

CHAPTER III

RESULTS

1. Study Populations

One hundred and seven pregnant women and 75 married women were studied. They were characterized according to age, education, socioeconomic status, number of previous pregnancies, and history of syphilis during pregnancy as shown in Table 4. It can be seen that the mean of the age was 25.2, the majority of the education was primary and secondary school (77.6%). Majority of the socioeconomic status was in the low and medium income, 48.6% and 36.5% respectively. Number of previous pregnancies: 51.4% was primigravida; 30.8% was secundigravida. All of the subjects were non-reactive for syphilis.

1.1 Pregnant Women Group Studied

There were 107 pregnant women in the study group, cervical swab and venous blood specimens were supposed to be collected three times from each subject at the first, the second and the third trimester, respectively. Not all of the subjects, however, had attended the Antenatal Care Unit as was suggested. Therefore, the subjects were grouped according to the following criteria:

Group 1: The specimens were obtained from 74 pregnant women, attending at the first, the second and the third trimester.

Table 4 Characteristics of the Women in This Study:
Pregnant Women and Married Women

Characteristics	Pregnant Women (n = 107)	Married Women (n = 75)
Age (years)		
Range	17-41	22-58
Mean	25.2	32
Education (%)		
No Education	3.7	-
Primary and Secondary School	77.6	41
College and University	13.1	59
Unknown	5.6	-
Socioeconomic Status (%)		
Low (<3,000 baht)	48.6	-
Medium (>3,000 - 6,000 baht)	36.5	-
High (>6,000 baht)	8.4	-
Unknown	6.5	-
Number of Previous Pregnancies (%)		
1 (Primigravida)	51.4	-
2 (Secundigravida)	30.8	-
3 (Tertigravida)	13.1	-
>3 (Multigravida)	4.7	-
History of Syphilis During Pregnancy	Non-reactive (VDRL)	Non-reactive (VDRL)

Group 2: The specimens were obtained from 15 pregnant women, attending at the first and the second, or the first and the third trimester. There were 11 pregnant women attended at the first and the second trimester and 4 pregnant women attended at the first and the third trimester.

Group 3: The specimens were obtained from 18 pregnant women, attending only at the time of the initial visit (the first trimester) in pregnancy.

In conclusion; there were three, two, and only one specimens obtained from each of pregnant women in group 1, group 2 and group 3, respectively.

1.2 Married Women Group Control

Only cervical swab specimens were obtained from 75 married women for virological study.

2. Virological Study

Recovery of CMV was performed by inoculating the cervical swab specimens, obtained from 107 Thai pregnant women and 75 married women, into human foreskin fibroblast cell cultures. CMV was recovered 16 of 107 (14.9%) pregnant women, and 4 of 75 (5.3%) married women. The cervical swab specimens containing HCMV produced characteristic of CPE within 3-4 days but usually appeared 1-2 weeks or longer after inoculation. Spread of infection in the monolayer was rather slow and produced typical characteristics of CMV infection such as clusters of affected cells, scattering all over the entire monolayer cell surface, and not many of affected cells were

seriously damaged and detached from the surface of vessel. The cells in the affected area were enlarged, rounded, and refractiled as shown in Figure 5, and were differed from the normal human foreskin fibroblast cells as shown in Figure 6.

The cell suspensions from all cultures with characteristic of CPE were passaged into both human foreskin fibroblast cells and Vero cells. These cell suspensions could induce CPE only in the human foreskin fibroblast cells but could not produce the same effect in the Vero-cells. The isolates were identified as CMV by their typical characteristic appearance and slow progression of CPE, in addition to the failure to demonstrate viral replication in non-human cells such as Vero-cells.

Besides the typical characteristic of CPE and the host range specificity which were used as the criteria for HCMV identification, the positive isolates were also confirmed by an Indirect Immunofluorescent Antibody Test (IFA-Test) using a specific antibody to CMV as shown in Figure 7.

2.1 Isolation of Cytomegalovirus from Cervical Excretions of Pregnant Women and Married Women

As shown in Table 5, CMV was isolated from 16 of 107 (14.9%) pregnant women and from 4 of 75 (5.3%) married women. In the comparison of the cervical excretions between these two groups of women, CMV isolation in the pregnant women was statistically significant higher than that in the married women (By Chi Square Test at $df = 1, P < 0.05$).

