 in which have directly correlate with other parameters (TNF- α , IFN- γ , IL-6 and IL-8) among miscarried women with HCMV as in figures (4-20), (4-21), (4-22) and (4-23) this result might be refer to that HCMV condition enhances production of TNF- α , IFN- γ , IL-6 and IL-8 in relation the expression of mir-US5-1.

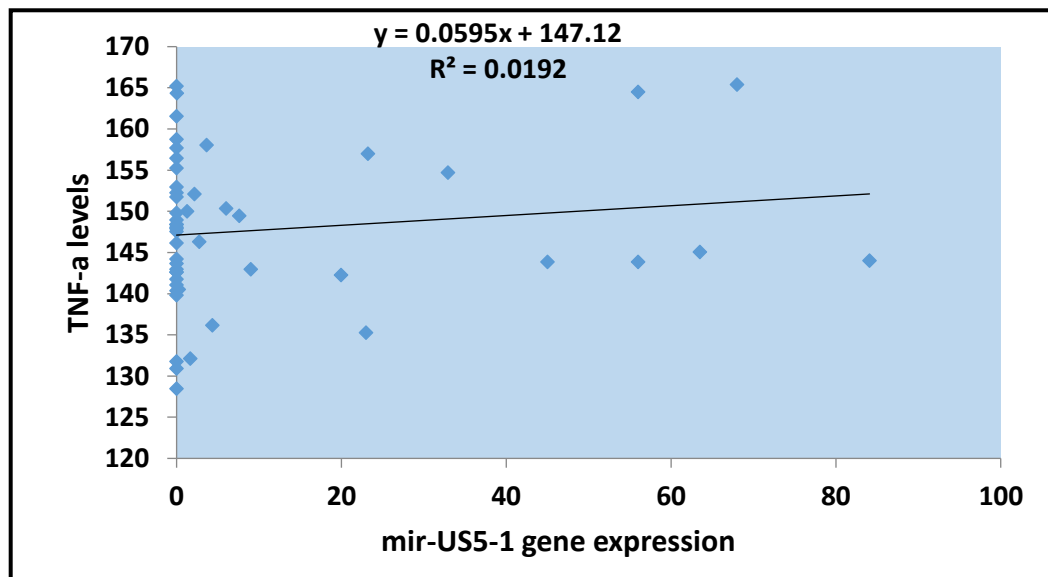


Figure (4-20): The Logistic scatter mir-US5-1 and TNF- α level among HCMV patients.

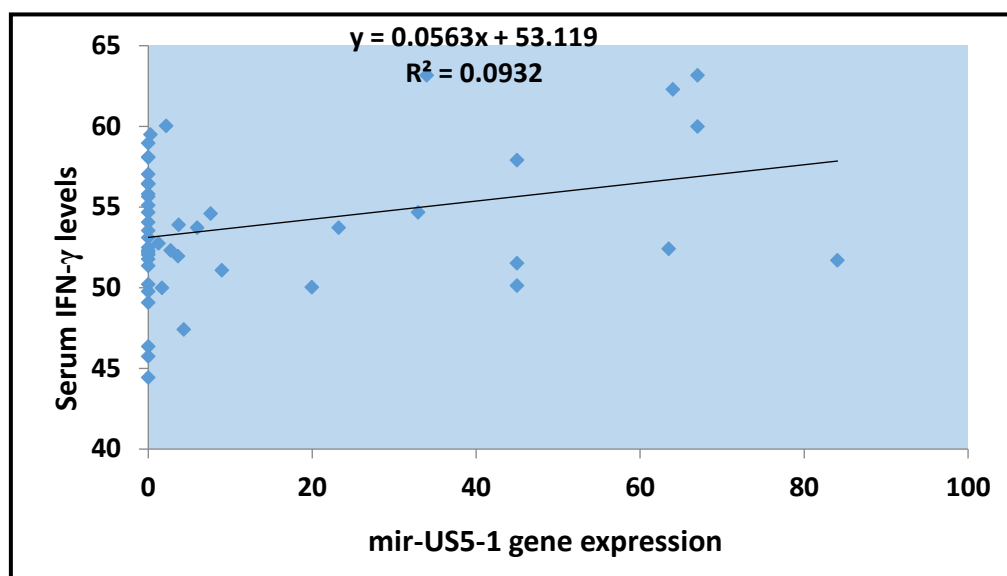


Figure (4-21): The Logistic scatter mir-US5-1 and IFN- γ level among HCMV patients.

