ORIGINAL ARTICLE

Cytokines Expression in Pregnant Women With Primary and Non-primary Cytomegalovirus (CMV) Infection

Nur Fazlin Akmal Muhammad Faizal[1,2], Ramlah Kadir[2,3], Zeti Norfidiyati Salmuna[1,2]

[1] Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan, Malaysia
[2] Hospital Universiti Sains Malaysis, Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan, Malaysia
[3] Department of Immunology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kota Bharu, Kelantan, Malaysia

ABSTRACT

Introduction: Cytomegalovirus (CMV) infection in pregnancy is the commonest cause of congenital infection worldwide. Primary CMV infection in pregnancy carries a higher risk of fetal transmission compared to non-primary infection. This study aims to determine the cytokines expression in pregnant women with primary and non-primary CMV infections in both types of infection. **Methods:** This prospective cohort study was conducted at Microbiology Laboratory, Universiti Sains Malaysia (USM) from January 2019 until June 2020. Seventy-four pregnant women with abnormal pregnancy outcomes with positive CMV IgG with or without IgM by electrochemiluminescence assay (ECLIA) were subjected to IgG avidity assay by ECLIA method to discriminate primary and non-primary CMV infection. Later, the sera were subjected to magnetic Luminex multiplex enzyme-linked immunosorbent assay for cytokine analysis to determine their concentrations in both primary and non-primary CMV infection. Cytokines and chemokines tested were IL-12, IL-2, IFN- γ, TNF-α, IL-1β, IL-6, IL-10, IFN- γ, TNF-α, MCP-1 (CCL-2), and IP-10 (CXCL-10). **Results:** Concentrations of IL-1β, IL-6, and MCP-1 (CCL-2) were significantly elevated in pregnant women with primary CMV infection with the p-values of (0.001, 0.035, and 0.002) respectively. The intensity of IFN-γ, IL-12, and IL-2 were higher in primary CMV infection with the p-values of (0.018, 0.004, and 0.007). **Conclusion:** The pro-inflammatory cytokines were expressed significantly in pregnant women with primary CMV infection together with MCP-1 (CCL-2), showing predominant Th1 response. The low level of cytokines in non-primary CMV infection might be due to the latent state of CMV in a host.

Keywords: Cytomegalovirus, Primary, Non-primary, Cytokines, Pregnant women

Corresponding Author:
Zeti Norfidiyati Salmuna, MPath (Med Microbiology)
Email: zetifidiyati@usm.my
Tel: +609-7676586 (Ext: 6252)

INTRODUCTION

Cytomegalovirus, belonged to the Herpesviridae family is a double stranded DNA virus. CMV infection is endemic, especially in developing countries, and the virus seroprevalence differed among populations. In a previous study conducted by Camalxaman and colleagues, the seroprevalence of CMV in Malaysia states of Selangor and Wilayah Persekutuan Kuala Lumpur was 92% (1). A systematic review showed that in developing countries, the maternal seroprevalence that refers to the presence of CMV IgG in pregnant and puerperal women were range from 84% - 100%, with congenital CMV (cCMV) prevalence range from 0.6% to 6.1% (2). High maternal prevalence of CMV were later reflected to higher prevalence of cCMV infection in developing countries in comparison with previous literatures which were 0.2% to 2.2%, (3)with an average of 0.65% (4,5). The difference in the prevalence of cCMV infection in developing countries from previous literatures were due to lower maternal seroprevalence that ranges from 44%-94% (5).

CMV infection can be diagnosed by evidence of seroconversion from negative IgM/IgG negative to IgM/IgG positive in two consecutive samples taken one to three months apart or detection of CMV DNA by polymerase chain reaction (PCR) method (6). CMV infection is also diagnosed by isolation of CMV virus or the existence of viral proteins or nucleic acid in body fluid or tissue (7). cCMV refers to congenital CMV infection diagnosed in neonates, via detection of CMV DNA using polymerase chain reaction (PCR) method from blood, urine or saliva samples within the first three weeks of life (8).