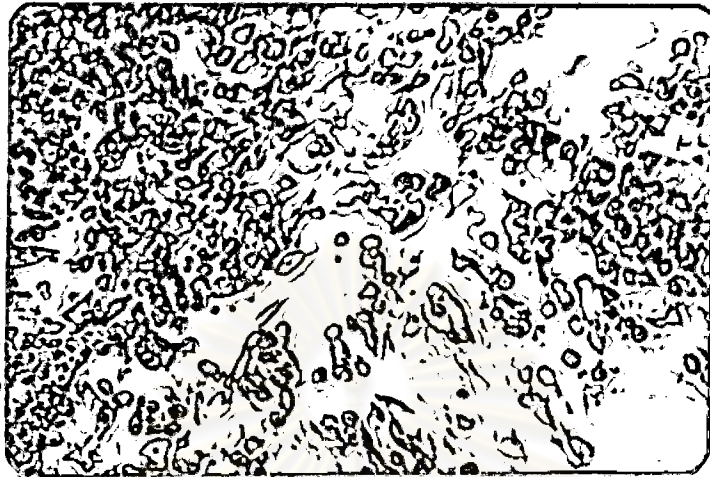


Figure 5 The cytopathic effect of human cytomegalovirus from clinical isolates in human foreskin fibroblast cells, 11 days after inoculation; unstained preparation. Magnification 100x

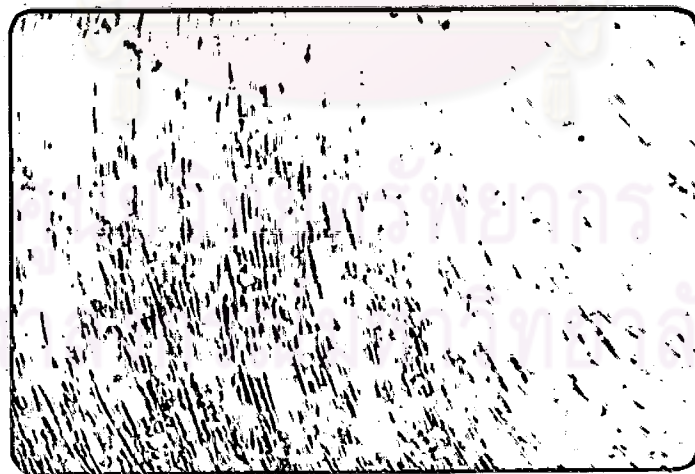


Figure 6 The monolayer of normal human foreskin fibroblast cells; unstained preparation. Magnification 100x



Figure 7 The typical fluorescence of infected human foreskin fibroblast cells induced by human cytomegalovirus from clinical isolates. Magnification 500x

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Table 5 Isolation of Cytomegalovirus from Cervical Excretions of Pregnant Women and Married Women

Study Populations	No. of Subjects Tested	No. of Culture Positive Subjects/ No. of Subjects Tested (%)
Pregnant Women Group Studied	107	16/107 (14.9)
Married Women Group Control	75	4/75 (5.3)

Chi Square Test at $df = 1, P < 0.05$

The characteristics of CMV-positive culture pregnant women were shown in Table 6. The mean of the age group was 24.69 and majority of the education was primary and secondary school (87.5%). In the vast majority of cases, the socioeconomic status was in the medium and low income, 50.0% and 37.5% respectively. Number of previous pregnancies was primigravida (56.25%) and Secundigravida (37.5%).

2.2 Isolation of Cytomegarovirus According to Age Group from Cervical Excretions of Pregnant Women

In pregnant women, all 16 CMV positive women were between 18 and 36 years of age. It was found as follows: 13.3% (2 of 15) in the ≤ 20 years of age, 14.9% (11 of 74) in the 21-30 years of age, 17.6% (3 of 17) in the 31-40 years of age, and none in the age group more than 40 years. However, this study showed that CMV isolation rate in any age group was no difference (By Chi Square Test, $P < 0.05$). Shedding of CMV related to age of these women was shown in Table 7.

