Republic of Iraq
Ministry of Higher Education and Scientific Research
University of Babylon
College of Science for Women

Study of Some Genetic Variation Associated with Cytomegalovirus Infection that Cause Recurrent Miscarriages in Babylon Province

A Thesis
Submitted to the Council of the College of Science for Women
University of Babylon in Partial Fulfilment of the Requirements for the Degree of Master of Science in Biology / Microbiology

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October 2023 A.D **Rabie Al-thaani 1445 A.H**

بِسْمِ اللهِ الرَّحْمنِ الرَّحِيمِ

﴿ يَرْفَعِ اللهُ الَّذِينَ آمَنُوا مِنكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ

دَرَجَاتٍ وَاللهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴾

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سورة المجادلة جزء من الآية 11

Supervisor Certification

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Dedication

To God of mercy and forgiveness

Who gave me strength, patience and knowledge

To my Great Teacher, messenger and ideal, Mohammad (Allah's peace and pray be upon him), who taught us the Important of knowledge and Education.

To my Great Country father of all civilization (Iraq).

To the flowers of my life who coloured my days (my lovely Nephews Karrar and Ruqayyah

To the person who carried me with his eyes, who protect me and who made me who I am today (my dear Father).

With my Love

Shahad Jamal.

Acknowledgments

I am deeply Grateful to almighty Allah for blessing and helping me to Achievement this research.

I would like to express Gratefulness to my supervisors Dr. Zainab A. Tolaifah for her helped and guidance throughout my study.

I would like to express my special thanks to Prof. Dr. Zaidan Almamouri for his assistance.

Deep thanks and appreciation are extended to the staff of Imam Al-Sadiq Hospital and to the staff of Babylon Teaching Hospital for women and children.

I would like to thank the patients for their help by allowing me to take blood samples because without this agrees, I couldn't ever do this research.

Finally, to all other who helped me in any way to finish this work, I would like to extend to my warmest sense of gratitude.

Shahad

2023

Summary

Recurrent miscarriages are defined as the loss of two or more previable (less than 24 weeks' gestation) pregnancies in succession. The current study aimed to detect the viral infection in women who suffer from recurrent miscarriages and determination if the variation in some genes is associated with the recurrent miscarriage. The study included 64 samples of aborted women who suffering underwent at least two recurrent miscarriages, as well as 50 samples of healthy women who underwent at least two births at the Babylon Maternity and Children Hospital and Imam AL-Sadiq Hospital in Babylon province.

Blood samples from both patients and control subjects were collected during the period from September of 2022 to January 2023. Sera were separated and stored in microfuge tubes and kept at -20^{o}C. DNA was extracted from the frozen blood and stored at -20℃. Sera samples from both patients and controls were subjected to serological teste to detect viral infection, the diagnosis was confirmed by (TORCH) test, mini-VIDAS technology and Chemiluminescence immunoassay Analyzer using IgG CMV kit, and DNA extraction. Tetra ARMS PCR Technique were used to amplify the IL 6 gene, and amplify the IFNAR2 gene, and the TLR9 gene by PCR, and then 60 samples (30 patients and 30 control) were sent to Korea for sequencing to identify these genes.

A comparison was made between the base sequences of the samples under study with the global genetic sequence stored in the NCBI Gene Bank, and it was found that there are mutations register SNPs (rs2236757 A>G) for the sequence of the IFNAR2(470bp) and one register SNP (rs187084 T>C) in the sequence of TLR9(644bp).

Nearly (87.1) % of aborted women were positive for anti CMV-IgG (50 out 64), with the highest percentage of infection found in the age group of 26 to

35 years (46) % and aborted women with blood group (O^+) (66) % being more susceptible to infection.

The PCR analysis of IL6 gene yielded results that indicate the presence of G and C alleles, with three distinct genotypes (GG, GC, and CC) and varying lengths (302, 206, and 152) bp. Additionally, the statistical analysis revealed a highly and significant difference between the patients and control groups.

The SNP of IFNAR2 (rs2236757 A >G) and TLR9 gene (rs187084 T>C) have indicated a significant correlation between the frequency of genotype and allele levels with abortion in CMV infected women.

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Abbreviations

Abbreviation	Definition
CIs	Confidence intervals
CTL	Cytotoxic T-lymphocyte
EDTA	Ethylenediaminetetraacetic Acid
ELFA	Enzyme-Linked Fluorescent Assay
ELISA	Enzyme Linked Immunosorbent Assay
ER	Endoplasmic reticulum
ESHRE	European society of Human Reproduction and Embryology
GB	Glycoprotein
HCG	Human chorionic gonadotrophin
HCMV	Human cytomegalovirus
HHV-5	Human Herpes Viruses type 5

IFN-1S	Interferon
IFNAR2	Interferon receptor2
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-6	Interleukin-6
IRL	Inverted repeats regions long
IRS	Internal repeat regions short
IUGR	Intrauterine growth restriction
MSMD	Mendelian susceptibility to mycobacterial disease
NCBI	National centre for biotechnology information
OD	Optical Density
ORs	Odd Ratio
PAMPs	pathogen-associated molecular patterns
PCR	Polymerase Chain Reaction
PE	preeclampsia
PL	pregnancy loss
PRRs	pattern recognizing receptors
RM	Recurrent miscarriage
RPL	Recurrent pregnancy loss
RPM	Round Per Minute
SNPs	Single nucleotide polymorphisms
TBE	Tris Bromate Ethylene

TLR 9	Toll-like receptor 9
TORCH	Toxoplasmosis, Rubella, Cytomegalovirus, and Herpes simplex virus
TRL	Terminal repeat long
TRS	Terminal repeat short
VZV	Varicella Zoster Virus

Chapter One
Introduction

1-1 Introduction

Recurrent Miscarriage, (RM), is a medical condition characterized by the occurrence of two or more pregnancy losses prior to the 20th week following the last menstrual cycle. This condition affects an estimated 1-3% of couples and is commonly referred to as recurrent pregnancy loss or recurrent miscarriage (van Dijk *et al*., 2020). Approximately 50% of cases of recurrent pregnancy loss (RPL) have an unknown etiology, despite various causal factors such as chromosomal abnormalities in parents, uterine abnormalities, infectious illnesses, endocrine abnormalities, and autoimmune defects being associated with RM (Ali *et al*., 2020a).

Viral causes are responsible for the majority of congenital infections that pose a substantial threat to fetal well-being. The human uterus is often infected by Rubella, Cytomegalovirus (CMV), and Toxoplasma, resulting in congenital abnormities, stillbirth, abortion, and premature infants (Pourroostaei *et al.*, 2022). Human Cytomegalovirus (HCMV), otherwise referred to as human herpesvirus 5, serves as the archetypal member of the Betaherpesvirinae family. As with all herpesviruses, it is capable of establishing latency and consequently endures for the individual's lifetime. HCMV infection is prevalent worldwide (Zuhair *et al*., 2019).

The prevalence of Congenital Human Cytomegalovirus (HCMV) infection is high. The clinical presentation of HCMV infection can range from an absence of symptoms to severe symptomatic manifestations. The latter may be characterized by petechiae, microcephaly, chorioretinitis, hepatosplenomegaly, and growth retardation (Bartlett *et al*., 2017; Xia *et al*., 2021). During pregnancy, the onset of congenital cytomegalovirus may ensue due to the reactivation of previously acquired cytomegalovirus or primary infection (Shimada *et al*., 2021). Some investigations have exhibited the significance of CMV-related infection in the unmanaged loss of pregnancy;

Studies have reported that infections caused by Cytomegalovirus are a possible causative agent in inducing pregnancy loss in women (Al-Dulaimi *et al.*, 2019). However, it has been suggested that immunogenetic variables have a significant contribution to the etiopathogenesis of recurrent miscarriage. The maintenance of a successful pregnancy has been associated with the balance of Th1 and Th2 cytokines (Chaouat *et al.*, 2002). Additionally, the physiological growth of the human fetus during gestation is linked to the decrease in Th1 cytokines, including IL6, TNFα, and IL-10 (Costeas *et al.*, 2004). A prolonged exposure to Th1 cytokines can stimulate a cell-mediated immune response that is harmful to the fetus, leading to pregnancy losses (Babbage *et al.*, 2001). The expression of cytokines in humans is controlled by their genetic constitution, thereby indicating the effect of polymorphisms on the rate of cytokine production (Fathi *et al.*, 2019).

Inteleukin-6 plays a pivotal role in the regulation of inflammation and exerts both proinflammatory and anti-inflammatory effects on the immune system (Al-lateef *et al.*, 2023). Within the IL-6 gene region, a plethora of single nucleotide polymorphisms (SNPs) exist that can modulate IL-6 expression, thereby influencing downstream target genes. Prior investigations have posited a plausible association between -634C/G and -174 G/C SNPs and modified IL-6 expression, as well as an augmented risk of RPL (Abo-alella *et al.*, 2021).

The innate immune response forms against viral infections through various mechanisms, which are regulated by interferon immunity genes (Zhang *et al.*, 2020; Zhang, 2020; Karacan *et al.*, 2022). IFN-Is are cytokines with potent inflammatory and antiviral functions. In humans, there are 17 IFN-Is (13 IFNα subtypes, IFNβ, IFNκ, IFNε, IFNω) (Malle and Bogunovic, 2021).

Toll-like receptors (TLRs) are belonged to the family of pattern recognition receptors (PRRs), of which TLR9 is located intracellularly and recognizes unmethylated cytosine-phosphate-guanine (CPG) DNA (Zhou *et al.*, 2021). Therefore, TLRs gene mutations may alter the expression or function of the corresponding encoded proteins, and affect the individual immune responses (Zhang *et al.*, 2023). TLRs, named after Toll proteins in *Drosophila melanogaster*, that detect microbial pathogens associated with molecular patterns to induce an immune response (Zhang *et al*,2021).

1-2 Aim of the Study:

In view of the of aforementioned introduction, this study was designed to detect the viral infection in women who suffer from recurrent miscarriages and determination if the variation in some genes is associated with the recurrent miscarriage.

1-3 Objective of the study:

1. To detect anti- human virus IgG antibodies by serological test.
2. To detect genetic polymorphism of IFNAR2, IL6 and TLR9 genes by tetra arms PCR technique and DNA sequencing.

Chapter Two

Literature Review

2-1-Recurrent Miscarriage

The loss of two or more pregnancies is known as recurrent miscarriage (RM) or recurrent pregnancy loss (RPL) (ESHRE Guideline Group on RPL., 2018; Practice Committee of the American Society for Reproductive Medicine, 2020). This reproductive disorder affects nearly 5% of couples worldwide (Ali *et al*., 2020a; Ali *et al*., 2021), with known causes including endocrine defects, chromosomal defects, immune disorders, uterine defects, genital infections, and vitamin D3 deficiency (Ali *et al*.2020a; Ali *et al*.2020b).

Recurrent miscarriage is estimated to affect 1–2% of women of reproductive age, depending on the definition used, and with the caveat that the actual prevalence is difficult to obtain owing to difficulty accessing data (Oliver *et al*., 2015; European Society of Human Reproduction and Embryology [ESHRE] Early Pregnancy Guideline Development Group, 2017; Rasmark Roepke *et al*., 2017; Woolner *et al*., 2020). The term used to describe the condition varies between countries and professional bodies (Youssef *et al*., 2019); for example, ESHRE uses the term ‘recurrent pregnancy loss’ (ESHRE Early Pregnancy Guideline Development Group, 2017), whereas the Royal College of Obstetricians and Gynaecologists (RCOG) in the UK uses the term ‘ recurrent miscarriage’ (RCOG, 2011).

Recurrent pregnancy loss (RPL) is defined as three or more consecutive pregnancy miscarriages (Hendriks *et al.*, 2019).

The most common and most critical health and social concern is pregnancy loss (PL), characterized as random or surgical uterine expulsion before fetal survival is possible (Kolte *et al.*, 2015; Mohammed, 2020). Recurrent Pregnancy Loss (RPL) has been redefined as the occurrence of two or more pregnancy losses (Ticconi *et al*., 2020).

Besides, a previous live birth does not preclude a patient from suffering recurrent pregnancy loss (No, 2011). Epidemiological trials indicate that recurrent pregnancy loss occurs in (1-3) % of mothers (Rajcan-Separovic, 2020; Sultana *et al.*, 2020). Accumulating evidence indicates that roughly (10-15) % of confirmed pregnancy raises the risk of further miscarriage with each successive pregnancy loss, hitting 45 % with three consecutive losses, and around 50 %, it is of unclear etiology (Nassour-Mokhtari *et al.*, 2020; Mohammed *et al.*, 2020). First-trimester miscarriage is the commonest complication of pregnancy. It is defined as a spontaneous loss within the first 12 completed weeks. Approximately, 5% of women will experience two consecutive first-trimester pregnancy losses, while 1% will experience three consecutive losses (Practice Committee of the American Society for Reproductive Medicine, 2012).

Viral intrauterine infections like herpes simplex viruses as etiological, causes of first trimester pregnancy loss of pregnant women at aged 20 -30 years (Naqid *et at*., 2020).

2-2-Factors of Abortion

Maternal infections play a key role in pregnancy loss and their incidence is a major factor in patients with bad obstetric history (Alsamarai *et al.*, 2013). Variety causes associated with early abortion include genetic, immunological, chemical toxicity, surgical, viral infection represented by herpes viruses, cytomegalovirus, and rubella virus, microbial infection, preeclampsia (PE), and endocrine factors such as insulin-dependent diabetes mellitus, thyroid disorders, maternal illness, luteal phase dysfunction, age, hematological, and semen or chromosomal defects and most of the time, you can't find specific underlying pathology in at least 50% of couples (Ji *et al.*, 2019; Lu *et al.*, 2019; Rajcan-Separovic, 2020), as shown in Figure (2-1).

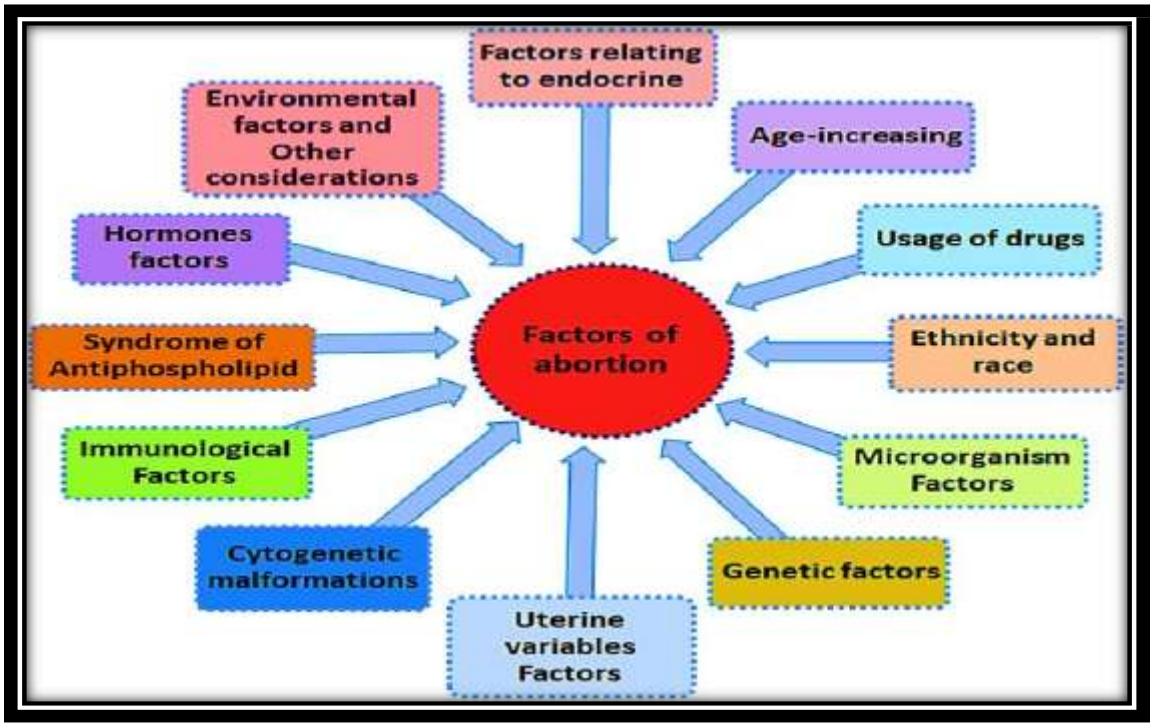


Figure (2-1): Factors of Abortion in Women (Rajcan-Separovic, 2020)

2-3-Causative Agent

Several microbial infections are correlated with maternal that can be transported by uterus if get throughout pregnancy causing abnormalities or diseases in the fetus or new-born and may lead to recurrent pregnancy loss by a wide variety of pathogens, represented by herpes viruses, cytomegalovirus, rubella virus, and *Toxoplasma gondii* (Adam *et al.*, 2019). Some infections others also commonly play a role in abortion conception as syphilis, hepatitis B, human parvoviruses, Coxsackieviruses, Epstein-Barr viruses, and Varicella-zoster virus. (Konikkara *et al.*, 2019). *Toxoplasma gondii*, which comes from uncooked vegetables, meat, or animal waste, the most widespread parasite globally that infects humans and livestock (Al-Labban, 2017; Mirza-Alizadeh, *et al.*, 2018). *Rubella* is a very common developmental condition that can lead to a miscarriage, stillborn, or severe congenital

disability, known as the Congenital Rubella Syndrome (Leung *et al.*, 2019; Terracciano *et al.*, 2020). These infectious diseases are all associated with congenital abnormalities caused by maternal infection.

There is a dramatically elevated chance of the infection, as the CMV is the most frequent source of congenital infectious diseases more than 70% within the first three months of pregnancy which can result in abortion (Mirambo *et al.*, 2016; Vasani and Kumar, 2019). Infection with CMV during pregnancy is important due to its impact on the health of pregnant mothers and fetal development. Maternal infection, especially in the first trimester, increases the risk of fetal infection and can lead to neurological, auditory, and visual disabilities, resulting in significant costs and challenges (Sharghi *et al.*, 2019).

CMV infection has been detected in 0.2 to 2.5% of new-born infants and is the most common identified cause of congenital infection, manifestations range from severe disease with any combination of intrauterine growth retardation, jaundice, hepatosplenomegaly, petechiae, thrombocytopenic purpura, myocarditis, pneumonitis, central nervous system abnormalities, deafness, and chorioretinitis to more limited involvement. Symptomatic infants may die of complications within the first months of life; more commonly, they survive but are neurologically damaged. It is now recognized that even congenitally infected infants who are asymptomatic at birth may develop sensorineural hearing loss, visual impairment, or psychomotor and/or intellectual disabilities later in life. Thus far, it appears that perinatal infected infants do not develop late neurologic sequelae of infection (Hodinka, 2015).

2-4-Viral Infections

The presence of pathogenic organisms in the placenta induces a maternal immune response to infection that could result in miscarriage. Although abortion has many causes, more than 50% of cases remain idiopathic (Homer, 2019; Sultana *et al*., 2020).

In the first trimester of pregnancy, the bulk of abortion happens in almost 80% of unintended fetal death due to symptoms such as bleeding and discomfort during pregnancy that contributes to extreme maternal anxiety (Vasani and Kumar, 2019; Hendriks *et al*., 2019; Bilibio *et al*., 2020). In most cases, the infection would be severe sufficient to cause fatal damage to a fetus than the mother.

These infections would enter the blood circulation of the fetus through the placenta (Zeb *et al*, 2018). TORCH infections (Cytomegalovirus CMV, Rubella virus and Toxoplasmosis) are considered the common causes of congenital infections and bad obstetrics outcomes worldwide. TORCH infections have been widely known to be associated with irregular and bad pregnancy outcomes. However, very little information is available about the effect of TORCH infections on pregnancy outcomes. The primary infections of CMV, Rubella virus, and *Toxoplasmosis* throughout the pregnancy could bring broad ranges of clinical symptoms during the trimesters of pregnancy. Furthermore, during the early stages of pregnancy; the result of TORCH infections may lead to fetal death, intrauterine growth restriction (IUGR) and malformations. Whereas, during the later stages of pregnancy the infections either be latent (asymptomatic) or may cause signs, broad ranges of clinical symptoms could be caused by TORCH infections, for instance, intracranial calcifications, jaundice, lymphadenopathy, hepatosplenomegaly and skin rash (Kakayi *et al*.,2021).

Over the last few decades, several studies were carried out on the pathogenesis and epidemiology of CMV-related infections, the most prevalent reason of congenital viral infections, in pregnant females. Congenital CMV, can develop during pregnancy as a result of reactivation of formerly acquired CMV or primary infection (Pourroostaei *et al.* ,2022).