There are two types of CMV infection, which are primary CMV infection, and non-primary CMV infection. Primary and non-primary CMV infection can be in active stages

evidenced by the presence of symptoms and CMV-specific IgM. Based on CMV IgG avidity testing, primary CMV infection is defined according to test kit used with recent primary CMV infection (within 3 months of exposure) is evidenced by low IgG avidity and non-primary CMV infection (after 6 months of exposure) evidenced by high IgG avidity (9). In pregnant women, primary CMV infection is defined as the presence of CMV IgG in a previously seronegative mother or the presence of CMV IgM antibody with low IgG avidity, and non-primary CMV infection refers to reinfection from an exogenous virus or reactivation of endogenous virus (latent) infection with high IgG avidity testing (10-12).

Although primary infection in pregnant women raises a significant risk of transmitting the virus to the fetus and diseases, the virus transmission to the fetus in women with non-primary infection is frequent (13). During pregnancy, the frequency of viral transmission increased from 30% during the first trimester to 70% in the third trimester (14-15). Primary CMV infection during the third trimester of pregnancy possess a greater chance of congenital transmission than in the first trimester of pregnancy (16). The risk of fetal transmission from mother with primary CMV infection was estimated to be about 30%- 40% and much more lower for mother with recurrent infection that was estimated to be 1.1% to 1.7% (4, 17-20).

CMV can cause severe diseases in the offspring of pregnant women with an active infection (21). Besides, it can cause sensorineural hearing loss in over 25% of infected infants (22). This virus is also responsible for mental retardation and intellectual impairment in infants (23). Other possible diseases caused by CMV in infants are jaundice, microcephaly, developmental delay, and hepatosplenomegaly (24).

Cytokines can either be pro-inflammatory or anti-inflammatory and classified according to their cell of origin or their mechanisms of action (25). Proinflammatory cytokines are important for up-regulation of inflammation and produced mainly by activated macrophages (26). Examples of proinflammatory cytokines are IL-2, IL-6, IL-12, IFN- γ, TNF- α and IL-1β. Anti-inflammatory cytokines control the response of proinflammatory cytokines (26). Examples of anti-inflammatory cytokines are IL-1 receptor antagonist, IL-4, IL-10, IL-11, as well as IL-13. There are some cytokines that can be either proinflammatory or anti-inflammatory depending on circumstances such as leukemia inhibitory factor, INF-α, IL-6, and TGF-β (26). Chemokines are cytokines that induce chemotaxis (26). Examples of chemokines are RANTES, monocyte chemoattractant protein [MCP-1 (CCL-2)], Interferon gamma-induced protein [IFN-γ IP-10 (CXCL-10)] and etc (26).

A study by Scott et al. in 2012 showed an increase in IFN-γ IP-10 (CXCL-10) only from sera of pregnant women with primary CMV infection compared to control (27). The expression level of IL-10 is higher during the CMV reactivation in immunocompetent critically-ill patients (28). During pregnancy, CMV infection cause dysregulation of Th1 and Th2 cytokine by altering the cytokine expression in placental cells and increases the pro-inflammatory cytokines expression in amniotic fluid of congenital CMV infection (27).

Currently, the roles of immunomodulator agents in the treatment of CMV infection are still unclear. A balance between the usage of antiviral agents such as ganciclovir or valganciclovir and immunomodulator agent (such as CMV immunoglobulin) need further studies. To date, only antivirals such as ganciclovir or valganciclovir is proven effective for prevention of sensorineural hearing loss (SNHL) in infants with cCMV (29). To ensure a successful pregnancy, Th2 cytokine phenotype expression is important, therefore in CMV infection that show skewness to Th1 cytokine phenotype may have a bad consequences to the developing fetus (30).