2.3 Number of Pregnancies and Cytomegalovirus Isolation from Cervical Excretions in Pregnant Women

The rate of CMV recovery in relationship to the number of pregnancies was shown in Table 8. All 16 of the cervical swabs positive for CMV were from the women who had three or fewer than three pregnancies. It was found in 16.4%, 18.2% and 7.1% of the women who were pregnant for the first, the second and the third time, respectively, while none of those pregnant more than three times had CMV positive cultures. This study showed that

Table 6 Characteristics of CMV-Positive Culture Pregnant Women

Characteristics	CMV-Positive Culture Pregnant Women (n = 16)
Age (years)	
Range	18-36
Mean	24.69
Education (%)	
No Education	0
Primary and Secondary School	87.5
College and University	12.5
Unknown	0
Socioeconomic Status (%)	
Low (\leq 3,000 baht)	37.5
Medium ($>$ 3,000 - 6,000 baht)	50.0
High ($>$ 6,000 baht)	6.25
Unknown	6.25
Number of Previous Pregnancies (%)	
1 (Primigravida)	56.25
2 (Secundigravida)	37.50
3 (Tertigravida)	6.25
$>$ 3 (Multigravida)	0

Table 7 Isolation of Cytomegalovirus According to the Age Group from Cervical Excretions of Pregnant Women

Age (years)	Pregnant Women Group Studied	
	No. of Subjects Tested	No. of Culture Positive Subjects/ No. of Subject Tested (%)
<20	15	2/15 (13.3)
21-30	74	11/74 (14.9)
31-40	17	3/17 (17.6)
>40	1	0
Total	107	16/107 (14.9)

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Table 8 Number of Pregnancies and Cytomegalovirus Isolation from
Cervical Excretions in Pregnant Women

No. of Pregnancies	Pregnant Women Group Studied	
	No. of Subjects Tested	No. of Culture Positive Subjects/ No. of Subject Tested (%)
1	55	9/55 (16.4)
2	33	6/33 (18.2)
3	14	1/14 (7.1)
>3	5	0/5 (0)
Total	107	16/107 (14.9)

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CMV was recovered more frequently from the cervix of the women with two or less pregnancies. However, CMV recovery rate of the women who had any gravida was no difference (By Chi Square Test, $P < 0.05$).

2.4 Cervical Cytomegalovirus Infection Rate According to Stages of Gestation

Viral excretion derived from the cervical secretion of the pregnant women group studied who had been followed throughout pregnancy were summarized in Table 9. All 18 of the cervical swabs positive for CMV were from the 16 pregnant women because only one of these women had viral shedding in all three trimesters. It was recovered from the first trimester in 2.8%, from the second trimester in 11.8%, and from the third trimester in 6.4% with an overall recovery rate of 14.9%. Unlike the previous prospective surveys among Japanese (Numazaki et al., 1970) and American women (Montgomery et al., 1972, Stagno et al., 1975b) which noted that viral excretion increased significantly with advancing gestation. In the present study showed that CMV was recovered frequently in the second trimester, the third trimester and the first trimester, respectively. However, there was no difference of CMV recovery rate in any stages of gestation of the pregnant women (By Chi Square Test, $P < 0.05$).

3. Serological Study

Two hundred and sixty-two serum samples were obtained from 107 pregnant women and were determined for the levels of total antibody against CMV by a Complement Fixation Test (CFT).

Table 9 Cervical Cytomegalovirus Infection Rate According to Stages of Gestation

Stages of Gestation	Pregnant Women Group Studied	
	No. of Specimens Tested	No. of CMV-Positive Specimens/ No. of Specimens Tested (%)
First Trimester	107	3/107 (2.8)
Second Trimester	85	10/85 (11.8)
Third Trimester	78	5/78 (6.4)

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3.1 Immune Status of the Pregnant Women

To determine the immune status of the pregnant women to CMV infection, 107 serum samples obtained at the first visit to the Antenatal Care Unit were tested for the presence of CF-antibody. The result showed that 26.17% (28 of 107) of these women were seropositive (CF-Ab-Titer \geq 1:8) and 73.83% (79 of 107) of those were seronegative (Table 10). The characteristics of the two groups were shown in Table 11.

As pregnancy progressed, serial serum samples were obtained from the pregnant women at the second and the third trimester. Subsequent serological determination revealed that 37.97% (30 of 79) of the previously seronegative women had seroconversion. It may suggest that they have experience with CMV infection during their pregnancy. The 43.04% (34 of 79) of them still had no CF-antibody to CMV. In addition, none of the seropositive women had CMV-antibody rising titer (Figure 8). The data of CMV-CF-antibody titer levels of 30 seroconversion pregnant women were shown in Table 12.

3.2 Immunity to CMV of Pregnant Women at Different Gestational Stages

Serum samples, collected from all pregnant women, were divided into three groups according to the age of gestation (the first, the second and the third trimester) in order to determine the relation between the antibody titer and the gestational stages. The result showed that the immunity to CMV infection of the pregnant women was different among the three gestational stages.