The effects of primary infection of CMV in pregnant women which can lead to the loss of pregnancy is still under study and analysis, and basic mechanisms are unknown. Some investigations discovered the high level of CMV related antigens in tissue from abortion and some findings revealed higher seropositivity. In addition, one study indicated an increased risk between pregnancy loss and CMV infection (Pourroostaei *et al.*, 2022), though the findings of other related studies did not compatible with it. Although some of these studies demonstrated the role of CMV related infection in uncontrolled pregnancy loss, the effects of CMV infection on RPL is unknown (Khudair and Al-Alwany, 2023).

Several studies have confirmed the role of some intrauterine infections (including listerosis, syphilis, CMV, HSV, adeno-associated viruses (AAVs) and parvovirus B19) as a cause of miscarriage, especially during the second trimester of pregnancy. However, the role of some other infections is still questionable (Sayyadi-Dehno *et al*, 2019; Khudair and Al-Alwany, 2023). several possible causes of miscarriage include genetic abnormalities of the fetus (more than 50% of miscarriages), anatomical abnormalities of the uterus, endocrine and immunological causes, environmental agents and infections (Khudair and Al-Alwany.2023).

2-4-1 Cytomegalovirus (CMV)

Ribbert first noted the distinctive cells of CMV disease in the kidneys and parotic glands of a syphilitic neonate in 1881, and first detected CMV from a congenitally infected infant in tissues in 1904 (Ribbert, 1904). After that, Farber and Wolbach in 1932 detected CMV in the salivary glands of a few autopsy children (Farber and Wolbach, 1932).

Human cytomegalovirus (HCMV) is a ubiquitous member of the Herpesviridae family, subfamily Betaherpesvirinae. (Revello and Gerna, 2004). The viral nucleocapsid containing a linear double stranded DNA of 236 kb and is surrounded by a proteinaceous tegument, which is itself enclosed by a loosely applied lipid bilayer (Davison *et al*., 2003).

Cytomegalovirus (CMV) is a wide-spread virus, commonly is associated with the salivary glands. CMV infection may be asymptomatic in healthy people, but it can be life-threatening in an immunocompromised patient. Congenital cytomegalovirus infection can cause morbidity and even death. (Ngai *et al*., 2018; Mozaffar *et al*., 2018; Kim and Lee, 2018; Gupta and Shorman, 2022).

Cytomegalovirus (CMV) is also called Human Herpes Viruses type 5 (HHV-5) according to (ICD) International Classification of Diseases. Cytomegalovirus can maintain hidden inside the body for a long time. Cytomegalovirus energizing is associated with hormonal changes and immunosuppression (Andrievskaya *et al*., 2015; Ghailan and Mohammed, 2020). Human Cytomegalovirus (HCMV) causes the first typical infection of the Betaherpesvirinae subfamily (Yeroh *et al*., 2015; Ghailan and Mohammed, 2020). CMV similar to all of the other herpes viruses founds hiding inside the human host. Primary HCMVI results in a latent or persistent contagious that can be found within endothelial cells,

different tissues, and (PBMCs) peripheral blood mononuclear cells. The majority of babies would have a subclinical infection (O'Connor and Murphy, 2012; Ghailan and Mohammed, 2020).

After infection, CMV often remains latent, but it can reactivate at any time. Eventually, it causes mucoepidermoid carcinoma, and it may be responsible for prostate cancer (Gupta and Shorman, 2022).

CMV infects between 60% to 70% of adults in industrialized countries and close to 100% in emerging countries. Of all herpes viruses, CMV harbors the largest number of genes dedicated to evading innate and adaptive immunity in the host. CMV represents a lifelong burden of antigenic T-cell surveillance and immune dysfunction. Congenital CMV is a leading infectious cause of deafness, learning disabilities, and intellectual disability (Gupta and Shorman, 2022). The high prevalence of congenital infections, which can lead to severe congenital abnormalities, is a major public health concern during puberty and adolescence (McCarty *et al*., 2020).

In addition, the indirect effects of CMV infection includes increased risk of mortality and morbidity(Raval *et al.*, 2021). The cytomegalovirus (CMV) is considered one of the opportunistic viruses with a worldwide distribution that can infect human at any stage of life (Gold and Nankervis, 2007) then the virus became dormant (Flowler and Boppana, 2006) and it is lifetime latency after primary infection and reactivation of the latent virus can re-occur in infected individuals at any time (Ziyaeyan *et al*., 2007).

2-4-1-1 History

Cytomegalovirus was first observed by German pathologist Hugo Ribbert in 1881 when he noticed enlarged cells with enlarged nuclei

present in the cells of an infant (Reddehase and Lemmermann, 2006). Years later, between 1956 and 1957, Thomas Huckle Weller together with Smith and Rowe independently isolated the virus, known thereafter as "cytomegalovirus (Craig *et al.*, 1975). In 1990, the first draft of human cytomegalovirus genome was published (Chee *et al.*, 1990) the biggest contiguous genome sequenced at that time (Martí-Carreras and Maes, 2019). The word "cytomegalovirus" is derived from the Greek roots Cyto and Megalo, meaning "huge cell" since Cytomegalovirus produces large cytomegalic inclusion bodies (Louten, 2016). Knowledge of the role of CMV as a major pathogen with different clinical manifestations in the 1970s and 1980s (Grosse *et al.*, 2009).

2-4-1-2 Classification

Human cytomegalovirus (CMV) belongs to a herpesviridae family and subfamily Beta-herpesvirinae also known as human herpesvirus 5 (HHV5) (Gerna *et al.*, 2019; Ghailan and Mohammed, 2020; Chen, Wang and Chen, 2020), as shown in Table (2-1).

Table (2.1): Classification of CMV

Group Group 1	(ds DNA)
Family	Herpesviridae
Subfamily	Betaherpesvirinae
Genus	Cytomegalovirus

CMV has worldwide prevalence and infects people of all ages and socio-economic backgrounds, without seasonal or disease cycles of transmission (Zuhair *et al.*, 2019). The virus can remain a life-long infection for certain cells as a result of latent infection (Reeves, 2020). The CMV infection occurrence is estimated to be over 80 percent on

average (Riis *et al.*, 2020). Some studies indicate that CMV is highly active in abortion that contributes to the high seroprevalence of CMV infection in women pregnant (Ebrahim *et al.*, 2015). Sero-positivity occurrence in adult women is ranging from 40 to 90%, with the highest frequency increasing in individuals who have fewer socio-economic backgrounds (Naing *et al.*, 2016; Biolatti *et al.*, 2018).

2-4-1-3 Structure

HCMV has more genetic variation than other herpes viruses that infect human beings (Kowalik, 2014; Sijmons *et al.*, 2015). Virion has the biggest genome approximately 235 kb and encodes more than 200 genes (Plosa *et al.*, 2012), the widely viral particulate has a diameter of 150-200nms (Blut, 2010). The double stranded linear DNA centre is wrapped in an icosahedral protein capsid (Makker *et al.*, 2016), which is surrounded by a protein sheet called tegument and a lipid envelope, as shown in Figure (2-2) (Hama and Abdurahman, 2013).

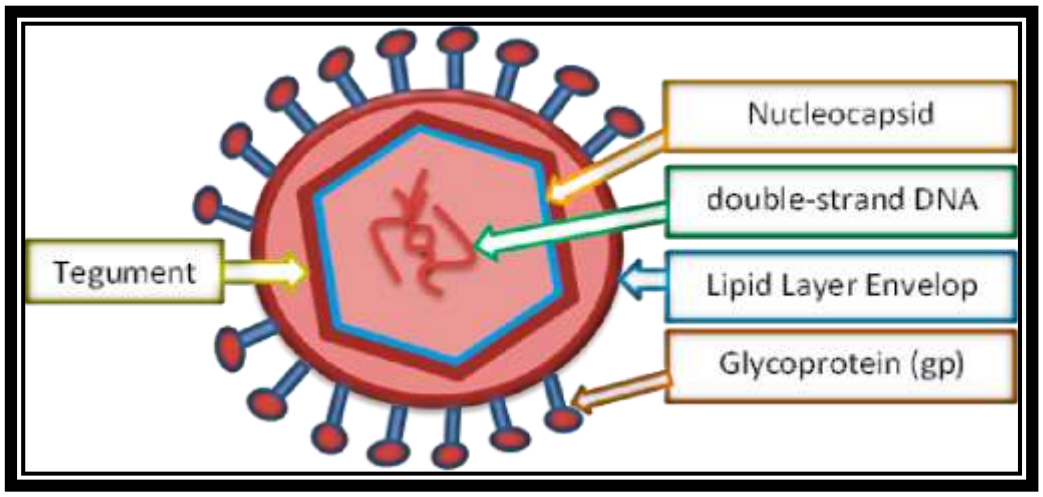


Figure (2-2): The Structure of Cytomegaloviruses (Hama and Abdurahman, 2013).

The tegument is a protein-rich membrane between the capsid and the envelope containing 50% of the overall viral protein of the virion and is

one of the dominant immune response targets for infection (Griffiths *et al.*, 2015). The research has shown that the HCMV genome comprises about 751 open reading frames (Patro, 2019; Ross *et al.*, 2020). GP contains three topological domains: an ectodomain, a transmembrane domain, and the cytoplasmic (or intraviral) domain (Burke and Heldwein, 2015; Gomes *et al.*, 2023). Importantly, GP is an immunodominant protein and an important target of neutralising antibodies. The humoral response against GP following natural infection is largely directed against five antigenic domains (named AD-1–5) with different immunodominance and distinct contribution to neutralising responses (Gomes *et al*, 2023).

2-4-1-4 Genome

It is the largest genome of any human virus (Kowalik, 2014) DNA genome enclosed in an icosahedral protein capsid, which itself is surrounded by a proteinaceous layer termed the tegument and finally, a lipid envelope (Figure 2-3) (Kalejta, 2008). The virus envelope is derived from cell membranes. There are about eight different viral glycoproteins are embedded in the lipid bilayer (Blut, 2010).

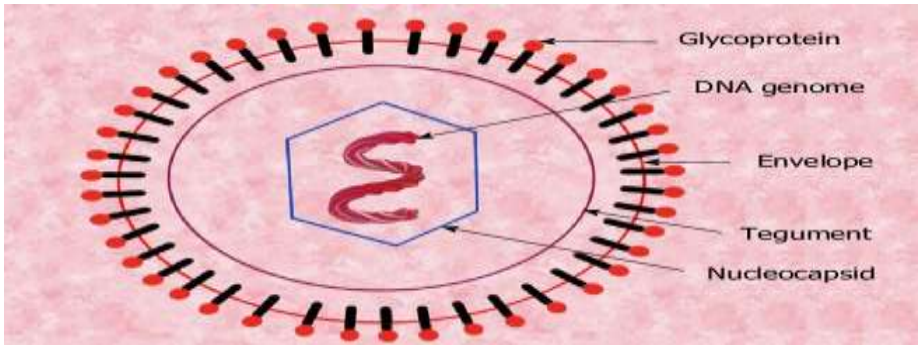


Figure (2-3): The Genome of CMV is Divided by Internal Repeat Sequences (IRS) into Two Region Termed Unique Long (UL) Region and a Unique Short Region. (Plosa‹ *et al*., 2012).

Human cytomegalovirus (HCMV; species Human herpesvirus 5) has the largest genome of any known human virus about 236 kbp in size encoding 200 genes within an enveloped icosahedral capsid (Chee *et al*., 1990). The genome is a linear, double stranded DNA molecule consisting of two unique regions each flanked by inverted repeats regions, internal repeat long and short (IRL, IRS) and terminal repeat long and short (TRL, TRS). In wild type HCMV strain Merlin (GenBank accession no. AY446894) the sizes of long unique regions (U L) and short unique regions (US) are 193.019 and 35.482 bp respectively (Dolan *et al*., 2004). Many studies have shown that HCMV strains are impressively divergent in a subset of genes encoding membrane-associated or secreted proteins (Dolan *et al*., 2004; Pignatelli *et al*., 2004). Although the functions of many genes are still unknown, the roles and functions of most HCMV genes in infective stages have been identified. HCMV infects 60–90% of the population worldwide (Stevenson *et al*., 2014) and can infect various human cells, including fibroblasts, epithelial cells, endothelial cells, smooth muscle cells, and monocytes (Gerna *et al*., 2019).

The capsid structure of herpesviruses was resolved by cryo-electron microscopy, which confirmed that the HCMV three-dimensional capsid structure is similar to the alpha- and gamma-herpesviruses' capsid structure in overall organization (Dai and Zhou, 2018; Sun *et al*., 2020). The HCMV genome is twice the size of the VZV genome and >50% larger than the HSV-1 genome (Baines, 2011). Each protein is specifically designed to associate with the final structure in a precise manner (Muller *et al*., 2021).

2-4-1-5 Replication:

The infection cycle of HCMV, as for other *Herpesviridae*, comprises various steps in the cell nucleus where genome replication and the assembly of capsids take place. The replication of the 230 kbp DNA genome is thought to occur by a rolling circle process and results in the formation of concatemers that are cleaved into unit-length genomes and packaged into a preformed capsid as shown in (Figure 2-4).

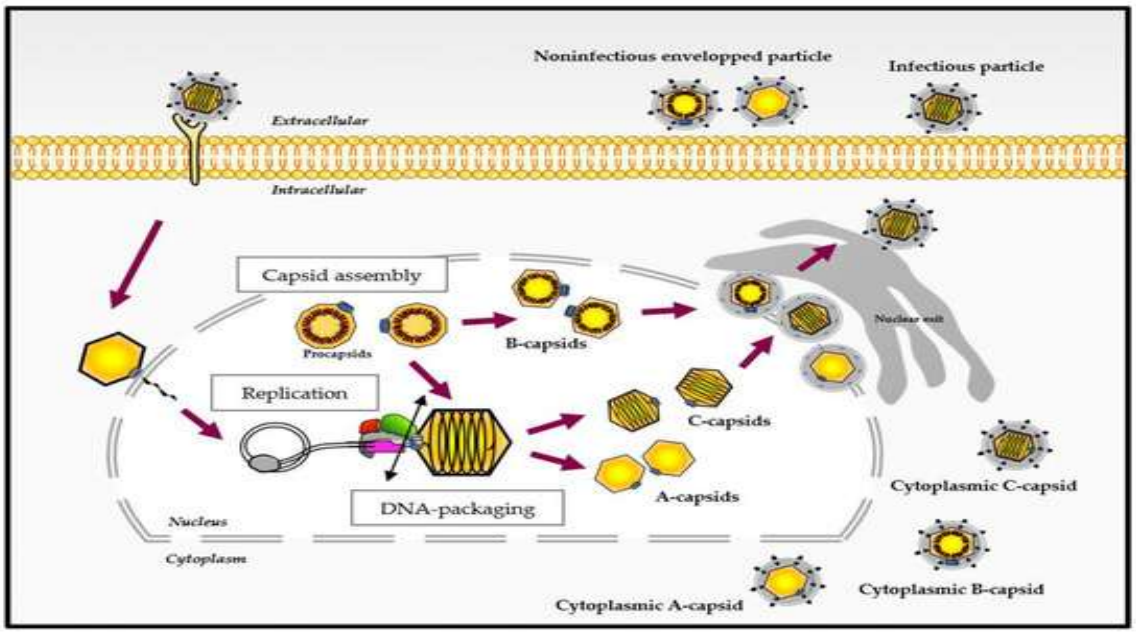


Figure (2-4): Different virus-like particles during the viral cycle of herpesviruses (adapted from (Ligat *et al*,2018; Ligat *et al*,2020). After binding and entry into the host cell, the capsid is transported to the nuclear pore and delivers viral DNA into the nucleus. After genome replication, the DNA packaging step occurs and ends with the cleavage of the concatemers (represented by the double-ended black arrow), releasing viral DNA into the capsid. Different capsid forms are present in the host cell nucleus during infection. These capsid forms, referred to as A-, B- and C-capsids, represent empty capsids, scaffold-containing capsids and viral DNA-containing capsids, respectively. The C-capsids are considered as a precursor of infectious virus.

The capsid assembly and DNA packaging are crucial steps for herpesvirus multiplication involving various viral proteins. Importantly,

these steps are highly specific to the herpesvirus family, have no counterpart in the human organism and thus represent a target of choice for the development of new antivirals (Muller *et al.*, 2021). HCMV infects and replicates in a diverse variety of cells, including dendritic cells, smooth muscle cells, epithelial, mucosal tissue cells, macrophages vascular endothelium, fibroblasts, and hepatic cells (Adam *et al.*, 2019; Gerna *et al.*, 2019). HCMV entry into the host's cell starts a viral lytic cycle stage following the breakdown of the external defences of the host and continues replication (Taisne *et al.*, 2019). Viral late (L) genes are mainly structural components of the virion that assist in the development and release of newly formed viral particles (Manandhar *et al.*, 2019), as shown in Figure (2-5).

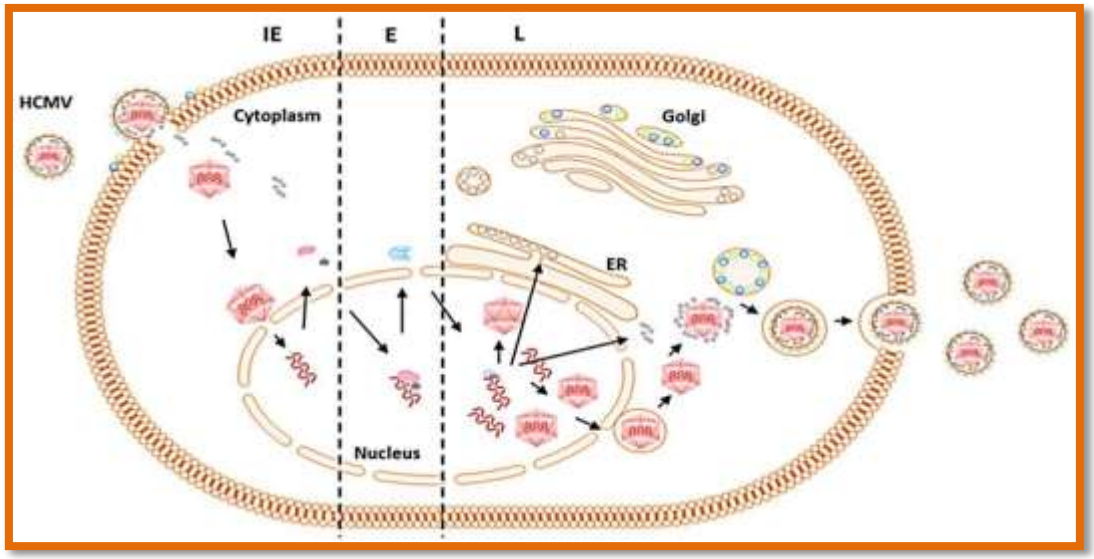


Figure (2.5): Diagram shows a Replication of Cytomegalovirus in Human Cells (Manandhar *et al*., 2019).

All herpesviruses share a characteristic ability to remain latent within the body over long periods. Although they may be found throughout the body, CMV infections are frequently associated with the salivary glands in humans and other mammals *(*Koichi *et al*., 2007).

2-4-1-6 pathogenesis

HCMV is capable of infecting most forms of cells and organs (Jain *et al.*, 2011). It can also lead to harmful high-stress medical effects by HCMV and the risk of chronic diseases, as can other family herpes viruses (Reed *et al.*, 2019). The primary viral cause of birth defects and life-threatening complications in immunocompromised persons ranging from asymptomatic to serious end-organ dysfunction (Stern *et al.*, 2019; Riis *et al.*, 2020; Azoulay *et al.*, 2020), and high risk in AIDS patients, neurosensory illness, transplant recipients, people confined to intensive care units and some degree, in elderly patients (Oiknine-Djian *et al.*, 2018). HCMV infection is generally asymptomatic in healthy individuals, while in immunocompromised individuals, such as organ transplant recipients or human immunodeficiency virus carriers, HCMV poses a life-threatening risk. (Jean Beltran and Cristea, 2014). HCMV infections are normally relatively benign. Infants infected in utero can be born with disseminated HCMV disease or be damaged in terms of neurologic development. Among the cell types infected are placental trophoblasts, which spread virus from mother to baby, and neurons and glial cells in the developing nervous system (Vanarsdall and Johnson, 2012). The virus can be present in various secretions of an infected person, such as discharge from the oropharynx, urine, vagina, semen, milk, and blood components. The virus spreads by direct contact with an infected person, vertically from mother to foetus, and parenteral, i.e., blood transfusions, organ or bone marrow transplants (Polz-Dacewicz *et al.*, 2013). CMV infection may be either latent (non-productive), lytic (productive), asymptomatic or symptomatic. HCMV is the most common virus transmitted from infected pregnant mother to child. Approximately one-third of women who have a primary HCMV infection during pregnancy

pass the virus on to the neonate. Thus, approximately one in 150 children is born with congenital HCMV infections. Congenital HCMV infection is defined by detection of the virus in the newborn's urine, blood or saliva within three weeks of birth. Children with congenital HCMV present with small body size, jaundice, petechiae, hypotonia and hepatosplenomegaly (Blaho, 2010).