Even though evidence of seroconversion is one of the confirmatory method to diagnose primary CMV infection, however it is not easily achieved as currently there is no health authority recommend it as a routine antenatal screening (31). This study used IgG Avidity assay to diagnose primary or non-primary CMV infection during pregnancy. IgG avidity test is a useful method to differentiate primary CMV infection from reactivation. Low avidity is highly sensitive and specific to diagnose primary CMV infection (19)(23). CMV IgM is a poor detection marker to diagnose primary CMV infection since CMV IgM is also produced during viral reactivation (32). Later, this study used the Magnetic Luminex® Multiplex technique to determine cytokines expression in both types of infection. Nine cytokines and chemokines were measured which were IL-2, IL-6, IL-12. IFN-γ, TNF-α, IL-1β, IL-10, MCP-1 (CCL-2) and IFN-γ IP-10 (CXCL-10).

MATERIALS AND METHODS

Samples

The sera samples used were archived samples collected from pregnant women with abnormal pregnancy outcomes sent to the Microbiology and Parasitology Laboratory, Hospital Universiti Sains Malaysia from June 2019 until July 2020 that has completed TORCHES screening (toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus) and positive for CMV IgG with or without CMV IgM. The approval and ethical clearance from the The Human Research Ethics Committee of USM (JEPeM) was attained upon commencement of the study [Reference No: USM/JEPeM/17110606]. Since the samples used for analysis were archived samples from pregnant women with abnormal pregnancy outcomes as our centre is one of a referral tertiary centre, no samples

from healthy control obtained. Besides, a study showed that CMV seropositivity among blood donors were 97.6% in our centre that indicates previous exposure of community to CMV (33). Therefore, we conclude that the comparison of cytokines expression between the two groups of CMV infection (primary and non-primary CMV infection) were more feasible.

Definition of variables used in this study

Primary CMV infection: low CMV IgG avidity (<45.0%) following manufacturer's guideline (9) that correspond with onset of infection of less than 90 days (34) with or without CMV-specific IgM positivity.

Gray zone: CMV IgG avidity of 45.0-54.9% following manufacturer's guideline (9) that may correspond with onset of infection of in between 90–180 days (34) with or without CMV-specific IgM positivity.

Non-primary CMV infection: high CMV IgG avidity (> 55.0 %) following manufacturer's guideline (9) that correspond with onset of infection of more than 180 days (34)with or without CMV-specific IgM positivity.

IgG Avidity assay (Roche Elecsys® CMV IgG avidity)

The sera sample with possible CMV infection were collected and proceeded with IgG Avidity assay (Roche Elecsys® CMV IgG avidity) to determine infection types, either primary infection or non-primary infection following the manufacturer's guideline using COBAS e-6001 analyzer. Less than 45.0 % avidity is interpreted as low avidity followed by 45.0-54.9% avidity as gray zone and lastly more than 55.0 % avidity as high avidity (9). If a gray zone result was obtained, no specific clinical interpretation should be given and a repeat sample should be taken within 2 to 4 weeks (9) as it may correspond to onset of infection in between 90-180 days of exposure (34) (Figure 1).

Magnetic Luminex® Multiplex ELISA

Multiplex ELISA was done to the sera balance using Magnetic Luminex Kit® (R&D Systems, Minneapolis,MN) in a Luminex 200 Analyzer to determine the level of cytokines present. Refer to Figure 1. The range of detection of all cytokines measured were provided in Table I in concentration (pg/ml). Median Fluorescence Intensity (MFI) reading was used if the concentration of cytokines measured fall below the detectable range. The Median Fluorescence Intensity (MFI) reading and concentration was collected. A similar method had been published that used MFI as valuable data for statistical analysis (35).

RESULTS

A total of 74 sera samples of pregnant women with abnormal pregnancy outcomes were included in this study. Among 74 samples that were positive for CMV-specific IgG or IgM, 5 were categorized into primary CMV infection group and 69 patients were categorized into non-primary CMV infection group by CMV IgG avidity testing. Five patients with low IgG avidity testing were only positive with CMV- specific IgG. On the other hand, out of 69 patients with high IgG avidity testing, only 1 patient positive for both CMV- specific IgM and IgG with the remaining 68 patients positive for CMV-specific IgG only. All 74 sera were sent for Magnetic Luminex® Multiplex ELISA for detection of pro-inflammatory cytokines, anti-inflammatory cytokines and chemokines (Figure 1).