Table 10 CMV-CF-Antibody Titer of Pregnant Women at First Trimester

CF-Antibody Titer	Number of Pregnant Women	
	Seropositive (%)	Seronegative (%)
0	-	79
8	21	-
16	4	-
32	1	-
64	1	-
128	1	-
Total	28 (26.17)	79 (73.83)

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Table 11 Characteristics of Pregnant Women with Seropositive and Seronegative at First Trimester

Characteristics	First Trimester	
	Seropositive Women (n = 28)	Seronegative Women (n = 79)
Age (years)		
Range	20-36	17-41
Mean	25.8	25.1
Education (%)		
No Education	7.1	2.5
Primary and Secondary School	75.0	78.5
College and University	14.3	12.7
Unknown	3.6	6.3
Socioeconomic Status (%)		
Low (\leq 3,000 baht)	53.5	45.5
Medium (>3,000 - 6,000 baht)	39.3	36.7
High (>6,000 baht)	3.6	8.9
Unknown	3.6	8.9
Number of Previous Pregnancies (%)		
1 (Primigravida)	35.7	56.9
2 (Secundigravida)	46.4	25.3
3 (Tertigravida)	7.2	15.3
>3 (Multigravida)	10.7	2.5

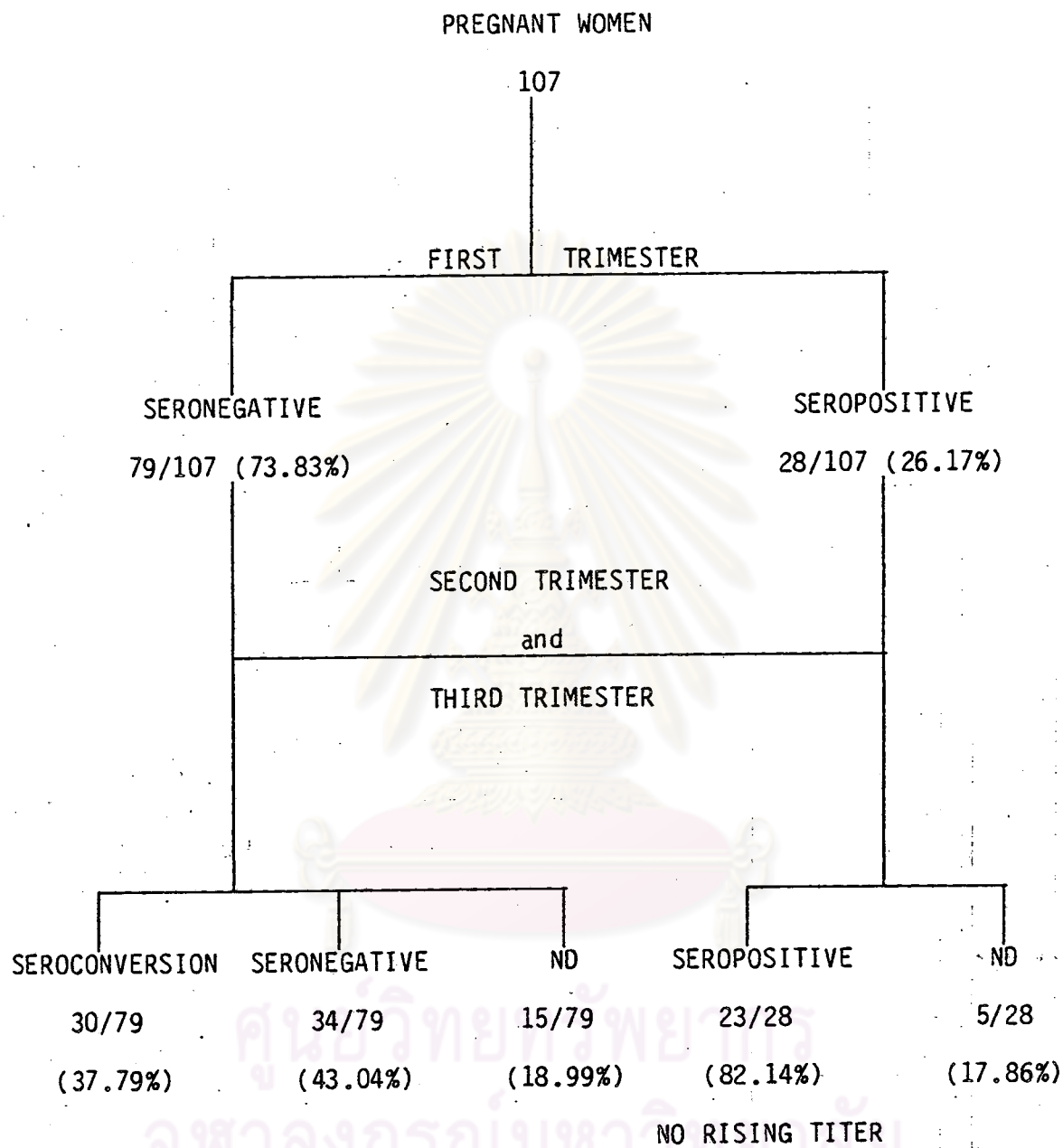


Figure 8 Immune Status of the Pregnant Women

Table 12 CMV-CF-Antibody Titer in 30 Seroconversion Pregnant Women

Study No. Seroconversion Pregnant Women	CMV-CF-Antibody Titer Related to Trimester		
	First	Second	Third
009	0	16	16
010	0	16	8
011	0	0	8(+)
017	0	0	8
023	0	0	8
027	0	16	16
030	0	8	ND
034	0	0	8(+)
036	0	8	8
038	0	8	16
043	0	16(+)	8
044	0	8(+)	8
049	0	8	8
051	0	8(+)	ND
055	0	8(+)	8
056	0	8	8
061	0	0	8(+)
062	0	8	8
064	0	8	16
066	0	ND	16
067	0	16	ND
068	0	8	8
070	0	8	8
078	0	8(+)	ND
079	0	8(+)	ND
087	0	8	8
099	0	8	8
100	0	8	8
108	0	8	8
110	0	8(+)	8

ND = Not Done

(+)= CMV-Positive Culture

If not indicated means culture negative

The immunity was 26.17% (28 of 107) during the first trimester and increased to 56.79% (46 of 81) in the second trimester and 60.81% (45 of 74) in the third trimester. It would mean that the pregnant women were more susceptible to CMV infection at the first trimester than at the second and the third trimester. This suggested that the prevalence of CMV infection often occurred during their pregnancy. The data of CMV-CF-antibody titer levels of the pregnant women at different gestational stages were shown in Table 13.