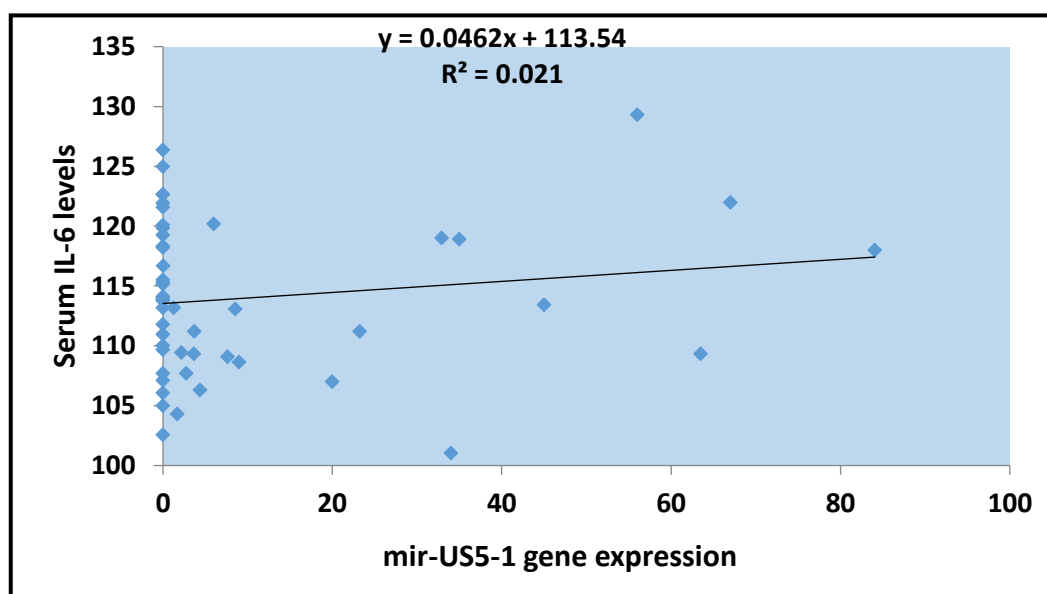


Figure (4-22): The Logistic scatter mir-US5-1 and IL-6 level among HCMV patients.