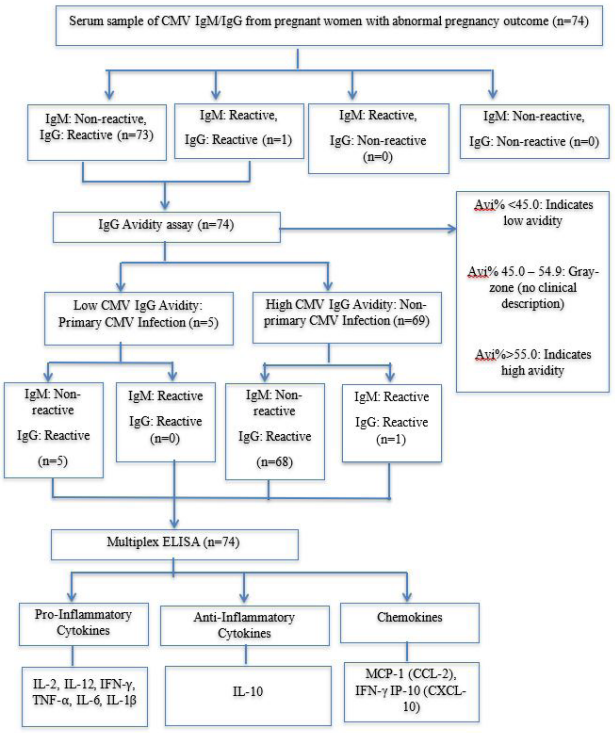

Figure 1: The flowchart of serological study

Table I Range of detection of cytokines and chemokines measured in concentration (pg/ml)

Cytokines/ chemokines	Range of detection (pg/ml)
Pro-inflammatory cytokines	
IFN-γ	10630.00 - 43.74
IL-12	34410.00 – 141.60
IL-2	9090.00 – 37.41
IL-1β	4180.00 – 17.20
IL-6	1130.00 – 4.65
TNF- α	2240.00 – 9.22
Anti-inflammatory cytokine	
IL-10	880.00 – 3.62
Chemokines	
MCP-1 (CCL-2)	7160.00 – 29.47
INF-γ IP-10 (CXCL-10)	250.00 – 1.03

Referring to Table II, there is a significance difference of IgG avidity in primary and non-primary CMV infection indicates that CMV IgG avidity testing can discriminate both types of infections. The median IgG avidity for primary CMV infection in this study was 43.20 (30.5 – 43.65), followed by non-primary CMV infection, 74.90

Table II: CMV IgG avidity in primary and non-primary CMV infection in pregnant women in USM (n=74)

Variables	Primary CMV Infection (n=5)	Non-primary CMV infection (n=69)	*P*-values*
	Median (IQR)		
IgG avidity	43.20 (30.5 – 43.65)	74.90 (71.90 – 87.90)	<0.001

*Mann-Whitney U test
A *P*-value of <0.05 is considered statistically significant

(71.90 – 87.90).

Table III shows the demographic characteristics of 74 pregnant women with CMV infection. The median age for both groups were 26 for primary CMV infection group and 30 years old for non- primary CMV infection group respectively. The ethnicity of all patients was Malay. There are no significant differences in both groups in terms of maternal age, gravidity and parity. However, there is a significant difference in gestational weeks of pregnancy in between the two groups which was unavoidable since the patients may be referred to tertiary center at any gestational age if presented with abnormal pregnancy outcomes. There is no significant differences in term of median CMV IgG titer in both groups.

Table III: Patients demographic data (n=74)

Characteristics	Primary CMV Infection (n=5)	Non-primary CMV Infection (n=69)	-value*
	Median (IQR)		
Age	26.0 (23.0 – 31.5)	30.0 (26.5 – 34.0)	0.177
Parity	0.0 (0.0 – 2.0)	1.0 (0.0 – 2.0)	0.293
Gravidity	1.0 (1.0 – 3.5)	3.0 (1.0 – 4.0)	0.217
Gestational weeks	39.0 (35.0 – 39.0)	33.0 (26.5 – 36.0)	0.002
CMV IgG Titer	174.5 (101.9 – 230.8)	405.7 (173.2 – 757.0)	0.064

*Mann-Whitney *U* test.
A *P*-value of <0.05 is considered statistically significant.