2-4-1-7 Epidemiology:

Epidemiological studies have documented an average high seroprevalence of HCMV infection in HIV-infected individuals worldwide between 50% and 85% (Zuhair *et al.*, 2019). Asia, South America, and Africa are more predominant, and North America, England, Australia, Western Europe, and Germany are less prevalent, where the infection is more common in poor and less health-conscious countries, compared to more developed countries (Hassan *et al.*, 2016; Sharghi *et al.*, 2019). HCMV DNA can be contained in 20% of fetal tissues or placenta, and it can be recommended that indirect effects of placenta infection with cytomegalovirus lead to adverse pregnancy outcomes or contribute to stillbirth (Rawlinson *et al.*, 2017; Aldorri, 2018). There are many genetically distinct strains of HCMV, but it is possible to be diagnosed with more than one strain of HCMV infection, this has been found in recipients by organ donors (Bate *et al.*, 2010).

The CMV is widespread in the world, common even in the general immune-competent population, 50-80% of the healthy adult population being infected with CMV (Kalejta, 2008), although significant differences in the seroepidemiology exist between countries, its primary infection was usually asymptomatic (Polz Dacewicz *et al.*, 2013), it infects humans of all ages, with no seasonal or epidemic patterns of

transmission. The seroprevalence of CMV increases with age in all populations and ranges from 40 to 100%; the virus is acquired earlier in life, and the prevalence is highest among lower socioeconomic groups in crowded living conditions (Hodinka, 2015). It was stayed latent in undifferentiated monocytes and is widespread in the body (Badami, 2014), the seroprevalence of HCMV in the human population ranges between 30% to 90% in developed countries, with seroprevalence increasing with age (Crough and Khanna, 2009). Infections are classified as being acquired before birth (congenital), at the time of delivery (perinatal), or later in life (postnatal) (Hodinka, 2015).

2-4-1-8 Transmission

In the general population, cytomegalovirus is a widely transmitted infection. The spread process requires a direct communication between the infected and the non-infected cell (Jackson and Sparer, 2018). Quite popular human pathogens are transmitted to humans through a close contact between individuals infected with CMV through saliva, sexual intercourse, breastfeeding, blood transfusion, strong organ transplantation, or hematopoietic stem cell transplantation (Zheng *et al.*, 2019;). The last study found that CMV is spread not only through the oral mucosal route but also through the vaginal mucosal (Cannon *et al.*, 2010). Vertical (mother-to-child) transmission can occur through a placenta that causes congenital infection of the child (Naing *et al.*, 2016), as shown in Figure (2.6).

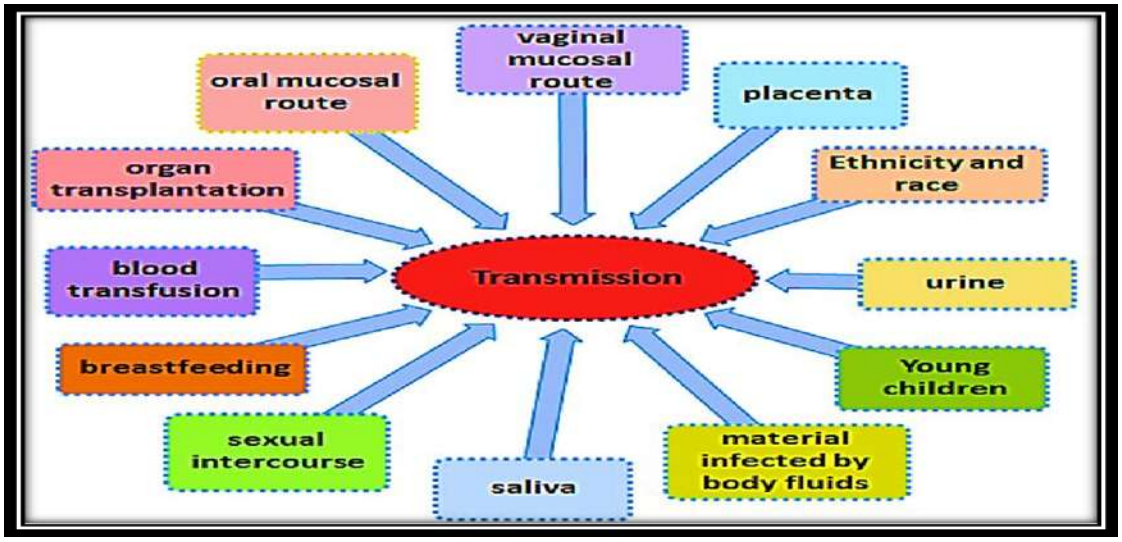


Figure (2-6): Cytomegalovirus Widely Transmitted Infection into Women Tissues (Naing *et al*., 2016).

Young children are the main cause of CMV infection and about (1-4%) of pregnancy occurs. Cytomegalovirus spreads quickly in environments where preschool age children are grouped, which transport in their urine and spit, as well as material infected by body fluids (e.g., utensils such as water cups, dummies/soothers) (Zheng *et al*., 2019). A cell infected with HCMV transport viral particles through the bloodstream to different tissues, leading to severe and chronic infection and induces mostly latent or permanent endothelial, multi-tissue and peripheral blood mononuclear cell (PBMC) inflammation. HCMV can enter and replicate the body in most cell types, (Stevenson *et al*., 2014). The mother-to-child transmission risk of cytomegalovirus is higher for primary maternal infection (50%) than for reactivated (not primary) or re-infection (2%) (Kagan and Hamprecht, 2017). Some experiments have found that abortion in the first trimester is a higher risk of CMV than the third trimester, sometimes CMV infection is harmless to the majority of people (Sherkat *et al*., 2014; Mirambo *et al*., 2016).

2-4-1-9 Transmission of CMV in pregnancy

Cytomegalovirus (CMV) infection during pregnancy is more complex than other infections, due to virus reactivation during the child bearing age and be transmitted to the fetus in spite of maternal immunity (Mukundan *et al*., 1977). Various ways of transmitting the virus to the fetus have been suggested, whereas the hematogenious spreading across the placenta with subsequent infection of placental and amniotic tissue seems to be the most common transmission way (Pass and Boppona, 1999). Trans placental transfers of virus in utero and fetal exposure to CMV secreted in the vagina during passage through the birth canal at delivery are the major modes of vertical transmission (Brown and Abernathy, 1998). In contrast to in utero (congenital) infection, per partum infection (either intra partum or postpartum) does not pose serious harm to the development of the neonate except in babies weighing less than 1500 gm. (Yeager *et al*., 1983). HCMV is a member of the TORCH group (*Toxoplasma gondi*, Rubella virus, Cytomegalovirus, Herpes simplex virus) of pathogens that can cross the placenta as illustrated in Figure (2-7) (Varada, 2014).

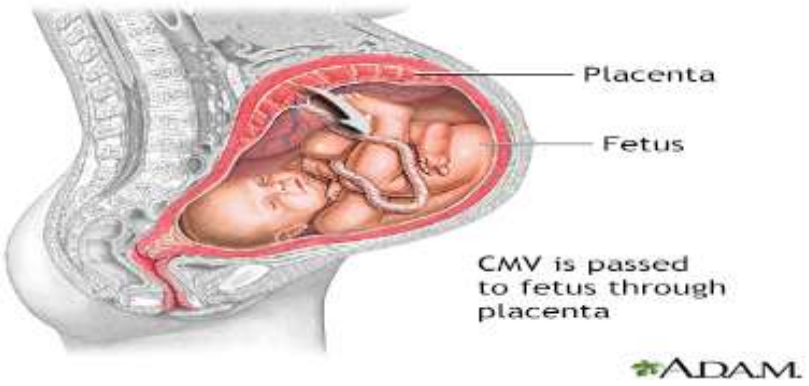


Figure (2-7): HCMV is Passed from Mother to Child through Placenta. (Varada, 2014).

2-4-1-10 Symptoms

Symptoms are typically non-specific, with flu-like symptoms (Habibi *et al.*, 2014). Chronic oral and neurological disorders, including periodontia, asthma, cardiac and neuropsychiatric issues, and other effects, such as weakness, cervical lymphadenopathy, fatigue, and muscle aches, can also be pathophysiologically associated with HCMV (Burgdorf *et al.*, 2019; Nauclér *et al.*, 2019). Higher levels of HCMV serum antibodies are also correlated with markers for aging and immunosenescence, atherosclerosis, and the elevated probability of all-cause deaths (Elwenspoek *et al.*, 2017; Stern *et al.*, 2019). Surviving infants may have hearing disability, hepatic and splenic growth, low fetal size, in particular head and brain development, repeated jaundice, and long-term mental delay (Alvarado-Esquivel *et al.*, 2018; Leung *et al.*, 2020). The discovery of certain specific genotypes shows severe symptoms in children with CMV infection (Hu *et al.*, 2021; Hu *et al.*, 2022).

A majority of these infections are a symptomatic as others and they are difficult to diagnose clinically (Sen *et al.*, 2012). Lastly, Nucleic acid HCMV proteins have eventually been identified in several tissue malignancies diverse cancers, such as cervical, colorectal, glioblastoma, neuroblastoma and breast cancer, diseases in the circulatory system, digestive system, and ophthalmic system (idiopathic)-they are all closely related to human cytomegalovirus (Liu *et al.*, 2020; Maple, 2020; Richardson *et al.*, 2020). The potential burden and magnitude of infectious diseases will increase if several viral strains of the dormant endogenous virus and/or superinfection were spontaneously reactivated (Plotkin and Boppana, 2019). Considering that the dose-restricted toxicity and immunity of regulated HCMV antivirals and diseases are limited

(Sinkó, 2019), in immunocompromised patients, ganciclovir and foscarnet have been licensed for use in life-threatening HCMV infections (Gratama *et al*. 2010). When a person is infected with CMV any time after birth it is known as an acquired infection. In normally healthy children and adults CMV infection is usually not a concern. 90% of the time it will not produce any signs or symptoms of infection. However, occasionally a flulike or mononucleosis type of illness may occur and produce symptoms such as spiking fever to 39-40c are not uncommon and can last for more than two weeks, sore throat, fatigue and swollen glands (Nesmith and Pass, 1995).

Clinical manifestations of CMV infection in immunocompromised patients include: prolonged fever over 38°C with (or without) leukopenia, hepatitis, pancreatitis, gastrointestinal disorders, pain with fever, inflammation of the oesophagus, dysphagia, disorders that may be associated with Candida infection, interstitial pneumonia, inflammation of the heart muscle, bladder inflammation, inflammation of the retina – frequently observed in AIDS patients (Boeckh and Geballe, 2011).

2-4-1-11 Role of Immunity of CMV:

T-cell mediated cellular immunity is the most important factor in controlling CMV replication. CMV induces a strong CD8 + cytotoxic T-lymphocyte (CTL) response; therefore, immunosuppression significantly contributes to the loss of CMV specific adaptive immune control.

However, the observation that only a fraction of patients with similar degrees of immunosuppression develops CMV infection suggests that other factors not yet defined contribute to susceptibility to reactivations (Vallejo *et al*., 2022). IgG and IgM antibodies to cytomegalovirus can be

considered as an easy tool for selecting patients who are at risk of cytomegalovirus infection. IgG was reflected the previous infection, presence of it doesn't prevent the reinfection or re activation but may reduce the severity of pathogenesis. While IgM immunoglobulin was considered as evidence of recent or acute infection which is formed immediately after infection and disappeared after short period 16-20 weeks (Goodrich *et al*., 2004).

The immune response to CMV is complex series of immunological processes, both innate and adaptive immunity are involved (Badami, 2014). When Cells infected with HCMV, its exhibit profound reprogramming of gene expression. The family of interferon-stimulated gene and inflammatory genes that included antiviral genes such as those for TNF-α, IL-1, IL-6, IL-8, IL-12, IL-18 and cyclooxygenase 2 (COXX-2) are hallmarks of innate immunity that contribute significantly to control infection (Lochmanova and Lochman, 2011) , CMV infection triggers activation of several arms of the immune system, activation of these arms can be both initiated and controlled by cytokines .Proinflammatory cytokines activate separate pathways that are involved in the immune response to CMV infection, whereas anti-inflammatory cytokines control the inflammation and turn off the response, Natural killer (NK) cells have been shown to be important sources of interferon (IFN-) during CMV infection (Geist and Hinde, 2001). Several distinct categories of cells shared to control of HCMV replication includes: natural killer (NK) cells, macrophages, B cells, αβ and γδ T cells but particularly T cells seem to play a key role in this process. Mostly T lymphocytes are of major importance in the initiation and maintenance immunity against viral infection (Lochmanova and Lochman, 2011).

2-4-1-12 Diagnosis

The most used approach to limit HCMV infected individuals is the detection of particular antibodies by identifying HCMV related antibodies. Many types of experiments are possible for the determination of an anti-HCMV titer with various susceptibility levels by techniques such as the enzyme-linked fluorescent assay is diagnosed (ELFA, applied in Mini-Vidas instrument), the direct immune fluorescence assays, or enzyme immune assays (ELISA) (Munro *et al.*, 2005; Rajaii *et al.*, 2009). Congenital CMV can also be detected by TORCH rapid test (Adler, 2011) and the amniotic fluid by culture and PCR as the best diagnostic for diagnosing an intrauterine CMV infection (Donner *et al.*, 1993; McCarthy *et al.*, 2009; Nayeri and Thung, 2013). The immunological diagnosis of primary maternal CMV disease is based on the existence in the blood of an early seronegative pregnant woman of a virus-specific immunoglobulin G (IgG) antibody or immunoglobulin M (IgM) antibody (Usta *et al.*, 2016). The positive results of IgG antibodies are usually an indicator of prior exposure to CMV and are indicative of old infection Significant (Carlier *et al.*, 2010; Ross *et al.*, 2011). The detection of HCMV (IgM) immunoglobulin has been used as a highly sensitive marker for active or recent CMV infections and can be detected in stage 4 to 8 weeks after primary infection (Lagrou *et al.*, 2009). In both primary and old infections, CMV IgG and IgM can also be found at the same time in women who are pregnant (Olumuyiwa *et al.*, 2019). However, false positives may occur due to cross-reactivity to other diseases such as autoimmune or viral infections (Usta *et al.*, 2016).

2-4-1-13 Treatment

If the likely cause of recurrent pregnancy loss can be determined treatment is to be directed accordingly. In pregnant women with a history of recurrent miscarriage, anticoagulants seem to increase the live birth rate among those with antiphospholipid syndrome and perhaps those with congenital thrombophilia but not in those with unexplained recurrent miscarriage (de Jong *et al*., 2013).

Nonetheless, the antiviral agents commonly used to treat CMV infections suffer from high hematologic, renal and neutropenia toxicity, low bioavailability and the development of drug resistant virus strains. The best way to prevent HCMV transmission is through behavior modification which emphasizes hygiene (Biron, 2006).

2-4-1-14 Control and Prevention

The principal sources of exposure for women of childbearing age are sexual contacts and children secreting the virus (Yow, 1989). Pregnant women also should be advised to avoid close contact with individuals likely to shed CMV such as adults with symptoms consistent with mononucleosis and toddlers attending group day care (Adler *et al*., 1998). Preventive precautions for pregnant women with congenital cytomegalovirus are adaptive, increases awareness, and do not lead to a major rise in anxiety (Lazzaro *et al*., 2019). Precautions for hygiene in pregnant women to avoid cytomegalovirus infection by not exchanging food, beverages, or utensils for the child, don't put a dummy/soother/pacifier in the child's mouth, clean hands for 15 to 20 seconds with soap and water particularly after changing diapers, feeding a small child, or rubbing the `that come into contact with the infection that

should be taken into consideration to prevent infection (Rawlinson *et al.*, 2017).

2-5 Genetic Susceptibility

Cytokines are defined as soluble small proteins (5–20 kDa) which bind to definite receptors on specific cells, start some internal cellular changes, and cause various genetic and chemical regulations (Ramadan *et al.*, 2020). Cytokines can be classified into a number of categories including tumor necrosis factors (TNFs), interleukins, lymphokines, chemokins, interferons, and transforming growth factors (TGFs) (Liu *et al.*, 2021). Secreted by phagocytic cells, such as many cells, macrophages, T lymphocytes, B lymphocytes, mast cells, endothelial cells, fibroblasts, and a single cytokine may be secreted by more than one type of cell (Mehta and Mahajan, 2006).

Cytokines are molecules of proteins groups which intervolved in several biological activities, like haematopoiesis, cellular differentiation & growth, and immunological functions, as inflammatory, apoptosis, fibrosis and necrosis, where they interact with the entire body, due to their production and controlling via multiple networks of molecular regulators, in addition to variation of types of cytokines; such as “Interleukins”; IL; “Chemokines”; CK; “Interferons”; IFN; and others. (Malek and Castro, 2010). The main function of cytokines is to regulate the differentiation of T lymphocytes. Differentiation T cell from Undifferentiation to T cells primary helper T lymphocytes Th1, and secondary helper T lymphocytes Th2, In addition to the regulation of interleukins (ILs) and interferons (INFs), (Babon and Nicola, 2012)

The response of host towards infections; especially viruses; includes several immunological components; mainly T – Lymphocytes; to regulate

immune cytokines, where "T – helper – 1"; Th1; type of cells secretes "Pro – inflammatory – Cytokines" and "T – helper – 2"; Th2; type secrets "Regulatory Humoral Immune Response Cytokines"; therefore; their elevated levels is associated with severity of infection, disease and type of causative agent. (Della-Torre *et al*., 2020).

When cytokines are over secreted; for some error that may cause this disaster; this can lead to trigging dangerous "Cytokine – Storm – Syndrome"; which may lead to severe events of adverse effect due to the wide source & igniter for cytokines; especially against viral infections; which cause acute immunological response by all ways of defense mechanisms that can response toward; Many cytokines appear to be important in immune control of CMV infection, although defining cytokines that may correlate either with protection or increased susceptibility to cytomegalovirus infection (Schleiss, 2013) such as Interleukin6(IL6), IFNAR2, TLR9.

2-5-1 Interleukin 6

Interleukins (IL) are important inflammatory molecules produced by blood monocytes and tissue macrophages. Leukocytes manufacture these cytokines, and these interleukins are secreted towards the target cell to bond on the surface when a human body is attacked by an infectious agent. This activates the signals within the target cells, thus, changing the behavior of the cells (Zubair and Ahmad, 2019). They are involved in several important cellular processes, including proliferation, maturation, migration and adhesion, and also participate in the activation and differentiation of immune system cells. The assessment of the levels of interleukins in the human body can be used as a diagnostic indicator of the development or progression of many diseases (Peluzzo and Autieri,

2022). An adequate and precise balance between pro-inflammatory and anti-inflammatory immune responses is required to effectively eliminate infectious pathogens while limiting immune-mediated damage to the host. This complex balance is particularly relevant in systemic infection when infection with pathogens that have not been contained can lead to a dysregulated systemic inflammatory response, leading to severe host pathology and high morbidity and mortality (Martinez-Espinosa *et al*., 2021).

Interleukin-6 (IL-6) is a cytokine which has a pleiotropic effect on T cell differentiation and a crucial role in the inflammatory response(Prins *et al*., 2012; Papamitsou *et al*., 2022). IL-6 is a 21–28 kDa glycoprotein, which is produced by various cells, but its primary sources are macrophages, T-lymphocytes and monocytes (Rasti *et al*., 2016). The gene encoding human IL-6 has been mapped on the short arm of chromosome 7 (7p21), containing 212 amino acids (Tanaka *et al*., 2014). The expression of this gene is observed in human endometrial tissues, and IL-6 is a multifunctional cytokine that is produced in the luminal epithelium in a periodical way. During the periods of implantation and menstruation, the levels of production are the highest, and conversely the levels of IL-6 are comparatively low in the proliferative phase, and remain steady all through the secretary phase (Papamitsou *et al*., 2022). A considerable number of polymorphisms in the IL-6 gene have been identified, a few of which are reported to modify its expression. The anti-inflammatory functions of IL-6 are well known, but its possible predictive value in pregnancy outcome remains to be clarified (Rasti, Nasiri and Kohan, 2016). Raised levels of IL-6 are evident in the altered cytokine profiles of RPL, unexplained infertility, preterm delivery and

preeclampsia. Numerous factors control the expression of IL-6, one of which is IL-1 (van Mourik *et al.*,2009; Papamitsou *et al.*,2022).

IL4 and IL10 cytokines are believed to be anti-inflammatory in function, on the other hand IL6 has been found to exhibit pro-inflammatory properties. Normally IL10 and IL6 are known as Th2 type cytokines. However, besides Th2 cells macrophages, Th1 cells and B cells produce IL10 and IL6 is formed by macrophages, fibroblasts, and B cells (Ali *et al.*,2020a).