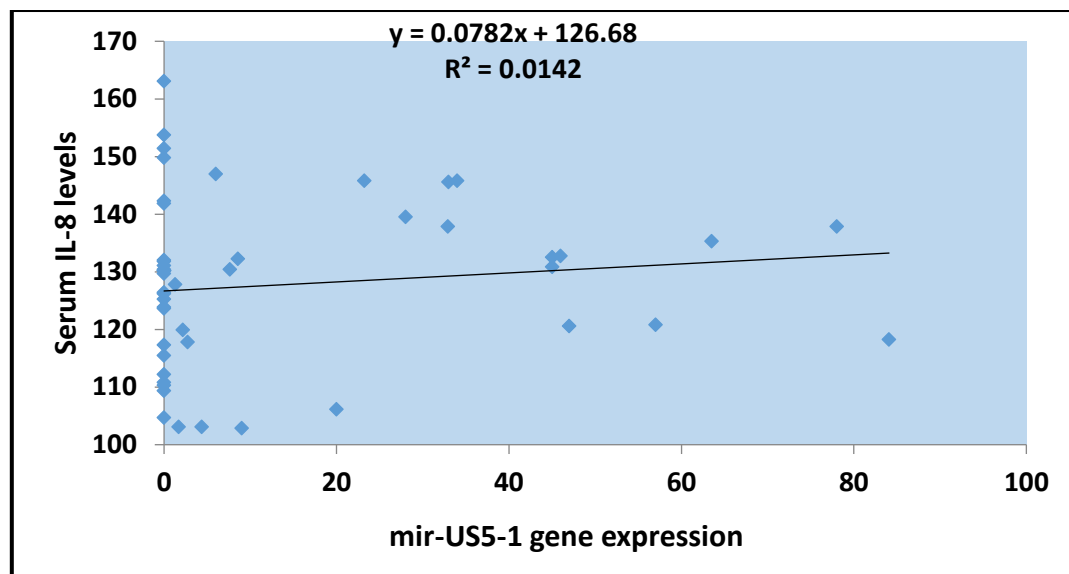
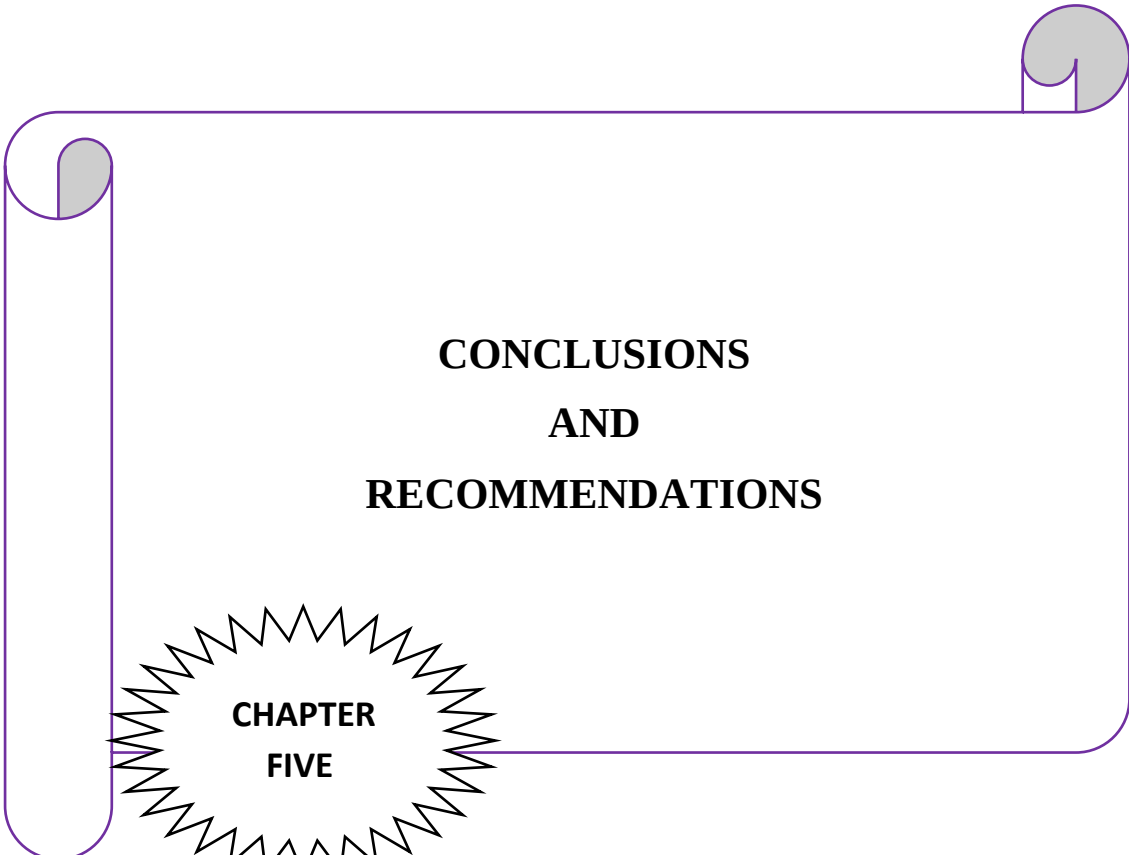