In Table IV, the gestational weeks of pregnant women with primary CMV infection (n=5) were all in third-trimester ranging from 35 to 39 weeks of pregnancy. Meanwhile, the gestational weeks of pregnant women with non-primary CMV infection ranging from the 1st trimester until 3rd trimester with (n=1/69) in first trimester, (n=19/69) in second trimester and (n=49/69) in the third trimester of pregnancy respectively.

In terms of the median concentrations of pro-inflammatory cytokines, IL-1β and IL-6 were found significantly higher in primary CMV infection group in comparison to non-primary CMV infection group, but not significant for TNF-α. Median concentration of MCP-1 (CCL-2), a chemokine was also found significantly higher in primary CMV infection group. However, there is no significant difference in median concentration of INF-γ IP-10 (CXCL-10) in both groups (Table V).

Referring to Table VI, some cytokines were expressed in very low level, such as IFN- γ, IL-12, IL-2 and IL-10, thus, Net-MFI (median fluorescence intensity was used as it is still able to identify the secreted cytokines but does not allow absolute quantification in concentrations (pg/ml). By using net-MFI, the pro-inflammatory cytokines such as IFN- γ, IL-12 and IL-2 can be detected in primary CMV infection group. The fluorescence intensity of IFN-γ, IL-12 and IL-2 were found significantly higher in primary CMV infection in comparison to non-primary CMV infection. In contrast, the fluorescence intensity of IL-10 (an anti-inflammatory cytokine) level was found not significant.

Table IV: Pregnancy trimesters in primary and non-primary CMV infections in pregnant women (n=74).

Pregnancy trimesters	Primary CMV infection	Non-primary CMV infection
1st (week 1 – week 12)	0 (0%)	1 (1.5%)
2nd (week 13 – week 28)	0 (0%)	19 (27.5%)
3rd (week 29 – week 40)	5 (100%)	49 (71.0%)
Total	5 (100%)	69 (100%)

Table V: Concentrations in (pg/ml) of cytokines and chemokines in primary and non-primary CMV infections (n=74)

Variables	Primary CMV Infection (n=5)	Non-primary CMV Infection (n=69)	P-values*
Pro-inflammatory cytokines	Median (IQR)		
IL-1	909.0 (225.2 – 1419.0)	0.00 (0.00 – 0.00)	0.001
IL-6	680.47 (0.00 – 327.0)	0.00 (0.00 – 15.26)	0.035
TNF-	0.00 (0.00 – 138.70)	0.00 (0.00 – 0.00)	0.672
Chemokines	Median (IQR)		
MCP-1 (CCL-2)	599.6 (304.7 – 2196.0)	183.8 (107.9 – 287.4)	0.002
INF-γ IP-10 (CXCL-10)	12.12 (8.16 – 41.98)	11.89 (8.96 – 16.66)	0.572

*Mann-Whitney U test
A *P*- values of <0.05 is considered statically significant

Table VI: Expression (net MFI values) of cytokines in primary and non-primary CMV infections (n=74)

Variables	Primary CMV infection (n=5)	Non-primary CMV infection (n=69)	P-values*
Pro-inflammatory cytokines	Median (IQR)		
IFN-	2.75 (0.13 – 9.38)	0.00 (0.00 – 0.75)	0.018
IL-12	0.75 (0.38 – 3.88)	0.00 (0.00 – 0.00)	0.004
IL-2	1.25 (0.50 – 9.13)	0.00 (0.00 – 0.25)	0.007
Anti-inflammatory cytokine	Median (IQR)		
IL-10	0.00 (0.00 – 17.13)	0.00 (0.00 – 1.625)	0.936