2-5-2 IFNAR2

Interferone-γ(IFN-γ) is a cytokine that provides protection against diseases by acting directly on target cells or through activation of the host immune system. IFN-γ can educate immune cells to recognize and destroy pathogens; thus, understanding these interactions with host immunity is of particular importance. Interferon-γ is the sole member of the type II interferon family discovered almost 60 years ago. E. Frederick Wheelock was the first to describe IFN-γ as a phytohemagglutinin-induced virus inhibitor produced by white blood cells after they have been stimulated (Jorgovanovic *et al.*, 2020).

IFN-γ is a pleiotropic cytokine with antiviral, antitumor, and immunomodulatory functions. Hence, it plays an important role in coordinating both innate and adaptive immune response (Mendoza *et al.*,2019). The production of IFN-γ is mainly regulated by natural killer (NK) and natural killer T (NKT) cells in innate immunity while CD8+ and CD4+ T-cells are major paracrine sources of IFN-γ during adaptive immune response (Burke *et al.*, 2019).

IFN-γ primarily communicates with the IFN-γ receptor (IFNR). IFN-γR is a tetramer composed of two IFN-γR1 strands in combination with two IFN-γR2 strands, where IFN-γR1 is the IFN-γR's ligand-binding tail and IFN-γR2 is the IFN-γ receptor's signal-transducing tail. MSMD stands for Mendelian susceptibility to mycobacterial disease, a category of primary immunodeficiency marked by higher vulnerability to mycobacteria. Mutation in approximately 19 genes involve in IFN-γ signaling pathway such as IFN-γR1, and IFN-γR2 lead to MSMD (Martínez-Barricarte *et al*., 2018; Ghanavi *et al*., 2021).

2-5-3 TLR9

It is small family of proteins that are among the earliest determinants of immune activation, have become the focus of biomedical research. Studies have suggested that genetic variants of TLRs play a crucial role in different autoimmune diseases, such as type 1 diabetes mellitus (T1DM), Graves' disease (GD), rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), and multiple sclerosis (MS) (Zhang *et al*., 2021). These genes can predict the severity of the disease as well as suggest the responsiveness of the disease to biological therapies (Zeggini *et al*., 2019).

Toll-like receptors (TLRs) are pattern recognizing receptors (PRRs) and play an important role in regulating human's immune system. The TLRs can recognize pathogen-associated molecular patterns (PAMPs) in both extracellular and intracellular environment. The TLRs have the ability to initiate signaling pathways that are responsible for activating both innate and acquired immune responses as well as the production of inflammatory cytokines. The members of TLRs family have been detected on human plasma membrane (TLR1, TLR2, TLR4-6) and in

leukocyte endosome (TLR3, TLR7-9) (Zhang *et al*., 2013; Chen *et al*., 2015).

Toll-like receptors (TLR) are crucial in the detection of viruses in circulation and the subsequent elicitation of an antiviral response. TLRs act as pattern recognition receptors of non-methylated viral CpG-containing DNA which signals the presence of CMV infection. TLR2 and TLR4 are cell surface receptors while TLR3, TLR7 and TLR 9 are endosomal receptors. TLRs facilitate viral attachment and entry resulting in CMV elicited signaling antiviral responses such as type 1 interferon activation of nuclear factor kappa β (NF-k β) and pro-inflammatory cytokine gene expression. Activation of the type 1 interferon producing cascade and production of cytokines form the major cellular antiviral mechanisms against CMV. Single nucleotide polymorphisms (SNPs) in the TLR2, TLR4, TLR7 and TLR9 genes were inconclusively reported to be associated with CMV infection (Mhandire *et al.*, 2020).

The phenomenon that may result from point mutations is called gene polymorphisms, which is also known as single nucleotide polymorphism (SNP) and refers to single nucleotide differences in some DNA sequences in the homologous interval (Dvornikova *et al*., 2020). SNPs occur in > 1% of the general population, unlike other mutations that generally occur in ≤1% of individuals (Ashton *et al*., 2010; Zhang *et al*., 2021).

Toll-like receptor-9 (TLR-9) acts as receptors of the innate immune system in addition to their role as a bridge between innate and adaptive immunity during their response against tumors (Bai *et al*., 2017). TLR-9 is located in a resting state on the endoplasmic reticulum membrane, while in a state of stimulation due to its binding to ligands it transports to the endosomal or lysosomal membrane in contrast to the rest of the

family of TLRs that form the membrane-bound pattern recognition receptors (Kutikhin, 2011).

Regarding the detection of SNPs that are ubiquitous during the study of human systems, the research focus has shifted to the identification of SNPs at the signaling and receptor levels, particularly those associated with key receptors of innate immunity, i.e., TLRs. TLRs, named after Toll proteins in Drosophila melanogaster, are a family of inherent immune recognition receptors that detect microbial pathogens associated with molecular patterns to induce an immune response (Zhang *et al.*, 2021). Furthermore, TLRs is the first protein family that conforms to the characteristics of the pattern recognition receptor (PRRs) predicted by Janeway. As germline-encoded proteins, PRRs recognize conserved microbial products, also known as pathogen-associated molecular patterns (PAMPs), can induce host defense activities and stimulate immune responses (Fitzgerald and Kagan, 2020). TLRs have been associated with a membrane protein involved in Drosophila embryogenesis and host defense. TLRs, in mammals, are synthesized in the endoplasmic reticulum (ER) after which they are transported to the plasma or endosomes (Fitzgerald and Kagan, 2020).

TLR9 is located on the human chromosome 3p21.2 with a 5kb gene length and encoded by TLR 9 gene, which has 2 exons, and the major coding region of the TLR9 gene is found on the second exon (Chen *et al.*, 2015)., the protein of TLR9 is about 150kDa in length and composed of 1,032 amino acids (Song *et al.*, 2019).

Finally, TLR9 possesses two DNA binding sites, the first is the CpG site, and the second is located at the 5' end, cooperative binding of them required for TLR9 dimerization and activation (Ohto *et al.*, 2018).

Chapter Three
Materials and Methods

3- Materials and Methods

3-1 Subjects

A Part of this study, which includes direct contact with patients and taking information in addition to collecting blood specimens, was done in the emergencies of Imam AL-Sadiq Hospital and the Babylon Maternity and Children Hospital as the basis study included all patient attended at the period from September of 2022 to January 2023. The research was approved and permitted by Hospital administration.

A total of 114 blood samples were divided into two groups. The first group included 64 samples of women who suffered from recurrent pregnancy loss, aged between (16-45) years. 50 positive samples of CMV were studied,14 negative samples of CMV were excluded and the second group included 50 sample of control women (CMV negative and had two successive pregnancy), with ages ranging from (19-43) years.

-A ***questionnaire*** template was included several important questions were asked and reported from patients , the patients' information includes :age , weight, chronic diseases and if the patient had operation, as mentions in the Table(3-1).

Table (3-1) Demographic Information Taken from Patients

NO.	
Age	
Chronic diseases	
Number previous abortion	
Blood group	

3-2 Materials

3-2-1 Laboratory Devices:

In order to complete the study, several devices were needed which are mentioned below in the Table (3-2).

Table (3-2) Laboratory Devices List

No.	Devices	The Industrial company
1-	Autoclave	Hermle, Japan
2-	Chemiluminescence immunoassay Analyzer	China
3-	Eppendrofe Centerfuge Hettich	Dragon
4-	Freeze -20℃	Concord
5-	Gel electrophoresis apparatus	Bioneer
6-	VIDAS device	Biomerieux Italian
7-	Vortex	Quality Lab System, England
8	Water Bath	China

3-2-2 Laboratory Materials:

Many materials were required to complete the study, several equipment (tools) were needed, and as mentioned in the Table (3-3).

Table (3-3) Laboratory Tools List:

NO.	Tool	The Industrial company
1-	EDTA Tube	Afco
2-	Eppendrof rack tube	Bioneer
3-	Eppendrof tube	Bioneer
4-	Gel Tube	China
5-	Micropipette from 100- 1000 microliter	Dragonlab
6-	Micropipette from 20-200 *microliter*	Dragonlab
7-	Micropipette from 5-50 microliter	Dragonlab
8-	Pipette tips (Yellow)	Afco
9-	Pipette tips (blue)	Afco

3.2.3 Chemical Materials

Several chemical materials were needed to accomplish this research, which mentioned in the Table (3-4).

Table:(3-4) Laboratory Chemicals List:

No.	Chemical material	Manufactured company
1-	Agarose	Promega
2-	DNA Ladder	Genedirex
3-	Ethanol 96%	Aljoud Iraq
4-	Ethidium bromide	Promega
5-	Green Master Mix	Promega
6-	Primers	Alpha DNA
7-	Proteinase K	Favorgen
8-	Nuclease free water	Promega
9-	TBE Buffer	Promega

3-2-4 Serological Diagnostic Kits

The laboratory kits used by the Company and their origin are shown in Table (3-5).

Table (3.5): Laboratory Kits are Mentioned:

NO.	Kits	Company
1-	CMV Test Kit	China
2-	Chemiluminescence immunoassay Analyzer Kit	China
3-	DNA Extraction Kit	Favorgen/ Taiwan
4-	Torch Test Kit	New York
5-	VIDAS Kit	

3-3 Methods

3-3-1-Blood Specimens

Blood specimens from patients were obtained by venipuncture, then about 2.5ml of blood was put in EDTA anticoagulant tubes in order to save it from coagulation, and the other part approximately 2.5ml was kept in tubes that did not contain EDTA for serological analysis, then it's kept in -20 ℃ until DNA extracted, as described in Scheme (3-1).

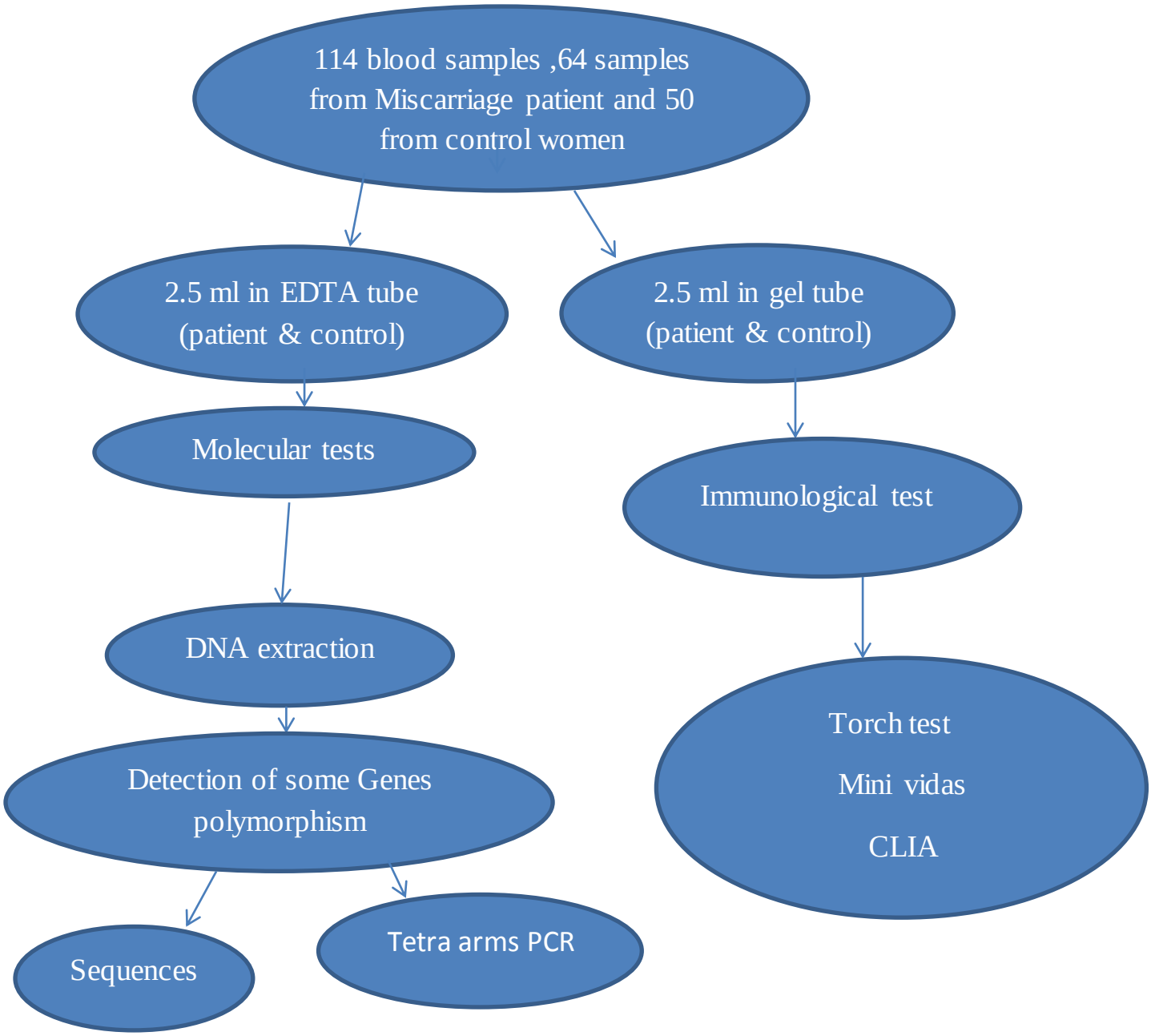


Figure (3.1): - The Study Design

3-3-2- Serological Diagnosis of CMV

Serological approaches are useful for the detection of HCMV antibodies in sera patients. Sera samples were insulated by centrifugation at 1000 rpm for 5 min in test tubes and kept at 2-8°C for 2 days or frozen for a long period at-20°C.

3-3-2-1 Diagnosis of the Cytomegalovirus in Healthy Women

Procedure test for 50 samples from healthy women with an age range between (19-43) years were diagnosed by adding 25µl of a serum sample to the hole cassette rapid test and adding 80µl of buffer directly to each well for diagnosis of HCMV antibodies. The findings were read after 15 minutes of the test procedure.

3-3-2-2 Diagnosis of the Cytomegalovirus in Patients Women

Procedure test for 64 samples of women suffering from recurrent pregnancy loss of 16-45 years of age. The Chemiluminescence immunoassay Analyzer was diagnosed (vidas and applied in Maglumi instrument). Maglumi devise for detecting IgG antibodies in sera samples by using IgG CMV Maglumi kits.

3-3-2-2-1 Diagnosis by One Step Torch Test

3-3-2-2-2Diagnosis by CMV Test

3-3-2-2-3 Diagnosis IgG-CMV by Chemiluminescence Immunoassay Analyzer

Table (3-6): Interpretation of seropositive and seronegative for CMV infection

Range value	Description
< 2 AU/Ml	Negative
≥ 2 AU/Ml	Positive

3-3-3 DNA extraction:

The DNA was extracted according to Favorgen DNA extraction company Kit and before the extraction two different solutions were needed to prepare in order to use in extraction which include:

1-Wash Buffer

For the preparation, 100ml of Ethanol (absolute) 96% to 100% has been added to wash -buffers container (when first open).

2-Proteinase K:

Proteinase K was prepared by adding 1ml of sterile distilled water to 10 mg of proteinase K container for making (10mg/ml) stocked solution , mixed it well by Vortex and ensured that proteinase K has been dissolved , storage of this solution at -20°C which then used to lyses the proteins.

3-Protocol of DNA Extraction:

Protocol DNA extraction was conducted through the following steps: as shown in (Figure3-2)

1. A 200µl of blood was transferred to microcentrifuge tubes (1.5 ml).
2. Added 20µl of proteinase K solution, and mixed with the pulse vortex and incubates at 58°C for 15 min.
3. Added 200µl of FATB buffer and mix with a vortex, then at room temperature left for 2min.
4. A 200 µl of ethanol (96-100%) was added and mixed well by vertexing for 10 seconds.
5. The spin columns were placed on collection tubes, and then the specimens were transferred into these columns. Full speed of centrifuge was use for 1 min at 13000 rpm.

6. A total 400 µl of W1 Buffer was added to the spin column. centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough. The spin column was placed back to the collection tube, the flow was discarded.
7. A 600 µl of Wash Buffer was added to the TG Mini Column.
8. Centrifuged at 11,000 rpm for 30 seconds and discarded the flowthrough, and the spin column was placed back to the collection Tube, centrifuged at full speed (12000 rpm) for an additional 3 min to dry the column.
9. The spin column was placed to a Elution Tube.
10. 100µl of elution buffer was used and left for 1 min at room temperature, then centrifuged at 13000 rpm for 1min.
11. The flow was collected after centrifugation into a sterile Eppendorf tube.
12. Spin columns were removed and the DNA was stored at - 20C° until used.

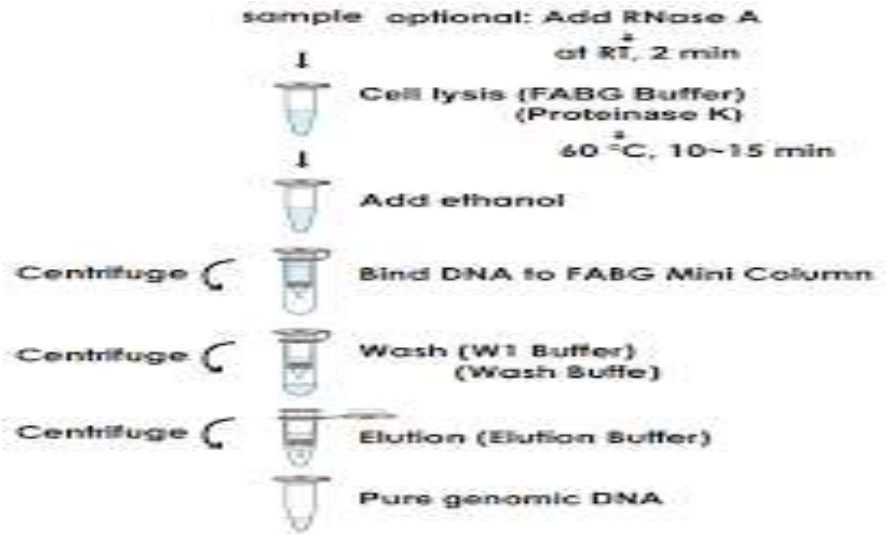


Figure (3-2) DNA Extraction Strategy (as to the instruction of manufactured company)

3-3-4 DNA Purity and Concentration Estimates:

The DNA intensity for all specimens is evaluated by placing (1µl) of the collected DNA for concentration detection (mg/µL) using the nanodrop spectral and observing clarity by noting the optical density ratio (OD) (260/280)nm to detect the purity of specimen's protein.

3-3-5 Polymerase Chain Reaction Technique

Used to improve PCR strategy and monitor the target genes ((IL6, IFNAR2, TLR9) of patients

3-3-6 Primers:

Three primer pairs were used to amplified targeted genes which associated with the recurrent miscarriage as shown in (Table 3-7).

Table (3-7) Set of Primers Used in Investigations

Name	Sequence (5'…….3')	Product Size(bp)	Reference
TLR9 F	TCATTCAGCCTTCACTCAGA	644bp	(Carvalho,*et al*,2008)
TLR9 R	CACATTCAGCCCCTAGAGGG		
IFNAR2F	GGCGCTAGACTAGATGTCATGG	470bp	Designed in this study (NCBI blastn)
IFNAR2R	AGGCTTGCCCTCAGAGTAAAA		
IL6Forwad outer primer	GACATGCCAAAGTGCTGAGTCACTAA	302bp	Designed in this study (Tetra arms PCR designed Tool)
IL6Reverse outer primer	GAATGAGCCTCAGACATCTCCAGTCC TA		
IL6Forward inner primer (G allele)	GCACTTTTCCCCCCTAGTTGTGTCTTC CG	206bp	
IL6Reverse inner primer (C allele)	ATTGTGCAATGTGACGTCCTTTAGCT TG	152bp	

3-3-6-1 Primer Preparation

The primers were synthesis in Macrogen the company (Korea). 100 pemole was prepared by added 300 µl of nuclease free water as stock solution), 10 pemole of each were performed by get10µl from stock solution of primers under interest and mixed with (90µl) nuclease free water the primer was kept at4c°.

3-3-6-2Amplification of Targeted Genes:

Utilized enhance the PCR technique and analyze the target site of DNA samples using specific primers (IFNAR2, TLR9) to determine a possible correlation between genetic mutation of genes and cytomegalovirus (CMV) infection in suffer women recurrent miscarriage.

3-3-7 PCR Reaction Mixture

The reaction mixture for gene polymorphism was formed by the following constituents, shown in Table (3-8).

Table (3-8): Contents of the Reaction Mixture (Promega) of PCR for gene Polymorphism.

Contents of reaction mixture	Volume µl
PCR PreMix	12
Forward primer*	1.5
revers primer**	1.5
Nuclease free Water (Promega)	8
Patient DNA template	2
Total volume	25

*Inner forward primer and inner reverse primer 1.5 µL for each in IL6 PCR.

3-3-7-1 PCR Condition for Amplification of the IL6 Gene Polymorphism

The PCR reaction as shown in Table (3-9).