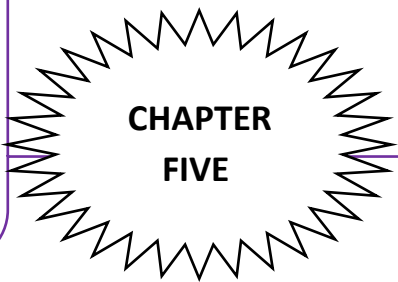
Figure (4-23): The Logistic scatter mir-US5-1 and IL-8 level among HCMV patients.

During miscarriage caused by HCMV infection, the expression of the viral microRNA US5-1 increases in parallel with the activation of Th1-type inflammatory cytokines, such as $\text{TNF-}\alpha$, $\text{IFN-}\gamma$, IL-6 and IL-8 which are a hallmark of the placental inflammatory response. mir-US5-1 directly inhibits the NF- κ B pathway by reducing the expression of $\text{IKK}\alpha$ and $\text{IKK}\beta$, which are key components in activating this inflammatory pathway (Choi *et al.*, 2023).

Although the virus uses these microRNAs as a mechanism for immune escape, this partial and delayed inhibition may not be sufficient to prevent the inflammatory storm that accompanies acute infection in pregnancy, leading to an immune imbalance between Th1 and Th2 cells within the placenta, therefore, the coincidence of elevated mir-US5-1 with cytokines suggests a failed attempt by the virus to suppress inflammation, contributing to immune damage and a dysfunctional gestational environment, and consequently, the increased rate of miscarriage (Hancock *et al.*, 2017).

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CONCLUSIONS AND RECOMMENDATIONS

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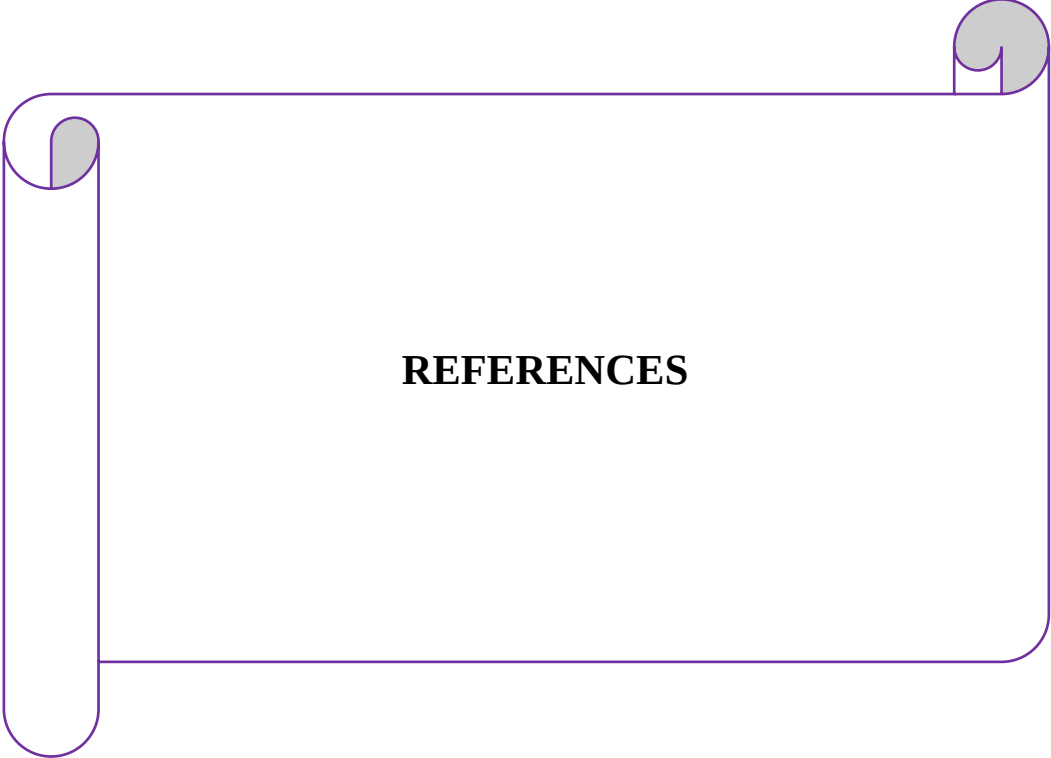
CHAPTER FIVE