*Mann-Whitney U test
P- values of <0.05 is considered statically significant

DISCUSSION

In this study, 74 archived serum samples from pregnant women with abnormal pregnancy outcomes were found seropositive for CMV. All sera were later subjected to CMV IgG avidity assay to determine the types of infection as it carries risk of infection to the fetuses (36). Referring to Table II, the CMV IgG avidity index (AI) was found out to be lower in primary CMV infection and higher in non-primary CMV infection and thus

able to discriminate the types of infection. This finding is consistent with several studies that also showed that mean AI was significantly lower in primary compared to non-primary CMV infection (37,38). IgG antibodies produced in primary CMV infection bind loosely with the antigen and exhibit low avidity. Those antibodies produced six months after infection bind tightly with the antigen and exhibit high avidity (23,39,40). Loosely-bind antigens are readily dissociate using protein denaturants such as urea, guanidine chloride, and potassium thiocyanate (32). Urea is the commonest dissociating agent used in ELISA and loosely-bind antigen-antibody complex readily dissociates using urea give low avidity result indicating recent infection and vice versa for the tightly-bind antigen-antibody complex (32).

After types of infection determined, the remaining sera samples were subjected for multiplex ELISA to determine the expression of cytokines and chemokines secreted in primary and non-primary CMV infection. Multiplex ELISA for determination of cytokine expression among pregnant women with primary CMV infection has been used before in previous study (41). The selection of cytokines and chemokines taken after analysis from literatures of pregnant women with CMV infection. The example of cytokines selected were pro-inflammatory cytokines (IL-1β, IL-6, TNF-α, INF-γ, IL-12, IL-2), anti-inflammatory cytokine (IL-10) and chemokines MCP-1 (CCL-2) and INF-γ IP-10 (CXCL10)].

By referring to Figure 1, around 6.8% (n=5/74) pregnant women with abnormal pregnancy outcomes have primary CMV infection in Universiti Sains Malaysia compared to 93.2% (n=69/74) with non-primary CMV infection. This finding is consistent with a study published in 2006 that showed that 97.6% of blood donors in USM were CMV IgG seropositive (33). Thus, expected that most patients attending USM in Kelantan are already exposed to CMV earlier before pregnancy. No recent study was found in Malaysia on the prevalence of primary and non-primary CMV infection among pregnant women with abnormal pregnancy outcomes.

There are no significant difference in age, parity, gravidity, and CMV IgG titre of all pregnant women with abnormal pregnancy outcomes in both groups (primary and non-primary CMV infection). Thus, both groups were comparable. However, there is a significant difference in the gestational weeks of pregnant women involved. Previous study showed that the rate of maternal transmission was correlated with gestational age. Picone and colleagues found out that the rate of transmission at the time of primary infection was 30.6% in first trimester, 34.1% in second trimester and 40% in third trimester (36). According to CDC, the risk of transmission to the fetus is higher in primary CMV infection acquired in the third trimester of pregnancy than the first trimester of pregnancy and severe congenital malformations and complications to the fetus are the greatest if primary CMV infection occurs in the first trimester of pregnancy (8).

All five pregnant women in the primary CMV group were in the third trimesters of pregnancy during the screening procedures. A systematic review showed that the fetal insult rate was only 0.4% if primary CMV infection was acquired in the third trimester of pregnancy (42).Three out of five fetuses from patients with primary CMV infection were delivered as small for gestational age (SGA) infants and 2 fetuses delivered as fresh stillbirth (one due to tight cord around neck and another one due to unknown cause) . Three infants will be on follow up for developmental and hearing assessment as cCMV infection may present with sensorineural hearing loss (SNHL) in later life (43). Even though a study showed that the risk of acquiring SNHL and neurologic sequelae are significantly linked to primary CMV infection in the first trimester of pregnancy,(44) a review showed that all women with low CMV avidity index, regardless of trimester, must be monitored for signs of fetal transmission (45).