Tables (3-9): Thermal Cycling Conditions for Primers IL6 Gene under Interest:

Primer pair for IL6			
Step Type	**Temperature °C**	**Time**	**Cycling**
Initial Denaturation	**95**	**3 min.**	**1**
Denaturation	**95**	**40 Sec.**	**30**
Annealing	**61**	**40 Sec.**	
Extension	**72**	**40 Sec.**	
Final Extension	**72**	**3 min.**	**1**

3-3-7-2 PCR Condition for Amplification of the IFNAR2 Gene Polymorphism

The PCR reaction is shown in Table (3-10).

Tables (3-10): Thermal Cycling Conditions for Primers IFNAR2 Gene under Interest:

IFNAR2			
Step Type	**Temperature °C**	**Time**	**Cycling**
Initial Denaturation	**95**	**3** min.	**1**
Denaturation	**95**	**40** Sec.	**30**
Annealing	**55**	**40** Sec.	
Extension	**72**	**40 Sec.**	
Final extension	**72**	**3 min.**	1

3-3-7-3 PCR Condition for Amplification of the TLR9 Gene Polymorphism

The PCR reaction is shown in Table (3-11).

Tables (3-11): Thermal Cycling Conditions for Primers TLR9 Gene under Interest

TLR9			
Step Type	**Temperature °C**	**Time**	**Cycling**
Initial Denaturation	**95**	**3 min.**	**1**
Denaturation	**95**	**40 Sec.**	**30**
Annealing	**57**	**30 Sec.**	
Extension	**72**	**30 Sec.**	
Final extension	**72**	**3 min.**	**1**

3-3-8 PCR Product Analysis by Electrophoresis

The PCR products of IL6, IFNAR2 and TLR9 genes were analyzed by agarose gel electrophorese in the following steps:

1. Dissolved the 1.5g of agarose powder into 100 ml of 1x TBE buffer in the microwave at 100C° for 15 minutes, after that, left to cool 50C°, then added 2µl of ethidium bromide stain.
2. The agarose gel solution was carefully poured into the tray after the comb was fastened for the preparation of holes and solidified for 15 minutes at room temperature to loading the DNA sample and after that was removed the comb gently.

3. In one well-added 5µl from DNA ladder, and 6µl from PCR product was added into each combs well.
4. The chamber for electrophoresis was packed with a 1x TBE buffer. Then electric current was performed at 100 volts for 45 minutes.
5. The UV transilluminator was used to image the PCR products

3-3-9 DNA Sequencing Method

DNA sequencing method was performed for genotyping detection of IFNAR2 and TLR9 polymorphisms according to the following step:

1. PCR products were purified by wizard® SV gel cleaning system and PCR to get rid of extra nucleotides and primers for genes (IFNAR2 andTLR9 polymorphism), that 60 samples were sent to Macrogen Korea and encoded with D for IFNAR2 samples and M for gene samples TLR9 in ice bag for DNA sequencing.
2. Identified IFNAR2 andTLR9 polymorphisms genes genotypes were submitted into of NCBI-GenBank to get GenBank accession number, and the sequences for the molecular IFNAR2 andTLR9 genes was matched with the gene sequences at the national centre for biotechnology information (NCBI-Blastn).
3. The molecular IFNAR2 andTLR9 polymorphisms genes analysis compared the observed DNA sequences of local samples with the retrieved neighbouring DNA sequences of the NCBI Blastn engine, then using Bioedit for multiple alignment sequences based on Editor software version 7. 2 (DNASTAR, Madison, WI, USA).
4. The identified SNPs were submitted for verification to the dbSNP database, and each SNP was re-positioned depending on its location in the reference genome, then determined the SNP by checking its related dbSNP location.

3-3-2-2-9 Statistical Analysis

The allele and genotype frequencies were dictated by direct counting. The Chi $\chi 2$ test was utilized to analyze allele and genotype frequencies in CMV infected women with recurrent miscarriages and control subjects. The Odd Ratio (ORs) and 95% confidence intervals (CIs) were determined with SPSS.

Chapter Four

Results and Discussion

4-1 Distribution of the studied groups

The 64 blood samples were obtained from women who experienced recurrent miscarriages and 50control group. Among the miscarriage and control women, the miscarriage women showed the highest percentage of seropositive to CMV for IgG (78.1%).

There is a significant difference between two groups of patients for CMV antibodies, these results are in accordance with Gaytant *et al*. (2002) and Munro *et al*. (2005), that studied the importance of virus infection to cause miscarriage

Toxoplasmosis, other infection, Rubella, Cytomegalovirus infection and a Herpes simplex (TORCH) infections are considering the common causes of congenital infections and can lead to an adverse outcome which are initially asymptomatic such as an embryo losing, stillbirth, and congenital defects of the child (Shashi *et al*., 2004). In other terms, microbial agents of TORCH can cross the placenta and infect the fetus during any trimester of pregnancy (Devi *et al*., 2008).

4-2 Sociodemographic properties of pregnant women

A total of 64 women with RM enrolled in this study, which were suffered from previous miscarriages, their ages ranged from 16-45 years old with different gestational age as illustrated in Table (4-1). 29.6% (19/64) of these women were employed whereas the rest 70% (45/64) were unemployed (Housewife), for TORCH infection CMV appeared in the highest percentage 78.1% (50/64) of infection followed by Toxoplasmosis 25% (16/64), then Rubella15.6% (10/64), herpevirus10.9% (7/64) that observed in the lowest percentage of infection because of the scanty of IgM positive result for CMV and there

are no IgM for Toxoplasmosis, herpesvirus and rubella, as shown in table (4-1 and 4-2).

Table (4-1): Some Sociodemographic Properties of Women with RM (n=64)

Properties	Variables	Number	%
Age groups (years)	16-25	23	35.9
	26-35	26	40.6
	36-45	15	23
Occupation	Housewife	45	70
	Employed	19	29.6
Miscarriage	Two	33	51.5
	Three	16	25
	More of three	15	23

Table (4-2): Seropositivity for TORCH infection in women with recurrent miscarriage.

Pathogenic agents (TORCH)			
CMV (IgG)	+Ve	50	78.1
	_Ve	14	21.9
Rubella (IgG)	+Ve	10	15.6
	_Ve	54	84.4
Herpesvirus (IgG)	+Ve	7	10.9
	_Ve	57	89.1
Toxoplasma (IgG)	+Ve	16	25
	_Ve	48	75

These findings coincided with the findings of a previous study which conducted in Serbia, which reported the highest seropositivity for CMV 96.2% and lowest for *T. gondii* 24.1% (Pribakovic *et al*., 2019). They also coincided with the findings of (Sen *et al*.,2012; Kakayi, Haji and Al-Daoody,2021). Another study in Iraq reported lower rates of infection, (10.87%) for CMV, followed by T. gondii4 (2.17%) and Rubella virus

(1.09%) (AL-Saeed *et al.*,2015). In Northern part of India, they reported positive cases of toxoplasmosis, Rubella virus and CMV which were 19.4%, 30.4% and 34.7%, respectively. Contradicted results were also obtained in some developed countries. For instance, (Han, 2008; Kakayi *et al.*, 2021) showed only 3.7% total seropositivity for toxoplasmosis in Korea and pointed out that in Europe and America relatively higher rates were recorded.

As it is clear from the results of this section, that different results were obtained from different investigations and may be due to the location or country in which study took place and the difference in the number of enrolled pregnant women. Furthermore, other reasons for different and similar seropositivity of TORCH infections in miscarriage women from the area to area might be due to the hygienic habits, culture differences related to feeding habits, education level, primary health care program and early diagnosis of infections (AL-Saeed *et al.*, 2015). The association between seroprevalence rates of anti-CMV IgM and IgG with repeated abortion is a real and present danger to pregnancy complications. CMV is the most frequent source of congenital infectious diseases; more than 70% within the first three months of pregnancy which can result in abortion (Vasani and Kumar, 2019).

4-3. Seroprevalence of CMV in Women with RM According to age

The age of all patients' women ranged from (16 to 45) years old are shown in Table (4-3):

Table (4-3): Incidence of CMV infected women with recurrent miscarriages according age

Age group/ years	No.	Percentage%
16-25	17	34%
26-35	23	46%
36-45	10	20%

The age (26-35) years old may be rearranged as the most age class which showed high prevalence of anti- HCMV antibodies IgG which represent 46% , while the age classes (16-25) years old showed the least prevalence of anti- HCMV IgG antibodies 34% As for the age classes (36-45) years old showed the least prevalence of anti- HCMV IgG antibodies 20% significant difference .This result considered to be comparable with Yasir (2012), who showed higher percentage of positively at ages (27-32) also Sotoodeh (2009) who showed 94% of positively at ages (25-34), this is because the chance of pregnancy is higher in younger ages (20-30) years than (31-40) years. These results considered to be comparable with Mahdi *et al.*, (2011), who showed that an increase in seropositive CMV IgG in relation with abortion and infection, this might be due to the effect of CMV on cellular metabolism and activation of other viruses that co-infect the cells inducing subclinical inflammation. Some studies found a higher risk of pregnancy loss with CMV infection (Griffiths and Baboonian, 1984) and other found high presence of CMV antigens in tissues from abortion (Spano *et al.*, 2002), despite these reports on the role of CMV infection in spontaneous pregnancy loss, the role of CMV infection in recurrent losses is less clear (Sherkat *et al.*, 2014).

In another hand the results of AL-Azawy *et al*. (2022), showed no significant between RPL group and control group.

4-4 Distribution of Patients According to Blood Groups

The ABO blood groups of the patients with Cytomegalovirus was determined in addition to the control group, then its compered to determine if there was a relation between the blood group and Cytomegalovirus infections as shown in Table (4-4).

Table (4.4): Incidence of CMV infected women with recurrent miscarriages according blood Group

Blood groups	Patients' infection with cytomegalovirus	%
A+	6	12
A^-	0	0
B^+	6	12
B^-	0	0
AB^+	4	8
AB^-	0	0
O^+	33	66
O^-	1	2
Total	50	100%

The most common blood group found in the patients infected with Cytomegalovirus was "O^+" with a percentage of 66%. The blood group phenotypes, "B^+" with the percentage (12%) in Cytomegalovirus patients, then it's followed by the blood group "A^+" with the percentage (12%). Finally, the blood group "AB^+" had the percentage (8%) for Cytomegalovirus patients respectively Figure (4-1).

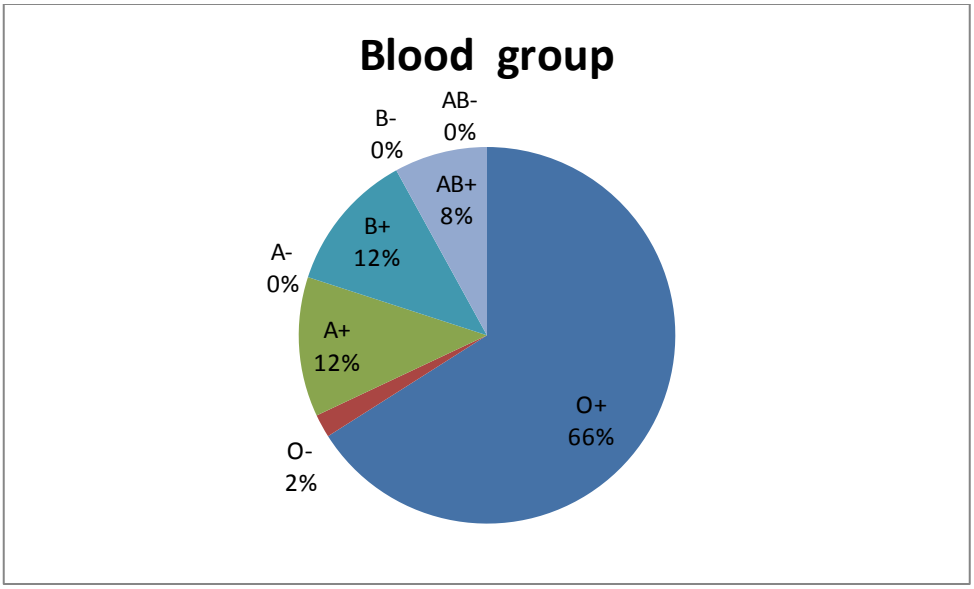


Figure (4-1): Distribution of the Patients Infected with Cytomegalovirus According to Blood Groups.

In this study, infection was appeared in patients that have blood group O^+ that mean blood group O^+ may have high risk is more than another group.

This result was differed with the previous study conducted by Jasim (2020) in Babylon City, while the findings from this research study consistent from the previous study advanced by Al Taie *et al.* (2018) in Iraq.

This result was contradicted to the previous study done by Freeman *et al* (2004), while the study done by Varga *et al.* (2011), did not find a significant difference between CMV seroprevalence with different blood groups.

According to Babker (2020) in Sudan, the rate of infection was high in blood type O compared to other blood groups.

Blood groups can play a direct role in infection by serving as receptors and/or co-receptors for microorganisms, parasites, and viruses. (Cooling, 2015).

These antigens may be interacting with virus envelope and this interaction leads to inhibition of fusion of virus envelope to cell target membrane (Banerjee and Mukhopadhyay, 2016).

4-5 Distribution of CMV Infection According to Miscarriage number

A correlation was recorded between CMV and the number of miscarriages with the highest rate of 50% (25/50) in case of two number of miscarriages, whereas correlation was recorded between CMV infection and the number of miscarriage Table (4-5).

Table (4-5): Prevalence of CMV According to Miscarriage:

TORCH / NO. OF Miscarriage	CMV	
	Number (%) for CMV+	Number (%) for CMV-
TWO	25(50)	8(12.5)
Three	12(24)	4(8)
More than three	13(26)	2(4)
Total	50	14

The study showed that, 50% of aborted women have only two miscarriage and 24% have 3 miscarriages while 26% more than three miscarriages , where cases miscarriage in the 1st trimester of pregnancy and nearly 1 third of cases haven't children yet.

Contradicted results were also obtained study Basra was and declared that most of the miscarriages due to TORCH infection caused by *T. gondii* 60% (Dawood, 2019), while these results were in disagreement with a study performed by (Al-Saeed *et al*., 2015) who investigated to determine the effect of TORCH agents on pregnancy outcome, and recorded 102 (55.4%), 50 (27.2%), 27 (14.7%) and 5 (2.7%) for the number of single, twice, triple, four and more miscarriages, respectively. *T. gondii* caused abortion either inhibits or triggers the apoptosis with excessive levels of Th1 cytokines, particularly IL-18 and IFN- γ (Nishikawa *et al*., 2002), and Rubella virus can reach the placenta and cause primary infections, which lead to spontaneous abortion (Chow *et al*., 2006). Whereas the mechanism by which CMV lead to miscarriage is not clear (Giakoumelou *et al*., 2016).

4-6 Molecular Study

Many cytokines appear to be important in immune control of CMV infection, although defining cytokines that may correlate either with protection or increased susceptibility to cytomegalovirus infection (Schleiss, 2013). In this study, three selected genes were studied that play a significant role in causing miscarriage in pregnant women. Interleukin 6, IFNAR2 and Toll Like Receptor 9 are among the genes that have been detected by using Tetra arm PCR Technique and sequencing techniques.

Cytokines and chemokines are the first line of defense against viral infections (McSharry *et al*., 2012). High post-transplant proinflammatory cytokine levels have been associated with the risk for developing CMV infection (Kato *et al*., 2014). The studies demonstrated that cytokine gene polymorphisms result in inter-individual differences in cytokine production (Mitsani *et al*., 2011).

Therefore, there are increasing evidences indicating that polymorphisms in genes coding for cytokines or chemokines and their receptors may modulate the susceptibility to, as well as the dynamics and outcomes, of CMV infections (Vallejo *et al*., 2022).

4-6-1Results of Interleukin 6 Polymorphism by Tetra ARMS PCR Technique

In this study, the PCR analysis yielded results that indicate the presence of G and C alleles, with three distinct genotypes (GG, GC, and CC) and varying lengths (302, 206, and 152) bp, as illustrated in (figures 4-2 and 4-3). The frequencies of the rs1800795 G>C polymorphism was found to be 46% and 24% for the homozygous genotypes GG and CC, respectively, in the patient group, and 30% for the heterozygous genotype GC. The healthy control group, on the other hand, exhibited polymorphic alleles GG, GC, and CC at frequencies of 80%, 6%, and 14%, respectively, as presented in (Table 4-6). These findings suggest a potential association between the rs1800795 G>C polymorphism and disease susceptibility. Additionally, the stratified analysis revealed a strong and significant difference between the patient and control groups.

Table (4-6) Genotypes and allele frequencies of IL6 − 174 G>C (rs1800795)

Model	Genotype	Case (50)		Control (50)		p-value	OR (95 and CI)
		N0.	%	N0.	%	Reference	
Codominant	GG	23	46	40	80		
	GC	15	30	3	6	**<0.001***	0.11(0.03-0.44)
	CC	12	24	7	14	**0.04***	0.33(0.11-0.97)
Dominant	GG	23	46	40	80	**Reference**	
	GC+CC	27	54	10	20	**<0.001***	0.21(0.08-0.51)
Recessive	CC	12	24	7	14	**Reference**	
	GC+GG	38	64	43	86	0.25	1.80(0.64-5.06)
Over-dominant	GC	15	30	3	6	**Reference**	
	GG+CC	35	70	47	94	**0.002***	6.71(1.80-24.99)
Alleles	G	61	61	83	83	**Reference**	
	C	39	39	17	17	**<0.001***	0.32(0.16-0.61)

Significant Statistically p < 0.05

CI: Confidence Interval; OR: Odd ratio

Recurrent miscarriage is a prevalent affliction that afflicts women, and it poses a significant issue for reproductive health as it affects roughly one in 300 pregnancies (Giakoumelou *et al*., 2016). The multifaceted nature of recurrent miscarriage requires an investigation into various factors to determine the underlying causes of this condition. These factors encompass coagulation factors, infection factors, immunological factors, anatomical issues, and chromosomal abnormalities (Soheilyfar *et al*., 2019). Some genetic-related factors, such as genetic polymorphisms,

have been linked to poor pregnancy outcomes, including the occurrence of recurrent pregnancy loss (RPL). Research has suggested that cytokines, which regulate immune responses, play a crucial role in maintaining a healthy pregnancy. Therefore, it is important to understand the genetic and immunological factors that contribute to RPL in order to develop effective strategies for its prevention and management (Makhseed *et al.*,2001). The presence of IL-6 and IL-10, which are anti-inflammatory cytokines, is considered crucial for maintaining a healthy gestation period and acting as the primary defense mechanism against viral infections. Functional SNPs with impact on interleukin-6 expression are crucial in the pathogenesis of recurrent pregnancy loss at various stages. The significant outcome of the research indicated a positive correlation between IL-6 SNP and the risk of RPL. The IL-6 -174C/G polymorphism is situated 174 base pairs upstream of the promoter of the IL-6 gene, which may regulate IL-6 transcription or posttranscriptional modification. Genetic predisposition may have an impact on the probability of, susceptibility to, or chronicity of CMV infection, along with the pace of RPL progress (Rafiee *et al.*, 2016). Several research studies have been conducted to explore the association between different cytokine polymorphisms and recurrent pregnancy loss (RPL). In addition, several other studies and investigations have not been successful in identifying a link between the common polymorphisms in the IL-6 SNP and the risk of RPL (Torabi *et al.*, 2009). Our findings demonstrate a significant correlation between the risk of recurrent pregnancy loss and the presence of the C allele in the IL-6 -174 gene among the cases under study. In contrast to the findings of Pourroostaei *et al*. (2022), it has been observed that there exists no association between the risk of recurrent pregnancy loss (RPL) and interleukin-6 (IL-6) -174 single nucleotide polymorphism (SNP). The observed discrepancies in the outcomes of the

aforementioned studies may have been influenced by ethnic variances or the influence of patients' enrollment criteria. Interleukin-6, a type of cytokine, has been shown to play a protective role in the immune system's response to bacterial and viral infections. Pathogenic agents must penetrate the fetal tissues or endometrium's intrauterine environment to induce an inflammatory response, which may culminate in a miscarriage (Salimi *et al.*, 2020). In the present investigation, our findings have demonstrated a noteworthy correlation between recurrent pregnancy loss (RPL) and patients who are positive for cytomegalovirus (CMV) with allele C in IL-6 -174. Based on the outcomes observed, we suggest that allele C in IL-6 -147 can escalate the risk of RPL both directly and indirectly, with indirect impact being through the increase in CMV infection. The latter, in turn, might exert significant influence in the occurrence of RM in women.

The results of this study were successful amplification the targeted partial sequence of IL6 included rs1800795 polymorphism by Tetra-arm PCR for control and patient women. The profile gel electrophoresis of PCR products for detection of IL-6 (- 174G/C) gene polymorphism was shown three genotypes: C allele 152bp, G allele 206bp and outer primers 302bp, as shown in Figures (4-2), (4-3).