Conclusions

1. Initial screening for HCMV infection was performed using a rapid diagnostic test ,which provided a quick and practical initial assessment, subsequently results were confirmed using the more accurate ELISA technique,the combined use of the two methods enhanced the validity of the results and reduced the likelihood of false positives.
2. Elevated levels of pro-inflammatory cytokines TNF- α , IFN- γ , IL-8, and IL-6 with a significant positive correlation between all cytokine parameters in aborted women infected with human cytomegalovirus (HCMV) compared to healthy pregnant women.
3. Real-time polymerase chain reaction (RT-PCR) is rapid , sensitive and is useful for diagnosing HCMV infection using viral and human microRNAs.
4. Detection of gene expression of miRNA -US5-1 and miRNA -181a , both of which were expressed during HCMV infection in miscarried women compared to healthy pregnant women.
5. The expression of miRNA-181a was directly correlated with that of mirRNA-US5-1 in miscarried women infected with HCMV, suggesting that viral infection promotes the production of miRNA-181a in relation to the expression of mir-US5-1.
6. The expression of miRNA-181a and mir-US5-1 is directly correlated with other parameters such as pro-inflammatory cytokines (TNF- α , IFN- γ , IL-8 and IL-6) in miscarried women infected with HCMV suggesting that HCMV infection enhances the production of pro-inflammatory cytokines.

Recommendations

1. Raising public awareness of the risks of HCMV through advertising campaigns, conferences and training of health care workers on early recognition of the symptoms and signs of HCMV infection in urban and rural areas.
2. Screening of pregnant women at high risk or with a history of miscarriage with education about viral infections during pregnancy within primary health care programs.
3. Conducting a study on the largest possible number of samples from aborted women with HCMV infection and from different regions to achieve the most accurate results.
4. Future studies should focus more on different types of microRNAs associated with humans, in addition to HCMV, for use as diagnostic biomarkers to track infection status or to predict preterm births and miscarriages.
5. Establishing a relationship between the prevalence of HCMV at different levels and the types of microRNAs that can be used and developed as effective treatments for the infection.
6. Studying the relationship between microRNAs and various pro-inflammatory interleukins and anti-inflammatory cytokines.



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الخلاصة:

تؤثر الميكروبات بشكل كبير على الصحة العامة، بما في ذلك مسببات الأمراض الانتهازية المعروفة باسم الفيروس المضخم للخلايا البشري، ويمكن أن تؤدي العدوى المباشرة للرحم والجنين إلى مضاعفات خطيرة لدى النساء الحوامل وأجنتهن مما قد يؤدي إلى الإجهاض لذلك، هدفت هذه الدراسة إلى تسليط الضوء على أهمية miRNA للفيروس المضخم للخلايا البشري (HCMV) لدى النساء المصابات بالإجهاض وتحديد المؤشرات الحيوية المناعية مثل بعض السيتوكينات المؤيدة للالتهابات: $TNF-\alpha$ و $IFN-\gamma$ و $IL-8$ و $IL-6$ ، حيث يزيد وجود الفيروس المضخم للخلايا البشري في عنق الرحم من خطر الولادة المبكرة أو الإجهاض التلقائي من خلال إطلاق بعض السيتوكينات الالتهابية.