3.3 The Association of Cervical CMV Recovery and the Presence of CMV-Antibody in the Pregnant Women

In those 16 pregnant women experiencing CMV-positive cultures, 15 (93.15%) of them had CF-antibody to CMV whereas the only one showed no detectable level of CF-antibody. The 15 culture positive women who possessed CF-antibody, ten (66.7%) had seroconversion, indicating the primary infection with CMV during their pregnancy. Three (20.0%) of them had CF-antibody at the first trimester and became CMV-positive culture during the following period, suggesting of reactivation infection. The remaining 2 pregnant women (13.3%), one of them had CF-antibody and viral shedding at the first trimester while the other women had CF-antibody and viral shedding in all three trimesters, which the type of infection was inconclusive. All of these data were shown in Table 14.

Table 13 CMV CF-Antibody Titer Levels of Pregnant Women at Different Gestational Stages

Stages of Gestation	No. of Serum Samples with CMV CF-Antibody Titer						No. of Positive Serum Samples/ No. of Serum Samples Tested (%)
	0	8	16	32	64	128	
First Trimester	79	21	4	1	1	1	28/107 (26.17)
Second Trimester	35	30	14	0	2	0	46/81 (56.79)
Third Trimester	29	31	12	1	1	0	45/74 (60.81)
Total	93	82	30	2	4	1	119/262 (45.42)

Table 14 CMV CF-Antibody Titer in 16 CMV-Positive Cervical Culture Pregnant Women

Study No. of CMV-Positive Culture Pregnant Women	CMV CF-Antibody Titer Related to Trimester			Evidence of Infection
	First	Second	Third	
011	0	0	8(+)	Primary Infection
034	0	0	8(+)	" "
043	0	16(+)	16	" "
044	0	8(+)	8	" "
051	0	8(+)	ND	" "
055	0	8(+)	8	" "
061	0	0	8(+)	" "
078	0	8(+)	ND	" "
079	0	8(+)	ND	" "
110	0	8(+)	8	" "
022	8	8(+)	ND	Reactivation
041	8	8(+)	8	"
111	8	16	8(+)	"
008	8(+)	16	16	Inconclusive
046	8(+)	8(+)	8(+)	"
091*	0(+)	0	0	"

ND = Not Done
 (+) = CMV-Positive Culture, If not indicated means culture negative
 * = The Seronegative Pregnant Women Possessing CMV-Positive Culture