Control group:

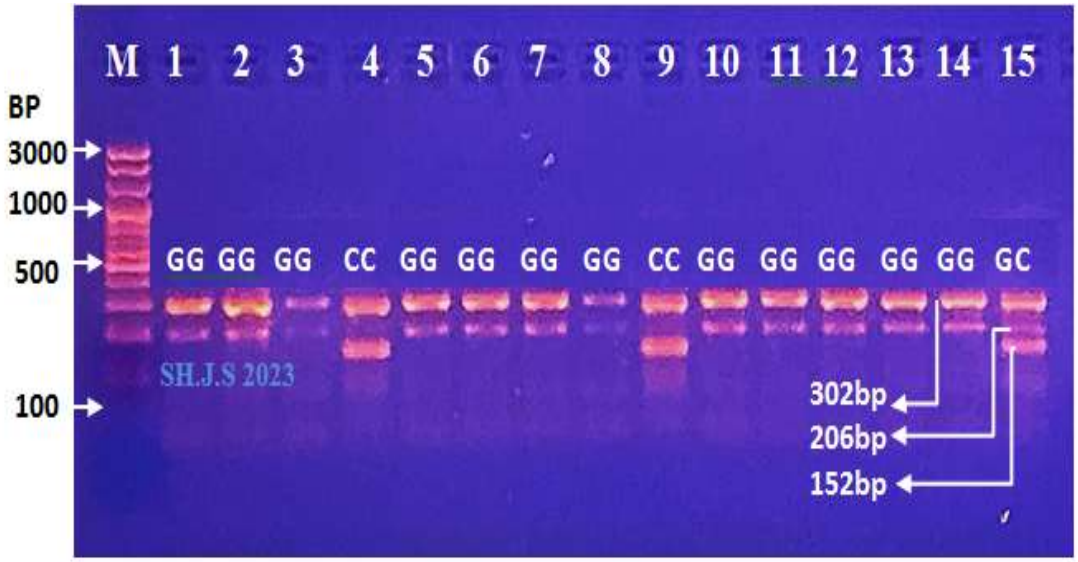


Figure (4-2): Electrophoresis of Arm- PCR product of IL6 gene included rs1800795 for Cytomegalovirus infected. Lane (15) heterozygote GC result (206bp). Lane (1,2,3,5,6,7,8,10,11,12,13,14) Homozygotes GG result (302bp) and Lane (4,9) Homozygotes CC result(152bp). 1.5% agarose, TBE buffer, Pre-stained by Ethidium Bromide. Time 45min./100 volt.

Patient group:

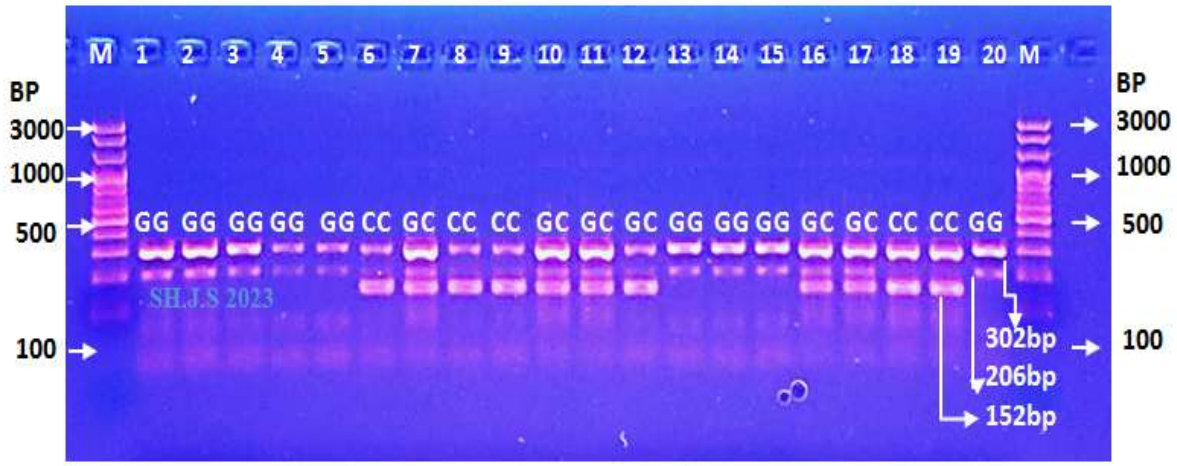


Figure (4-3): Electrophoresis for PCR product of IL6 gene for Cytomegalovirus infected. Lane (7,10,11,12,16,17) heterozygote GC result (206bp). Lane (1,2,3,4,5,13,14,15,20) Homozygotes GG result (302bp) and Lane (6,8,9,18,19) Homozygotes CC result(152bp). 1.5% agarose, TBE buffer, Pre-stained by Ethidium Bromide. Time 45min./100 volt.

4-6-2 Location of Interferon Alpha and Beta Receptor Subunit 2 (IFNAR2):

This IFNAR2 gene encoded the protein type I membrane protein that forms one of the two chains of a receptor for interferon alpha and interferon beta. Binding and activation of the receptor stimulates Janus protein kinases, The protein belongs to the type II cytokine receptor family. Mutations in this gene are associated with Immunodeficiency Figure (4-4).

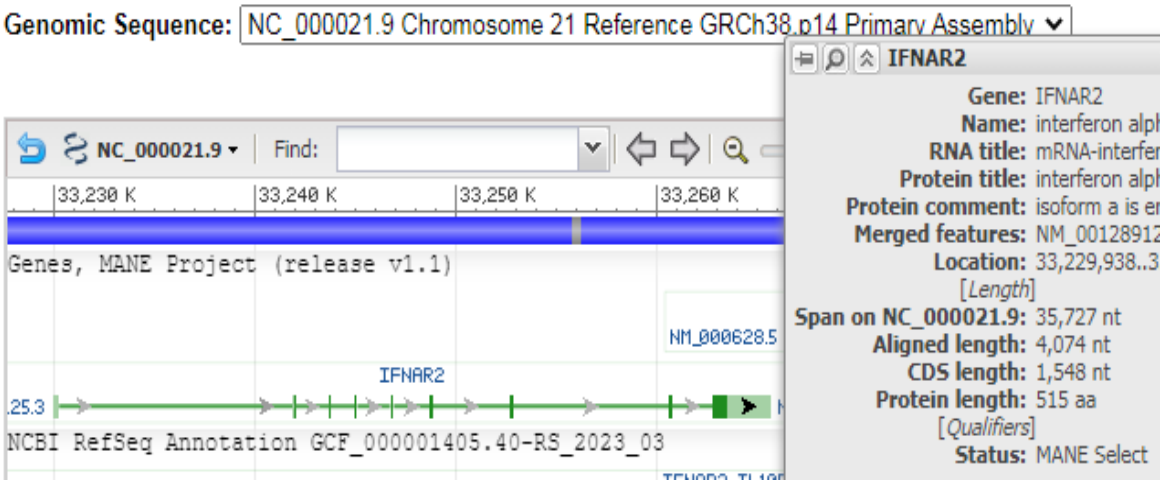
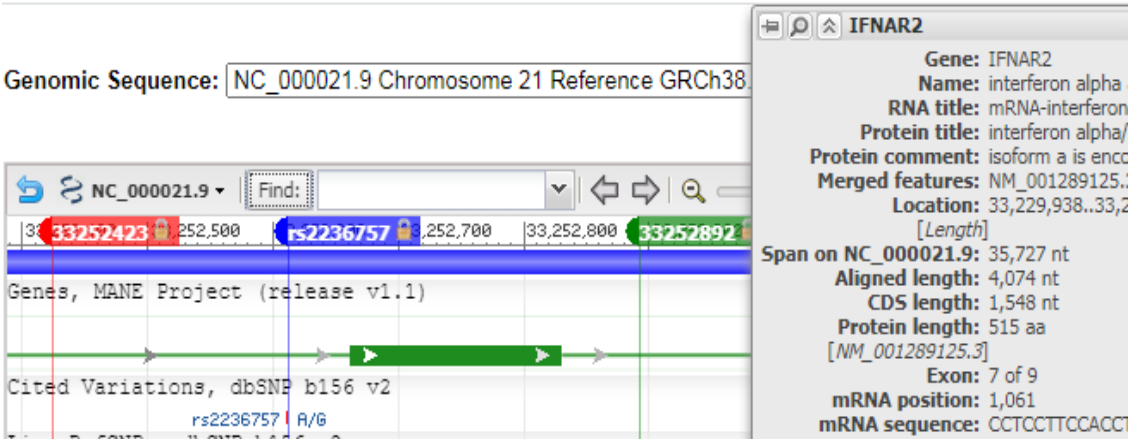


Figure (4-4): Location of IFNAR2 Gene on Chromosome 21.

4-6-2-1 Amplicon and primers covering SNP rs2236757A>G:

The Figure (4-5) illustrated site of SNP rs2236757A>G and rounded by forward primer site33252423 and reverse primer site 33252892 located on chromosome 21.



Figure(4-5) : Amplicon of partial sequence covering SNP rs2236757G>A scanted by sites of primer pair on chromosome 21.

4-6-2-2 Amplification of Partial Sequence of IFNAR2 Gene.

The result showed PCR products of amplicon included primer pair flanking targeted region of IRNAF2 gene. The PCR products of target of IRNAF2 gene showed 470bp for 22 patients with CMV infection and 16 control group as shown in (Figures (4-6) & (4-7)).

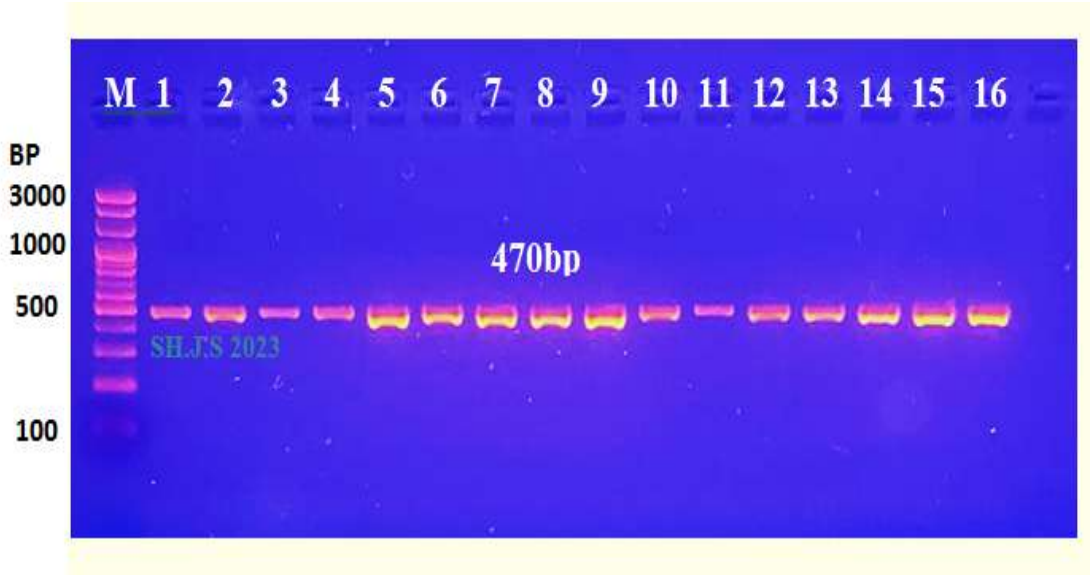


Figure (4-6): Electrophoresis of partial sequence of IFNAR2 gene PCR products on an agarose gel amplicon product in lane 1–16 refer to the control samples, M refers to the DNA size marker (100bp), the Volte of electrophoresis is 100 V for 45 minutes, the concentration of agarose is 1%, the size of bands is (470bp).

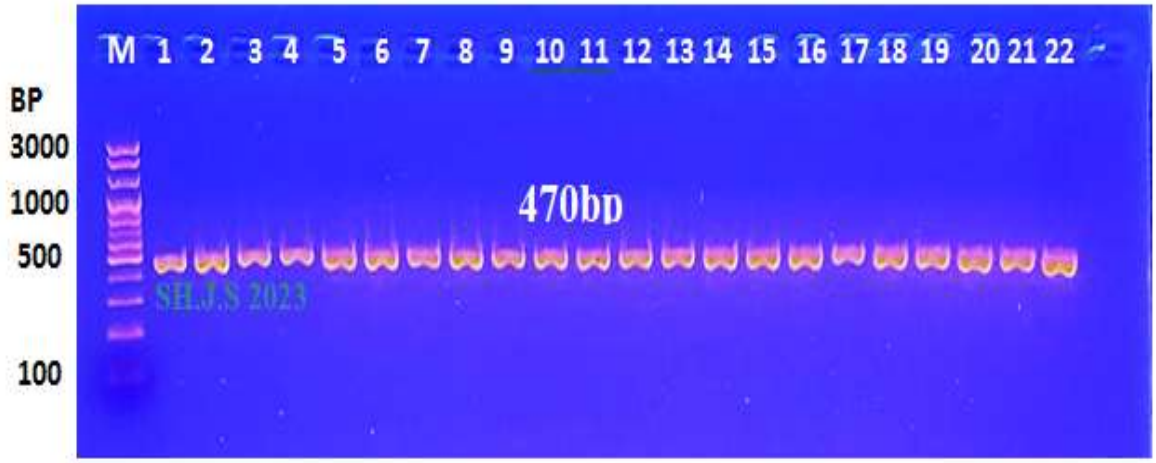


Figure (4-7): Electrophoresis of partial sequence of IFNAR2 gene PCR products on an agarose gel amplicon product in lane 1–22 refer to the patient samples, M refers to the DNA size marker (100bp), the Volte of electrophoresis is 100 V for 45 minutes, the concentration of agarose is 1%, the size of bands is (470bp).

4-6-2-3 Polymorphism of IFNAR2 Gene

The targeted region of IFNAR2 gene was shown three genotypes in SNP rs2236757 A>G, they are AA, AG and GG. The multiple alignment of chromograms sequence lines for patients and control group as shown in Figure (4-8) and (4-9).

4-6-2-4 Multiple Alignments of Chromatograms for Patients and Healthy Groups:

The results of multiple alignment of patients with CMV covering partial sequence of targeted region 33252324-33252892 was shown one SNP rs2236757 A>G. the results were shown high frequent of G risk allele correlated with disease as shown in Figures (4-8) & (4-9).

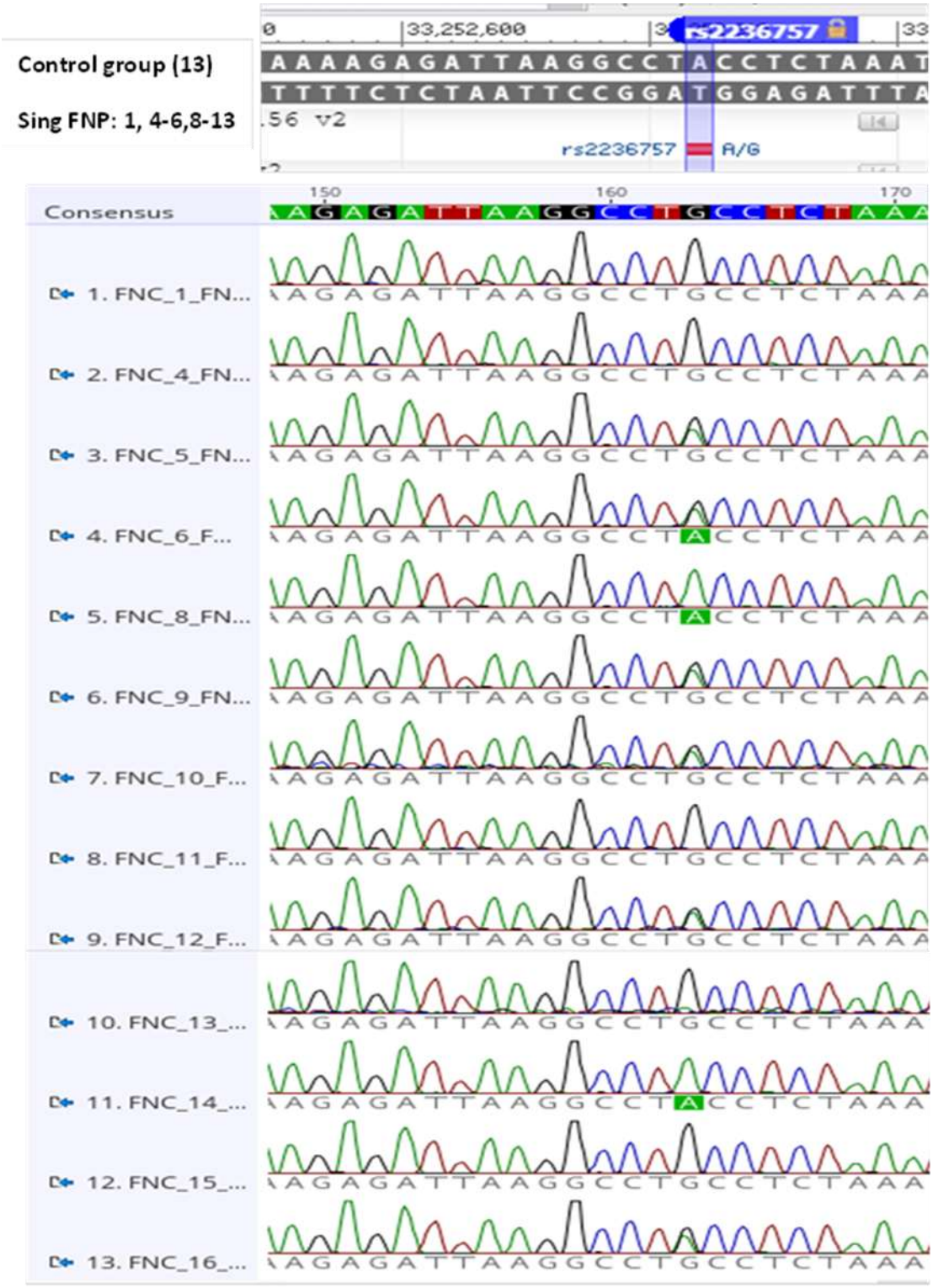


Figure (4-8): Multiple alignment of chromatograms sequence of IFNAR2 gene in healthy women for detection polymorphism in SNP rs2236757.based on genius prim software.

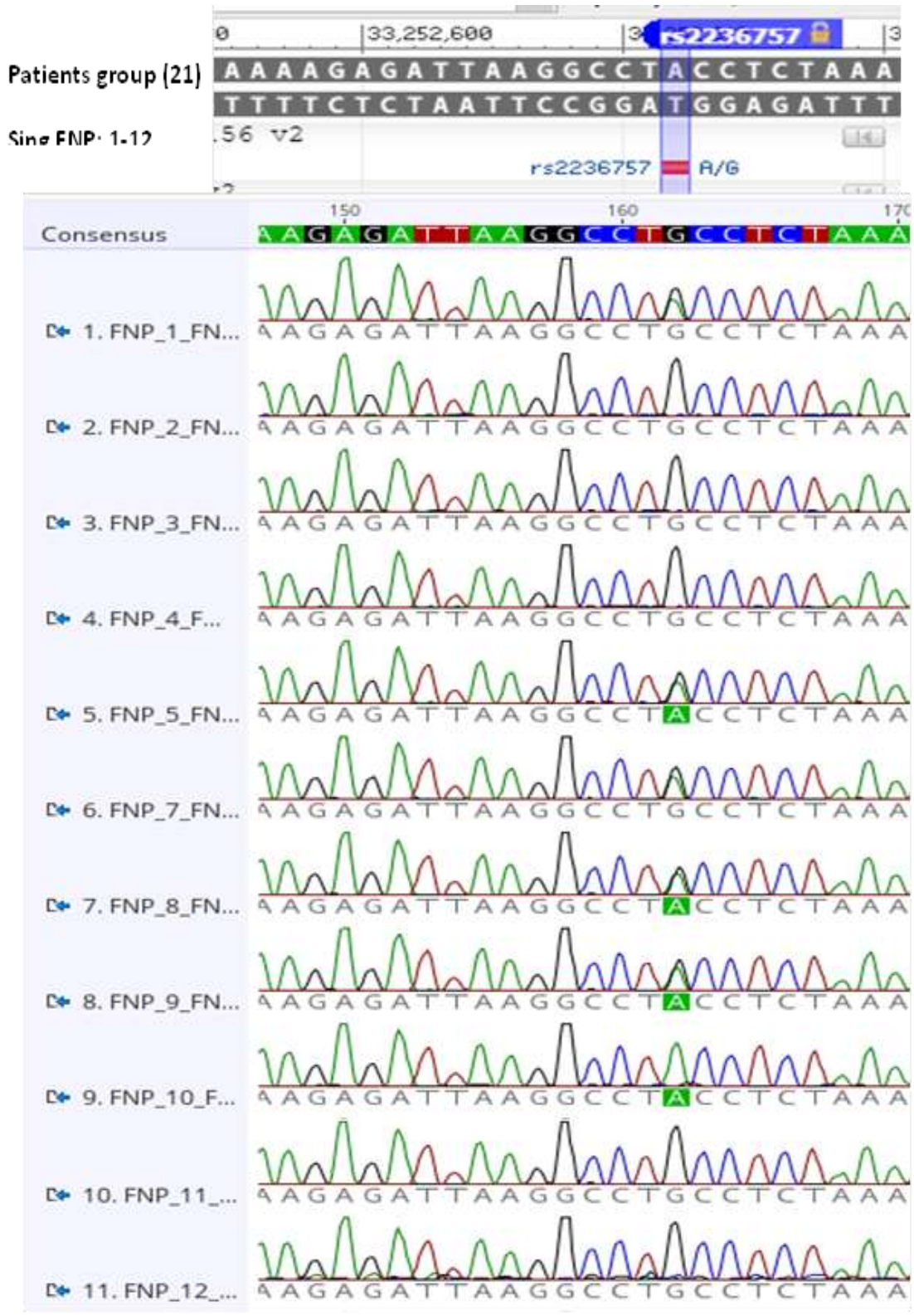


Figure (4-9): Multiple alignment of chromatograms sequence of IFNAR2 gene in miscarriage women for detection polymorphism in SNP rs2236757.based on genius prim software.