أجريت الدراسة على النساء اللواتي راجعن مستشفى الولادة والأطفال التعليمي بالإضافة إلى بعض المختبرات الطبية والعيادات الخارجية في محافظة الديوانية خلال الفترة من 15 كانون الثاني 2024 إلى 15 كانون الأول 2024، وبلغ عدد المشاركات 160 امرأة (110 امرأة مجهزة و 50 امرأة حامل سليمة كمجموعة ضابطة).

تم جمع عينات الدم واختبارها للفيروس المضخم للخلايا البشري باستخدام اختبار التشخيص السريع (الكاسيت)، وأظهرت النتائج أن 50 (45.5%) من النساء اللواتي تعرضن للإجهاض كانت نتائج اختبارهن إيجابية لفيروس المضخم للخلايا البشري بينما كانت نتائج اختبار 60 (54.5%) من النساء اللواتي تعرضن للإجهاض وكذلك جميع المجموعة الضابطة سلبية لفيروس المضخم للخلايا البشري، وبعد إجراء اختبار سريع تم التحقق من الإصابة باستخدام اختبار المتميز المناعي المرتبط بالإنزيم (ELISA) لتأكيد وجود IgG و IgM، وأظهرت جميع النساء اللواتي تعرضن للإجهاض بفيروس المضخم للخلايا البشري 50 (100.0%) نتائج إيجابية لـ IgG وأظهرت 20 (40.0%) من النساء اللواتي تعرضن للإجهاض نتائج إيجابية لـ IgM، بينما أظهرت جميع النساء الحوامل الأصحاء 50 (100.0%) نتائج سلبية لكل من IgG و IgM.

تراوحت أعمار النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري من 15 إلى 45 سنة، وبلغ متوسط العمر 28.82 ± 6.27 ، وكانت أعلى نسبة في الفئة العمرية 21-25 سنة، 16 (32.0%)، بينما كانت أقل نسبة في الفئة العمرية 41-45 سنة، 4 (8.0%).

أما بالنسبة لتوزيع الإقامة، فقد شملت مجموعة الإجهاض 35 مريضة (70%) من المناطق الحضرية و15 مريضة (30%) من المناطق الريفية، في حين شملت المجموعة الضابطة 40 امرأة حامل سليمة (80%) من المناطق الحضرية و10 نساء حوامل سليمات (20%) من المناطق الريفية.

أظهرت نتائج الدراسة أن عدد حالات الحمل الكلية للمريضات اللاتي لديهن أقل من 4 حالات حمل بلغ 24 حالة (48.0%) و26 حالة (52.0%) من المريضات اللاتي لديهن 4 حالات حمل أو أكثر، بينما أظهرت النتائج أن معظم النساء الأصحاء اللاتي لديهن أقل من 4 حالات حمل بلغ 38 حالة (76.0%). علاوة على ذلك، بلغ عدد حالات الإجهاض لدى المريضات 29 حالة (58.0%) من المريضات المصابات بالفيروس المضخم للخلايا البشري كان لديهن إجهاض واحد، و17 حالة (34.0%) من المريضات المصابات بالفيروس المضخم للخلايا البشري كان لديهن إجهاضان و4 حالات فقط (8.0%) من المريضات كان لديهن ثلاث حالات إجهاض أو أكثر.

بلغ عدد النساء اللاتي أجهضن بسبب الفيروس المضخم للخلايا البشري وأصبن بفيروس كوفيد-19 29 (58.0%)، بينما بلغ عدد النساء اللاتي أجهضن وأصبن بالفيروس المضخم للخلايا البشري ولكن غير مصابات بكوفيد-19، 21 (42.0%) وبلغ عدد النساء الحوامل الأصحاء المصابات بكوفيد-19 26 (52.0%)، بينما بلغ عدد النساء الحوامل الأصحاء غير المصابات بكوفيد-19، 24 (48.0%).