Most patients in this study are in the non-primary CMV infection group. Only one mother with positive IgM was found in this study, and she was having non-primary CMV infection in the first trimester of pregnancy as shown in Table IV. However, she had a miscarriage. A study in Malaysia showed that 17 pregnant women positive for both CMV IgG and IgM had miscarriages (46). Our findings were consistent with several studies showing that most pregnant women were in the non-primary CMV-infected group (37)(47)(48)(49). The risk of transmission to the fetus is much lower (~ 3%) in non-primary CMV infected-mother (8). Previous study also demonstrated that only 0.54% (7/1287) pregnant women with non-primary CMV infection delivered newborns with cCMV (50). Pregnant woman with high CMV IgG avidity in first trimester of pregnancy can be given counselling and reassurance that risk of fetal transmission is low and urgent sampling prenatally is not needed (40). However, pregnant women with high CMV IgG avidity in second or third trimesters of pregnancy must be monitored to rule out intrauterine transmission as possibility of primary infection after conception can't be rule out (32). Therefore, it is important to know when is the first time the pregnant women tested for CMV IgG avidity to determine risk of fetal transmission.

Previous studies mostly compare the cytokine level between seropositive CMV and seronegative CMV patients. However, due to the inability to get the seronegative CMV samples, we compare the primary with non-primary CMV infected patients. Our study turned out to be the first one that investigates cytokines' expression in primary and non-primary CMV infection, especially in pregnant women.

The concentrations and expression of pro-inflammatory

cytokines (IL-1β, IL-6, IFN-γ, IL-12, and IL-2) were found higher than anti-inflammatory cytokines (IL-10) in primary CMV infection (Refer to Table V and VI). However, the expression level of TNF-α in both types of infection was barely detected and not statistically significant. It showed that the cytokine balance in CMV infection during pregnancy was complex. One study among primary CMV- infected transplant patients showed an increase in expression of IFN-γ, which is consistent with our finding but contradictory for INF-γ IP-10 (CXCL-10) (51). A study among sera from ten newborns and their mothers with CMV infection fpund out that 9 out of 10 mothers that were categorized into primary CMV infection group showed a predominant Th1 response that is also consistent with our research evidenced by expression of IL-2, IL-8, IL-12, and IFN-γ (41). A study of cytokine expression among women infected with CMV compared with healthy controls showed high level of IL-1, IL-6, and IL-12 showing Th1 predominance (52). A study comparing placentae of CMV-infected stillborn babies with normal placentae showed higher expression of MCP-1 (CCL-2) and TNF-α in favor of Th1 predominance (53). IFN-γ and IL-12 were closely related to each other. Both were elevated in this study. IFN- γ is the sole type II interferon secreted by natural killer (NK) cells in response to innate immunity and by CD4+ Th1 lymphocytes and CD8+ cytotoxic lymphocytes (54). The production of IFN- γ was controlled by IL-12 and IL-18, cytokines secreted by antigen-presenting cells (APCs) (54). Macrophage that recognizes pathogen secretes IL-12 and chemokines will attract NK cells to the site of inflammation for secretion of IFN-γ . IL-12 is also elevated in primary CMV infection that will stimulate the production of IFN-γ that is also significantly elevated in our study. This finding is consistent with a study in the 2019 that documented an increased in IFN- γ among CMV-seropositive pregnant women (55).

The study limitation apart from inability to find healthy control for pregnant women to compare with CMV seropositive pregnant women due to the usage of archived samples and high seroprevalence of CMV in Kelantan, is the selection of few cytokine markers, representing the most dominant cytokines and chemokines in CMV due to budget constraints.

CONCLUSION

In conclusion, in pregnant women with primary CMV infection, the pro-inflammatory cytokines (IL-1β, IL-6, IFN-γ, IL-12 and IL-2) were expressed significantly together with MCP-1(CCL-2) chemokine, which showed the predominance of Th1 response. The low level of cytokines in non-primary CMV infection might be due to the latent state of CMV in a host. Thus, it does not trigger the immune host cells reactivation. A balance expression between pro-inflammatory and anti-inflammatory cytokines is important for a healthy pregnancy. By analysing the cytokine profile in primary and non-primary CMV infection will help the scientists to find a solution on the balance between the usage of antivirals and immunomodulating agents in CMV infection.

ACKNOWLEDGEMENT

This study was funded by USM Research University Grant (Grant No: 1001.PPSP.8012293).

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