The results showed three genotypes: AA, AG and GG showed more distribution in patients undergo CMV infection compared with control group, the values of Odd Ratio (OR) were supporting that G allele in GG was considered as risk allele. The Odd Ratio was higher in genotype GG: OR= 1.4(0.68-2.9), and the allele frequency was higher in with G allele 21 (61.7%) in patient group with high value of OR=1.4(0.68-2.9), p.value = 0.3, while the allele frequency low 34 (16.7%) in control group Table (4-7).

In the study, IFN-ɣ +874 gene polymorphism this result was matched to the previous study done by Vu *et al*. (2014) and Mitsani *et al*. (2011).

Our results were consistent with other studies had shown the same results of other diseases such as Tuberculosis (Wei *et al*., 2017), Bipolar 1 Disorder (Nayeri *et al*., 2019), chronic hepatitis B (Kadi and Monem, 2017).

Table (4.7): Genotype and Allele frequencies of IFNAR2 gene polymorphisms among patients with Cytomegalovirus and healthy women's

Genotypes rs2236757 A>G	**Patients 30**		**Control 30**		**P-value**	**OR =95% CI**
	NO.	**%**	**NO.**	**%**		
AA	6	20	7	23.3	Reference group	
AG	9	30	12	40	0.3	0.6(0.22-1.8)
GG	15	50	11	36.7	0.7	**1.4(0.68-2.9)**
Alleles frequency	**NO.**	**%**	**NO.**	**%**	**P-value**	**OR =95% CI**
A	21	61.7	26	83.3	0.3	0.7(0.3-1.4)
G	**39**	**38.3**	34	16.7	0.3	**1.4(0.68-2.9)**

4-7-3 Location of Toll like Receptor 9 (TLR9):

The TLR9 gene was encoded the protein of member of the Toll-like receptor (TLR) family, which plays a fundamental role in pathogen recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) that are expressed on infectious agents, and mediate the production of cytokines necessary for the development of effective immunity. The location of TLR9 was illustrated in Figure (4-10).

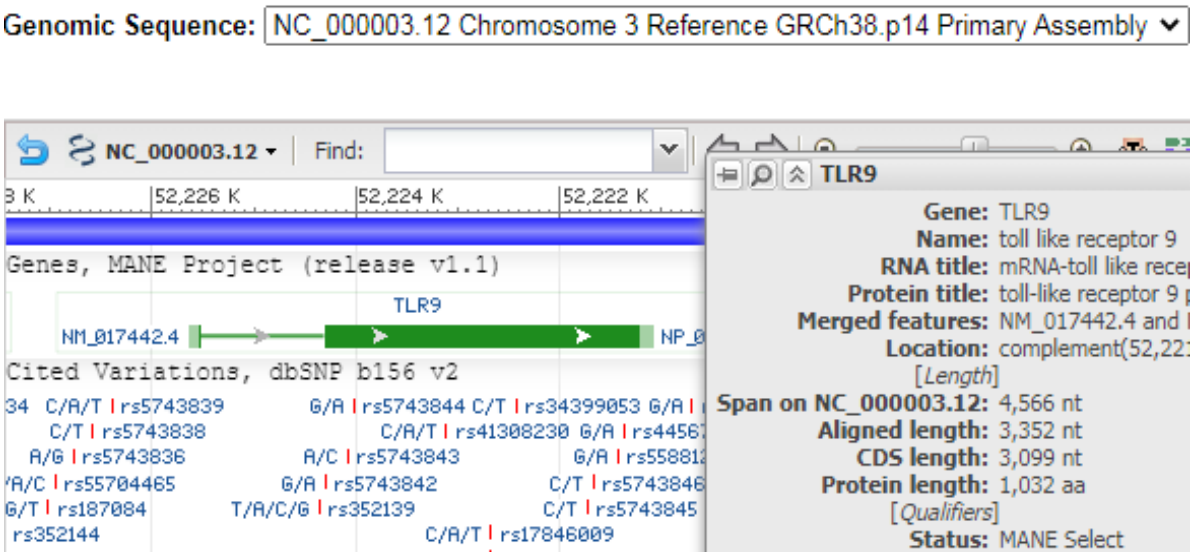


Figure (4-10): Amplicon of partial sequence covering SNP rs187084 T>C on downstream DNA strand scanted by sites of primer pair on chromosome 3.

4-7-3-1 Amplicon and primers covering SNP rs187084A>T:

The Figure (4-11) illustrated site of SNP rs187084 T>C on downstream DNA and rounded by forward primer site52226517 and reverse primer site 52227160 located on chromosome 3.

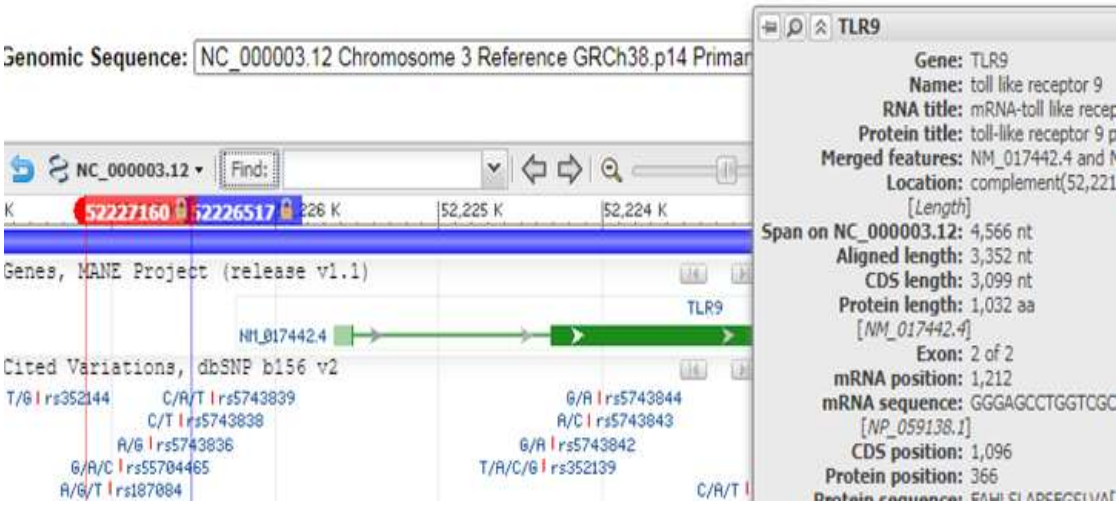


Figure (4-11): The location of TLR9 gene on chromosome 3.

4-7-3-2 Amplification of Partial Sequence of TLR9 Gene.

The result shows PCR products of amplicon included primer pair flanking targeted region of TLR9 gene. The PCR products of target of shown 644bp for 22 patients with CMV infection and 16 control group as shown in Figures (4-12) &(4-13).

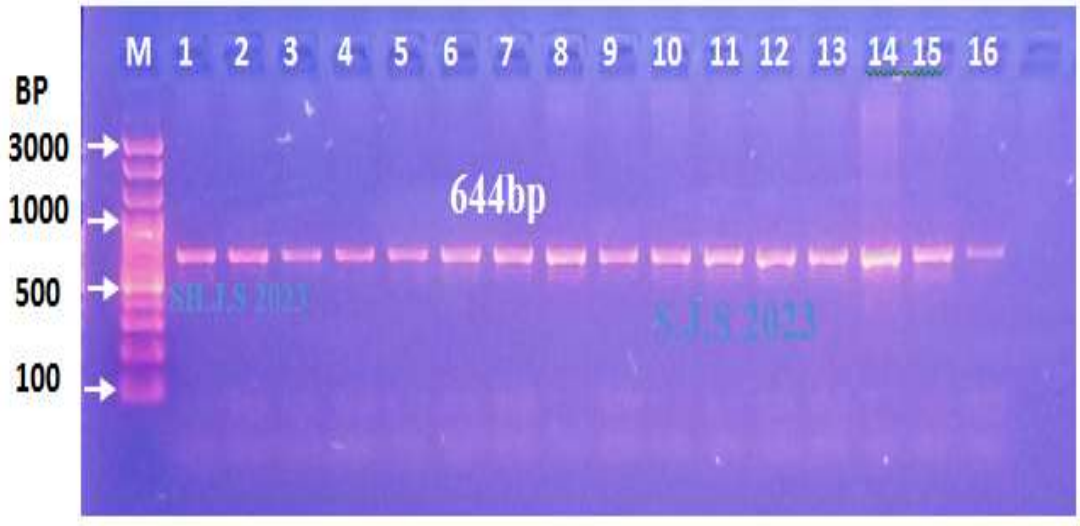


Figure 4-12: Electrophoresis of TLR9 gene PCR products on an agarose gel amplicon product in lane 1–16 refer to the healthy samples, M refers to the DNA size marker (100bp), the Volte of electrophoresis is 100 V for 45 minutes, the concentration of agarose is 1%, the size of bands is (644bp).

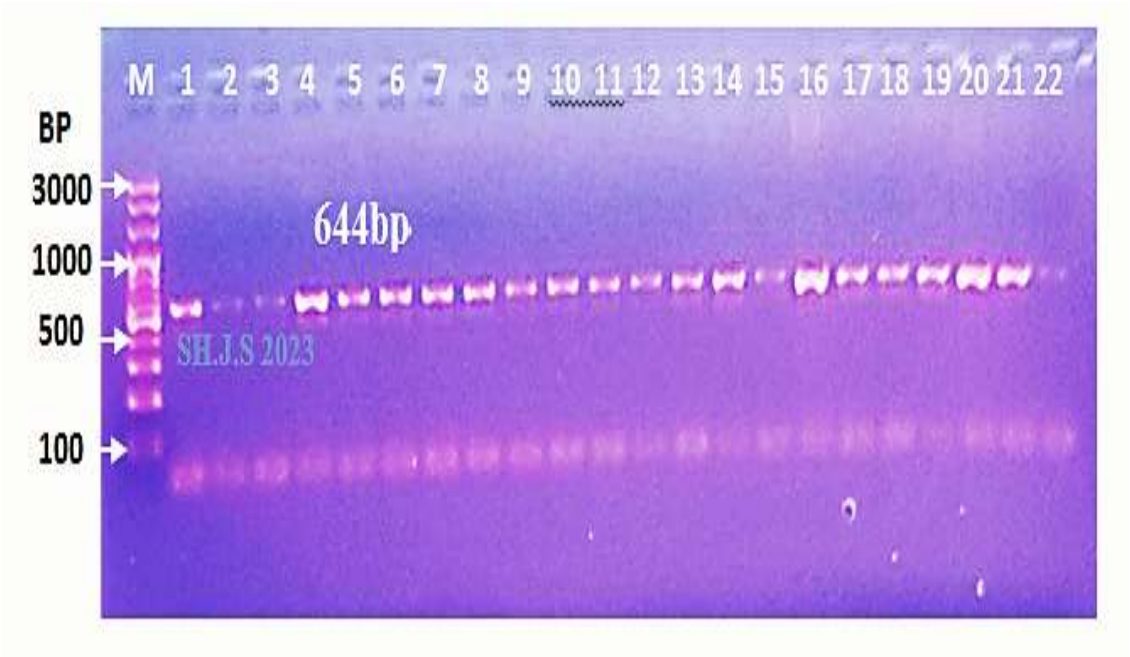
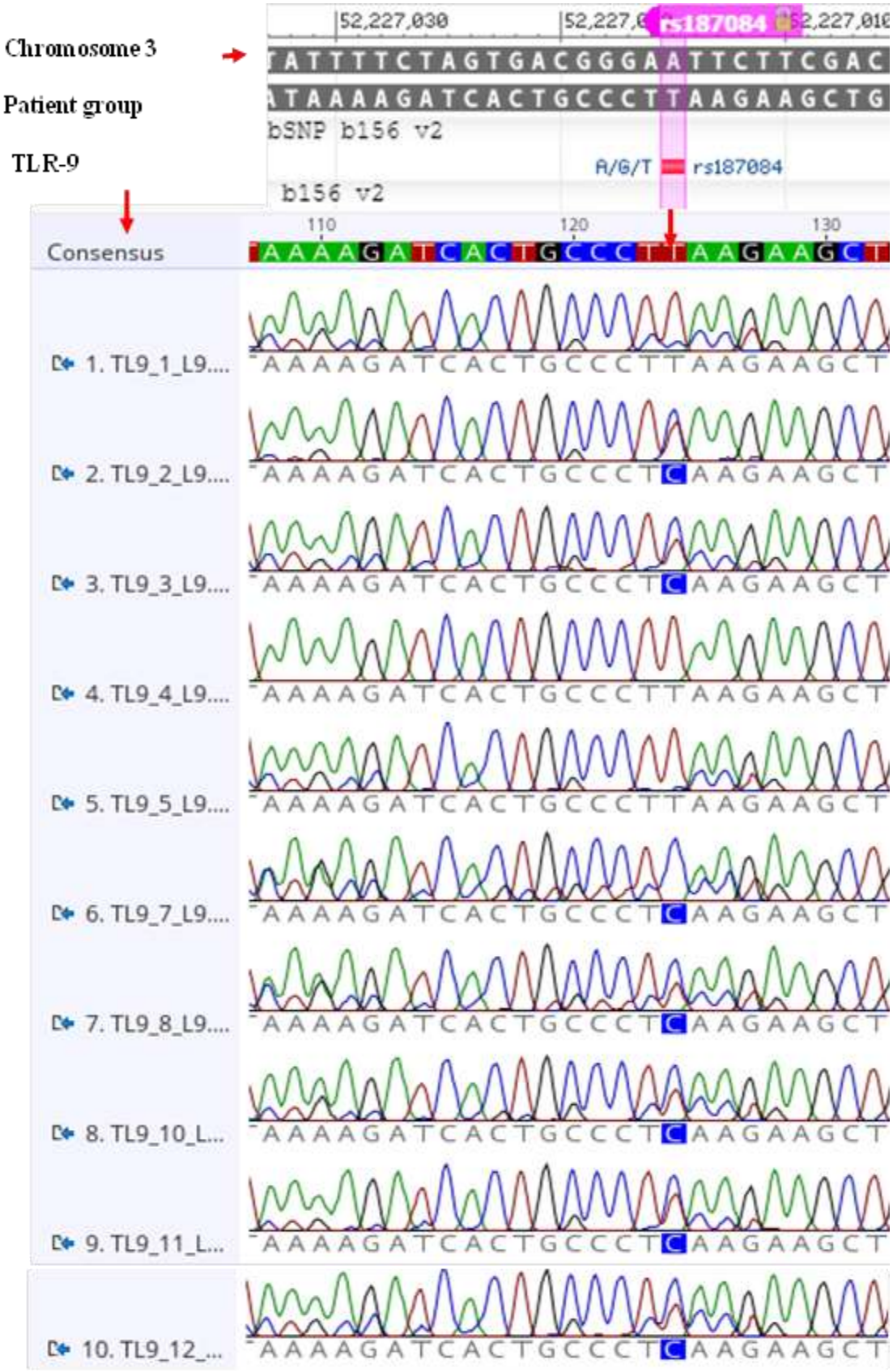


Figure (4-13): Electrophoresis of TLR9 gene PCR products on an agarose gel amplicon product in lane 1–22 refer to the patient samples, M refers to the DNA size marker (100bp), the Volte of electrophoresis is 100 V for 45 minutes, the concentration of agarose is 1%, the size of bands is (644bp).

4-7-3-3Multiple Alignments of Chromatograms for Patients and Healthy Groups:

The results of multiple alignment of patients with CMV covering partial sequence of targeted region 52226517-52227160 was shown one SNP rs187084 T>C on downstream DNA. the results were shown high frequent of C risk allele correlated with disease as shown in Figure (4-14) and (4-15).


Chromosome 3
Patient group
TLR-9
52,227,030
rs187084
bSNP b156 v2
A/G/T rs187084
b156 v2
110
120
130
Consensus
1. TL9_1_L9....
2. TL9_2_L9....
3. TL9_3_L9....
4. TL9_4_L9....
5. TL9_5_L9....
6. TL9_7_L9....
7. TL9_8_L9....
8. TL9_10_L...
9. TL9_11_L...
10. TL9_12_...

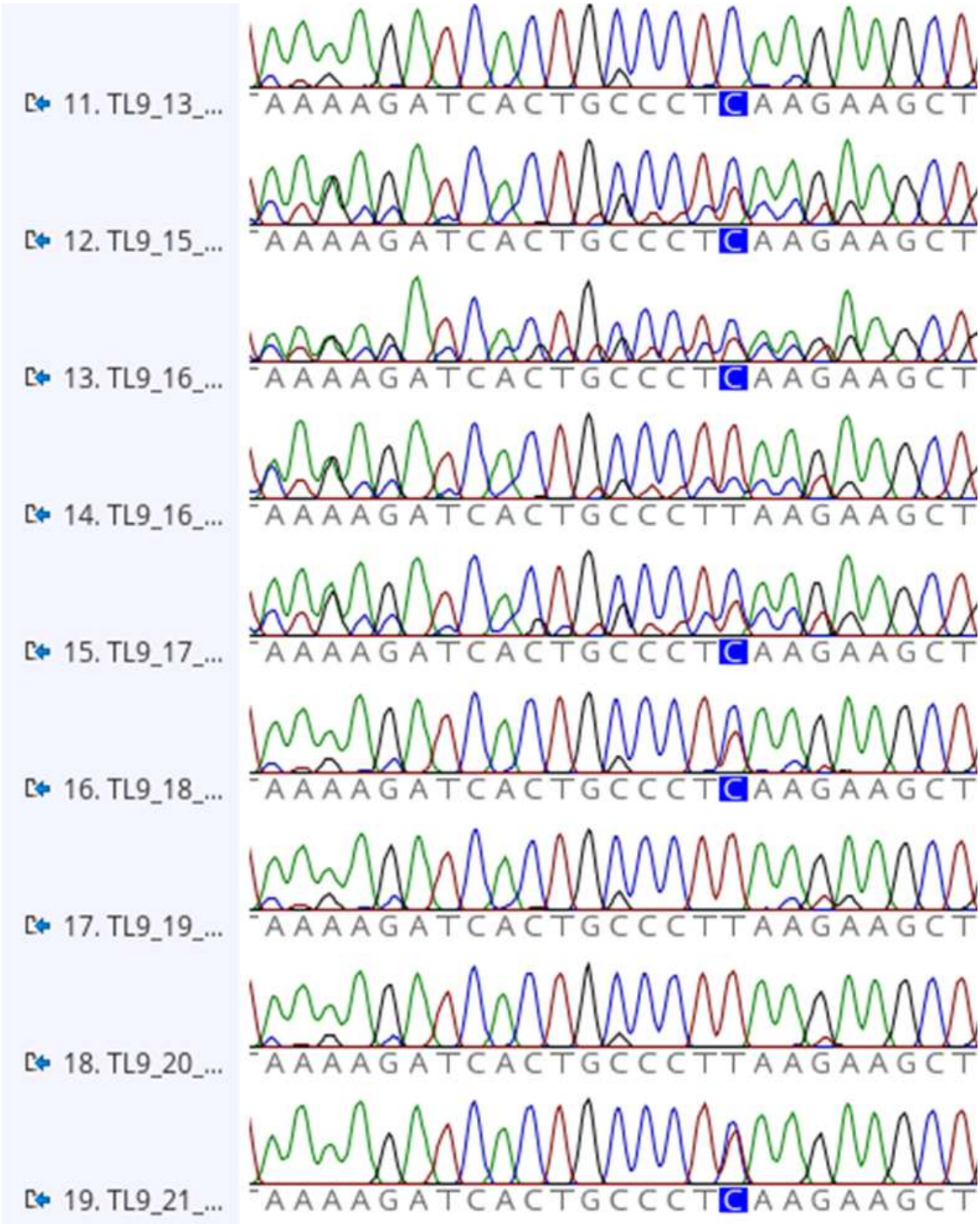


Figure (4-14): Multiple alignment of chromatograms sequence of TLR9 gene in miscarriage women for detection polymorphism in SNP rs187084.based on genius prim software