تم استخدام اختبار الممتز المناعي المرتبط بالإنزيم لتحديد مستويات السيتوكينات في مصل كل من المرضى والضوابط الأصحاء، وأظهرت نتائج التحليل المناعي أن مستويات عامل نخر الورم ألفا (TNF- α) في مصل النساء المصابات بالفيروس المضخم للخلايا البشري اللاتي أجهضن كانت أعلى من تلك الموجودة في المجموعة الضابطة الصحية (8.99 ± 172.72 و 8.93 ± 61.74 على التوالي، كما أظهرت أيضاً أن النساء اللاتي أجهضن وأصبن بالفيروس المضخم للخلايا البشري كان لديهن مستويات أعلى من الإنترفيرون جاما ($IFN-\gamma$) من المجموعة الضابطة الصحية بمستويات 4.81 ± 53.87 و 6.80 ± 36.65 .

علاوة على ذلك، كان مستوى IL-8 أعلى لدى النساء اللاتي أجهضن وأصبن بالفيروس المضخم للخلايا البشري من النساء الأصحاء (10.80 ± 127.74 مقابل 8.26 ± 66.17) وكانت مستويات IL-6 أعلى أيضاً لدى النساء المصابات بالفيروس المضخم للخلايا البشري مقارنة بالنساء الأصحاء، عند 6.22 ± 113.36 مقابل 6.08 ± 35.24 .

أظهرت نتائج الدراسة وجود ارتباطات بين معايير السيتوكينات لدى النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري حيث كان هناك ارتباط إيجابي معنوي بين $TNF-\alpha$ و $IFN-\gamma$ ($r=0.610, p=0.001$) ، و $TNF-\alpha$ و $IL-6$ ($r=0.549, p=0.001$) ، و $TNF-\alpha$ و $IL-8$ ($r=0.633, p=0.001$) ، و $IFN-\gamma$ و $IL-6$ ($r=0.561, p=0.001$) ، و $IFN-\gamma$ و $IL-8$ ($r=0.681, p=0.001$) ، و $IL-6$ و $IL-8$ ($r=0.510, p=0.001$)

وتضمنت الدراسة أيضاً التحقيق في التعبير الجيني لـ $miRNA$ باستخدام تفاعل البوليميراز المتسلسل في الوقت الحقيقي، وكان متوسط التعبير عن $miRNA-181a$ أعلى بشكل ملحوظ في النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري مقارنة بمجموعة الضابطة الصحية وكان 9.07 ± 1.40 و 1.0 على التوالي وكان متوسط التعبير عن $miRNA-US5-1$ في النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري 5.49 ± 1.20 ولكن لم يتم التعبير عن هذا الجين في المجموعة الضابطة الصحية ، وأظهرت الدراسة أيضاً وجود علاقة مباشرة بين التعبير الجيني لـ $miRNA-181a$ و $miRNA-US5-1$ ، بالإضافة إلى ارتباطهما المباشر بمعلّات أخرى ($TNF-\alpha$ و $IFN-\gamma$ و $IL-8$ و $IL-6$) في النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري .

استنتجت الدراسة الحالية أن النساء المجهضات المصابات بالفيروس المضخم للخلايا البشري قد أظهرن مستويات أعلى من الأجسام المضادة IgG و IgM مقارنة بالنساء الحوامل الأصحاء، فضلاً عن مستويات مرتفعة من السيتوكينات المؤيدة للالتهابات التي تضمنت $TNF-\alpha$ و $IFN-\gamma$ و $IL-8$ و $IL-6$ ، وكان هناك ارتباط إيجابي قوي بين هذه السيتوكينات، كما ارتبط التعبير عن $miRNA-181a$ ارتباطاً مباشراً بالتعبير عن $miRNA-US5-1$ ، وكان التعبير عنهما مرتبطاً بشكل مباشر بزيادة السيتوكينات الالتهابية، مما يشير إلى دور مشترك لهذه الجزيئات في عملية الإجهاض المرتبطة بفيروس المضخم للخلايا البشري.



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التعبير الجيني عن miRNA البشري والفيروس المضخم للخلايا البشري وتأثيرهما المناعي لدى النساء المجهضات

اطروحة مقدمة إلى

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