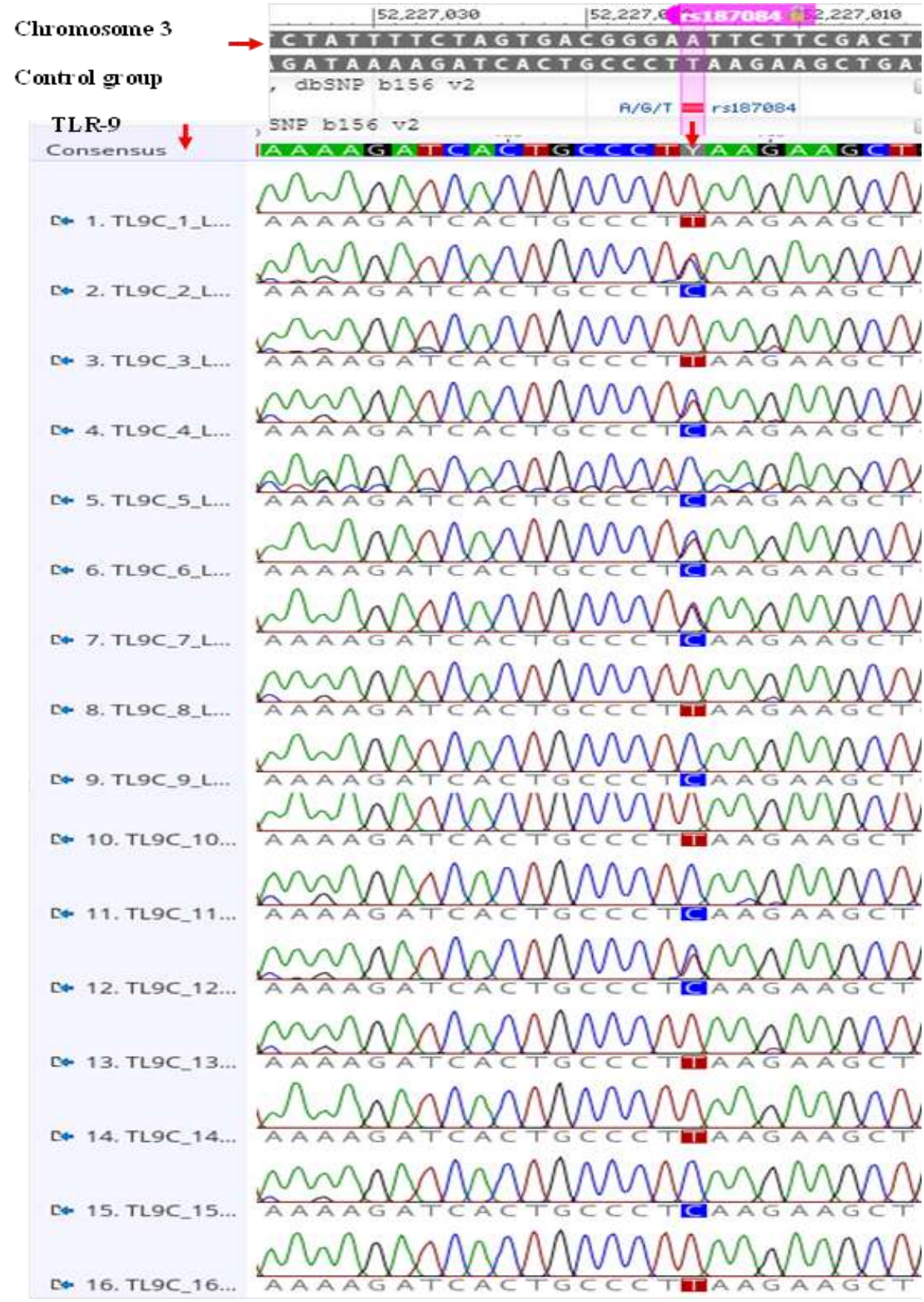


Figure (4-15): Multiple alignment of chromatograms sequence of TLR9 gene in healthy women for detection polymorphism in SNP rs187084.based on genius prim software.

This result agrees with Previous studies showed this SNP to be absent in Asian population, such as Taiwan (Cheng *et al.*, 2007).

The results shown three genotypes: TT, TC and CC were shown more distribution in patients undergo CMV infection compared with control group, the values of Odd Ratio (OR) were supporting that C allele in TC was considered as risk allele. The Odd Ratio was higher in genotype TC: OR= **2.3(0.8-6.7)**, and the allele frequency was higher in with C allele 27 (61.7%) in patient group with high value of OR=**1.3(0.63-2.7)** p.value = 0.4, while the allele frequency low 23 (38.3%) in control group as shown in Table (4-8).

Table (4-8): Genotype and Allele frequencies of TLR9 gene polymorphisms among patients with Cytomegalovirus and healthy women's.

Genotypes: **rs187084** A>G	Patients N=30	Control N=30	OR	P-value
TT	9(30%)	14(46.7)	Reference group	
TC	15(50%)	9(30%)	**2.3(0.8-6.7)**	0.1
CC	6(20%)	7(23.3%)	0.82(0.23-2.8)	0.7
T	33(55%)	37(61.7%)	0.7(0.36-2.7)	0.4
C	**27(45%)**	23(38.3%)	**1.3(0.63-2.7)**	0.4

There was a significant difference in TC genotype between patient and control group (p=0.1), while there was not significant difference in TT, CC genotype between them, and the allele C is significant between patient and control group (p=0.4). The C allele is significantly associated with an increased risk of CMV infection, while the TT genotype was associated with CMV viral clearance. in the study TLR9 (rs352139T >C)

polymorphisms were associated with an increased risk of CMV infection (Mhandire *et al.*, 2020).

Final finding, this study was highlight for the first time the role of polymorphism of three SNPs in three genes IL6, IFNAR2 and TLR9 in patient women undergo CMV infection. The results were conducted that all SNPs: rs1800795G>C, rs2236757A>G, rs187084 T>C were had risk alleles correlated with CMV infection.

Chapter Five

Conclusions and Recommendations

5-1 Conclusions

The current study revealed the following conclusions

1- CMV infection have essential role in recurrent miscarriages women.
2- The age ranged from 26-35 years considers as more reproductive age.
3- Blood group O^+ acts as risk factor for CMV infection in women with recurrent miscarriages.
4- The rs1800795 G>C polymorphism of IL6 gene may exert a noteworthy impact on the susceptibility to CMV in women with recurrent miscarriages.
5- IFNAR2 SNPs (rs2236757 A>G) and TLR 9 (rs187084 T>C) indicate a significant correlation between the frequency of genotype and allele levels with abortion in CMV infected women.

5-2 Recommendations

It is recommended that:

1- Conducting an extensive study of IL 6, IFNAR2, TLR9 gene sequences to identify all mutations in these genes that cause pregnancy disorders in women infected with CMV, Herpes, Rubella, *Toxoplasma* and other infections.
2- Expend the study of more genes and genomic loci and their association with abortion in women infected with CMV.
3- Conducting a study to investigating the relationship between infections with these viruses with different environmental conditions
4- Gene expression study will be necessary to confirm the effect of IL 6, IFNAR2, TLR9 gene polymorphism in the cause of pregnancy disorders in women infected with CMV.
5- Hospital laboratories should be encouraged to introduce diagnostic tests for cytomegalovirus CMV infection.
6- Early diagnosis and timely action will help to treat these cases effectively and will reduce the risk of morbidity and mortality.

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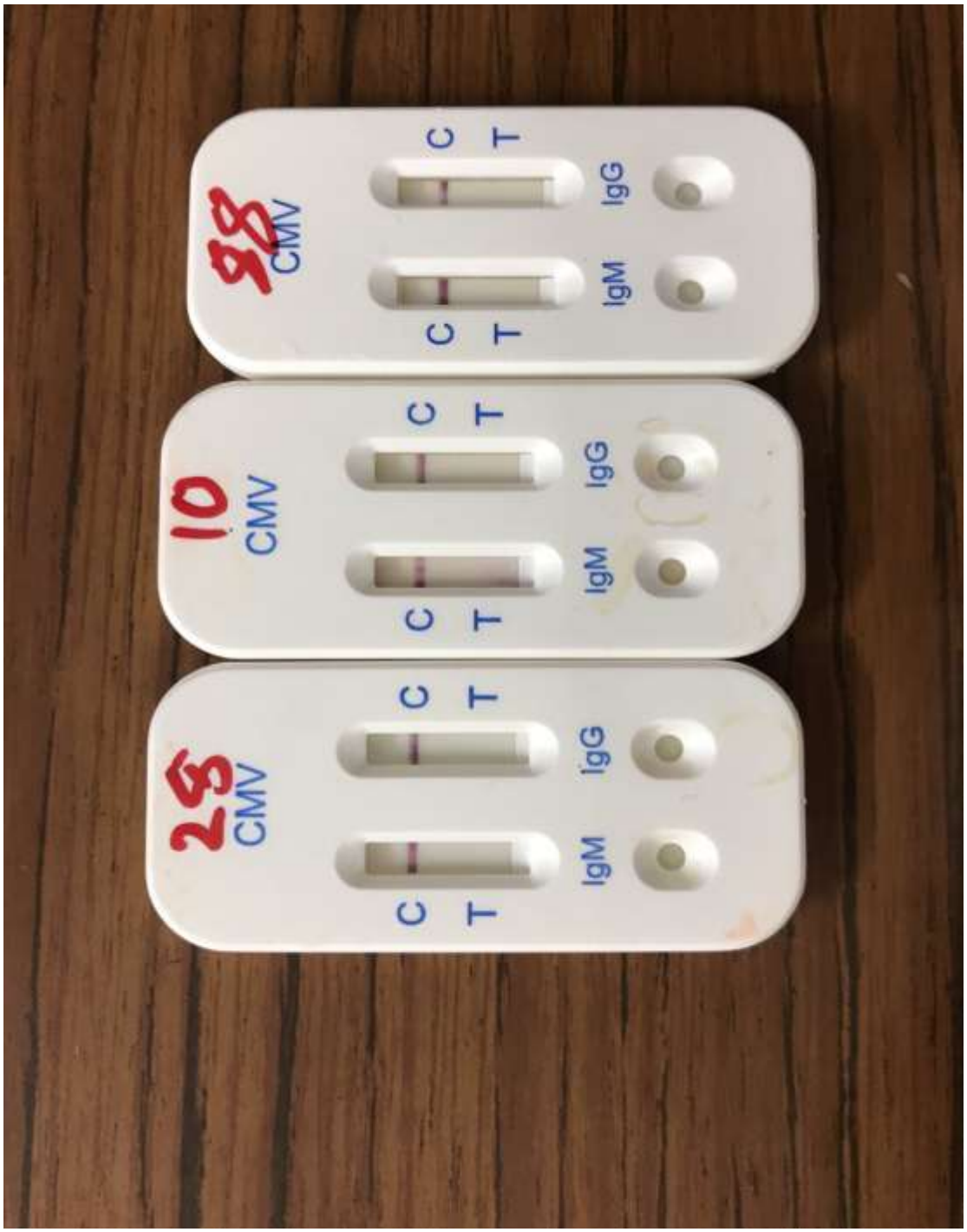
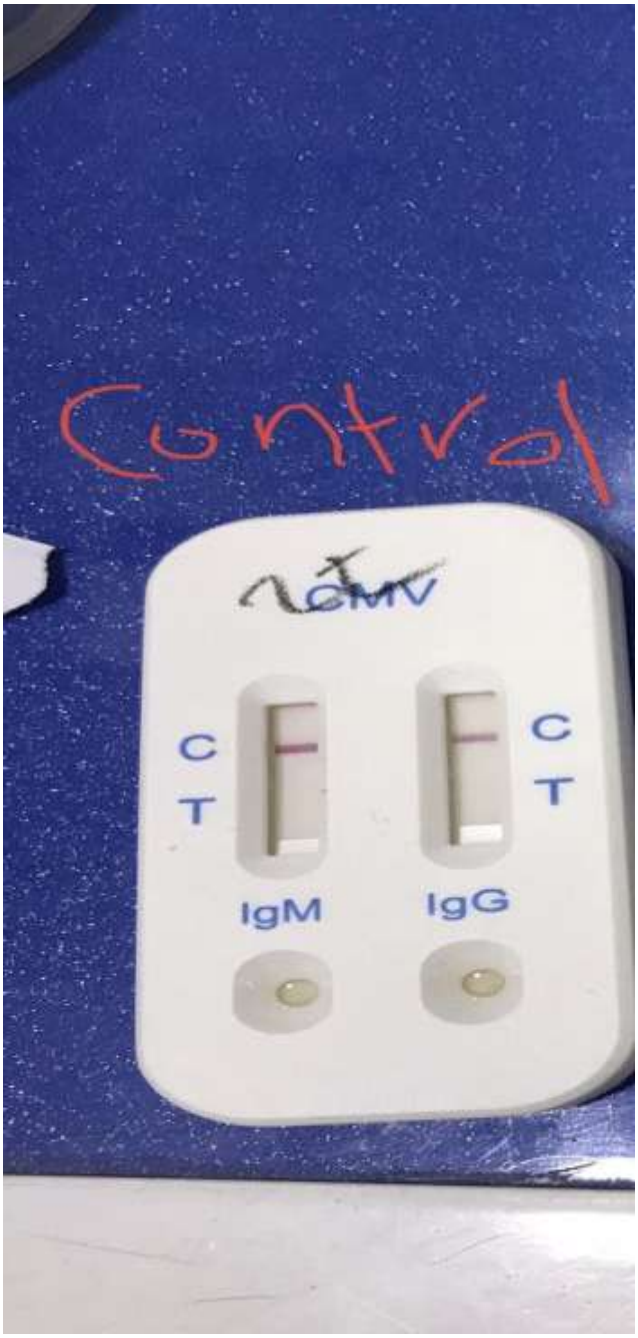
Appendix

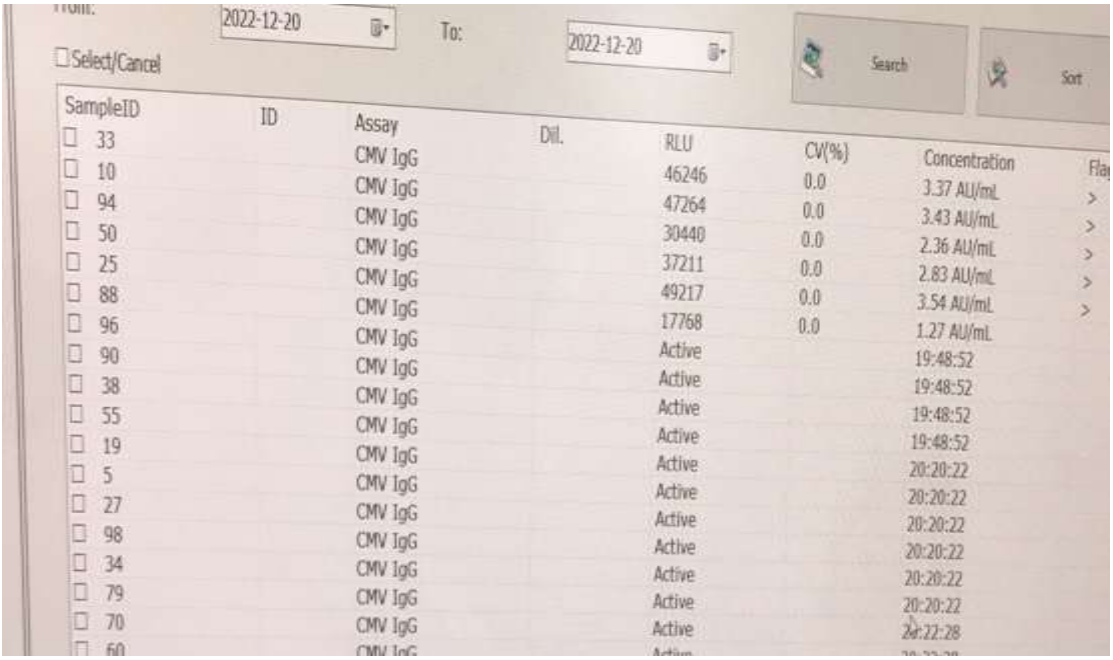
Appendix (1) the positive results of CMV test(patient).



Appendix (2) the negative resultsof CMV test(patient).



Appendix (3) the negative results of CMV test(control).



Appendix (4) the percentage of the cytomegalovirus (CMV)

الخلاصة

يعرف الإجهاض المتكررة على أنه فقدان مرتين أو أكثر من حالات الحمل السابقة (أقل من 24 أسبوعًا من الحمل) على التوالي. تهدف الدراسة الحالية إلى الكشف عن العدوى الفيروسية لدى النساء اللاتي يعانين من الإجهاض المتكرر وتحديد ما إذا كان التباين في بعض الجينات مرتبطًا بالإجهاض المتكرر. شملت الدراسة 64 عينة من النساء المجهضات اللاتي تعرضن لإجهاضين متكررين على الأقل، بالإضافة إلى 50 عينة من النساء الأصحاء اللاتي خضعن لولادتين على الأقل في مستشفى بابل للولادة والأطفال ومستشفى الامام الصادق في محافظة بابل.

تم جمع عينات الدم من كل من المرضى والمجموعة الضابطة خلال الفترة من ايلول 2022 إلى كانون ثاني 2023. تم فصل الأمصال وتخزينها في أنابيب ميكروفيوج وحفظها عند -20 درجة مئوية. تم استخراج الحمض النووي من الدم المجمد وتخزينه في درجة حرارة -20 درجة مئوية. تم إخضاع العينات المصلية من كل من المرضى والضوابط للفحص المصلي للكشف عن العدوى الفيروسية، وتم تأكيد التشخيص عن طريق اختبار (TORCH)، وتقنية mini-VIDAS ومحلل المقايسة المناعية الكيميائية باستخدام مجموعة IgG CMV، واستخلاص الحمض النووي. تم استخدام تقنية Tetra ARMS PCR لتضخيم جين IL 6، وتضخيم جين IFNAR2، وجين TLR9 بواسطة PCR، ومن ثم تم إرسال 60 عينة (30 مريضا و30 سيطرة) إلى كوريا لتسلسلها للتعرف على هذه الجينات.

تم إجراء مقارنة بين التسلسلات الأساسية للعينات قيد الدراسة مع التسلسل الجيني العالمي المخزن في بنك الجينات NCBI، وتبين أن هناك طفرات تسجل (rs2236757 A>G) SNPs لتسلسل IFNAR2 (470 زوج قاعدي) . وسجل واحد (rs187084 T> C) SNP في تسلسل TLR9 (644 زوج قاعدي).

ما يقرب من (78.1)٪ من النساء المجهضات كانت نتائجهن إيجابية لمضادات CMV-IgG (50 من 64)، مع أعلى نسبة إصابة وجدت في الفئة العمرية من 26 إلى 35 سنة (46)٪ وسجلت النساء المجهضات ذوات فصيلة الدم O^+ أعلى نسبة للاصابة (66%).

أدى تحليل PCR لجين IL6 إلى نتائج تشير إلى وجود أليلات G وC، مع ثلاثة أنماط وراثية متميزة (GG، GC، وCC) وأطوال مختلفة (302، 206، و152) زوج قاعدي. بالإضافة إلى ذلك، كشف التحليل الإحصائي وجود فرق معنوي بين المرضى ومجموعة الضابطة.

أشار SNP لـ IFNAR2 (rs2236757 A > G) وجين TLR9 (rs187084 T > C) إلى وجود علاقة معنوية بين تواتر النمط الوراثي ومستويات الأليل مع الإجهاض عند النساء المصابات بفيروس CMV.

جمهورية العـــراق

وزارة التعليـم العالي و البحـث العلمـي

جامعـة بابـل / كليـة العلـوم للبنـات

دراسة بعض المتغيرات الوراثية المرتبطة بعدوى الفايروس المضخم للخلايا التي تسبب الإسقاطات المتكررة في محافظة بابل

رسالة مقدمة الى

مجلس كلية العلوم للبنات /جامعة بابل

وهي جزء من متطلبات نيل درجة الماجستير في العلوم

علوم الحياة /أحياء مجهرية

من قبل الطالبة

شهد جمال صاحب جاسم الصيكـــل

(بكالوريوس علوم حياة/ أحياء مجهرية / جامعة بابل / كلية العلوم / 2018)

بأشراف

أ .م. د. زينب عبد النبي طليفح

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