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**CLINICO-MICROBIOLOGICAL STUDY OF CMV IN PREGNANT FEMALES WITH BAD OBSTETRIC HISTORY (BOH)****<sup>1</sup>Neha Singh, <sup>2</sup>Anant Dattatray Urhekar, <sup>3</sup>Sareena Rao**<sup>1</sup>Associate Professor, Department of Microbiology, M.G.M Medical College, Vashi, Navi Mumbai.<sup>2</sup>Ex- Professor & HOD, Department of Microbiology, M.G.M Medical College, Navi Mumbai.<sup>3</sup>Tutor, Department of Microbiology, M.G.M Medical College, Vashi, Navi Mumbai**Corresponding Author****Neha Singh**

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**ABSTRACT**

**Background:** CMV is a virus of paradoxes & can be a potential killer or a silent companion lifelong. It is probably one of the most common infections known to humans & is characterized by self-limiting infection in healthy individual. In addition to inducing severe birth defects, CMV causes a wide spectrum of disorders in older children & adults, ranging from an asymptomatic sub-clinical infection to a mononucleosis syndrome in healthy individuals to disseminated disease in immunocompromised patients. The aim of the study is to assess the seroprevalence of IgG and IgM antibodies for CMV in women with normal pregnancy, pregnant women with one abortions, age, parity and trimester of pregnancy.

**Materials and Methods:** Serum samples were collected from pregnant women attending antenatal clinic with demographic data. Patients were divided into three groups- Normal pregnant women without any history of abortion (N), pregnant patients with one abortion (A1) and those with two or more abortions (A2) that is those with a Bad Obstetric History (BOH). 71 serum samples were subjected for determination of CMV IgM antibodies and 63 for CMV IgG antibodies. The study was carried out over a period of two years from November 2012 to October 2014.

**Results:** Research findings indicated that CMV IgM prevalence was 12.67% and that of IgG was 92.06%. The distribution showed increase of IgG and IgM antibodies with increase in the number of abortions. In normal group patient's (N) IgG was mostly positive for patients with gestational age 3rd trimester (29-40 weeks). The distribution also showed that in this group IgG was most positive for those in the age group 20-25 years. In group A1 patient's IgG was found to be equally positive for those with gestational age 2nd trimester (13-28 weeks) and 3rd trimester (29-40 weeks).. In this group IgG was mostly positive for those in the age group 20-25 years. The distribution for group A2 patient's showed an increase of IgG antibodies for those with gestational age 3rd trimester (29-40 weeks). The patients of this group also showed increased IgG in the age group 20-25 years.

Conclusion: High prevalence of IgG indicates largely sub-clinical infection. The overall IgM prevalence was 12.67% which indicated recent exposure of the patients. In normal group patients, only 1 was found to be positive for IgM antibodies. She belonged to age group 36-40 years and gestational age 1st trimester (1-12 weeks). In group A1 patients IgM was found to be equally positive for those with gestational age 2nd trimester (13-28weeks) and 3rd trimester (29-40weeks) .In this group IgG was mostly positive for those in the age group 20-25 years. The distribution for group A2 patients showed an increase of IgM for those with gestational age 3rd trimester (29-40 weeks) and age group 20-25 years.

**Keywords:** CMV, mononucleosis syndrome, BOH

## INTRODUCTION

CMV is a DNA virus. Fetus is affected by transplacental route in about 30% - 40% cases. The consequences of infection include—abortion, stillbirth, IUGR, microcephaly, intracranial calcification, hepatosplenomegaly, thrombocytopenia, chorioretinitis, mental retardation & deafness.(1)

Maternal CMV is the commonest viral infection in the perinatal period and is the leading cause of Congenital CMV infections.(2)Intrauterine transmission of CMV to the baby can occur irrespective of prior maternal exposure. The incidence of Congenital CMV ranges from 0.5-3.0% in all live births. CMV is also linked to late abortions and still births.(3) In different parts of India, serological surveys have shown 80-90% prevalence of CMV IgG antibodies in women of child bearing age. Risk of seroconversion during pregnancy averages 2.0–2.5%.

Gestational age has no influence on risk of intrauterine transmission; however, clinical consequence for infected offspring appears to be worse when infection takes place before 20 weeks. (4,5)

## AIM

The aim of the study was to assess the seroprevalence of IgG and IgM antibodies for CMV for women with normal pregnancy, pregnant women with one abortion and pregnant women with two or more abortions (BOH) Bad Obstetric History. The sero-positivity was analysed according to number of abortions, age, parity and trimester of pregnancy.

## MATERIAL AND METHODS

The study was an experimental and analytical study carried out in the Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai over a period of two years from November 2012 to October 2014. Ethical clearance was obtained from institutional ethics committee prior to study. Blood samples were collected from pregnant women attending antenatal clinic and patients admitted in antenatal ward of the hospital after taking written informed consent to perform ELISA test for CMV IgM and IgG on the serum samples.

The IgM and IgG Elisa kit was manufactured by Delta Biologicals a subsidiary of Erba Diagnostics and supplied by Trans Asia Biomedicals Ltd. The CMV IgM Elisa Kit bearing lot no 261-C with expiry date 2015-06 had a sensitivity of 89.3% and specificity of 91.1%. The CMV IgG Elisa Kit with lot no-210 and expiry date 2015-09 had both sensitivity and specificity of 100 %. The test was performed as per instructions of the manufacturer.

IgG Elisa-The controls consisted of one negative control and 5 calibrators as positive control Validity and test results were calculated as per literature of the manufacturer.

Validity Criteria-

- a) OD of negative control was  $<0.6$  times the OD of calibrator 2
- b) OD of C2 was more than 0.16 at 450 nm.
- c) OD of C5 was more than 1.5

Cut off corresponds to calibrator 2. Ratio between OD value of sample and that of cut off was  $\geq 1.2$  and was considered as Positive result.

IgM Elisa- The controls consisted of two negative controls (NC), two positive controls (PC) and two cut off calibrators. The mean absorbance of NC, cut off calibrator and PC was calculated.

#### Validity Criteria-

- a) Blank well-Blank absorbance was  $<0.050$  at 450/630.
- b) NC-Mean absorbance after subtracting blank absorbance was  $<0.150$ .
- c) Cut off calibrator- Mean absorbance after subtracting blank absorbance was  $>0.150$  and  $<0.400$ .
- d) PC- Mean absorbance after subtracting blank absorbance was  $>0.500$ .

The index value was calculated to obtain the qualitative specimen results

The cut-off value was obtained by subtracting the blank absorbance from the mean absorbance of the cut off calibrator. The index value was calculated by dividing the specimen absorbance by the cut off value.

Index value was  $>1.1$  and was considered Reactive.

A predefined proforma was used to collect the data regarding age of the patient, gestational age (trimester) and number of abortions. The result of ELISA test was then subjected to statistical analysis.

## RESULT

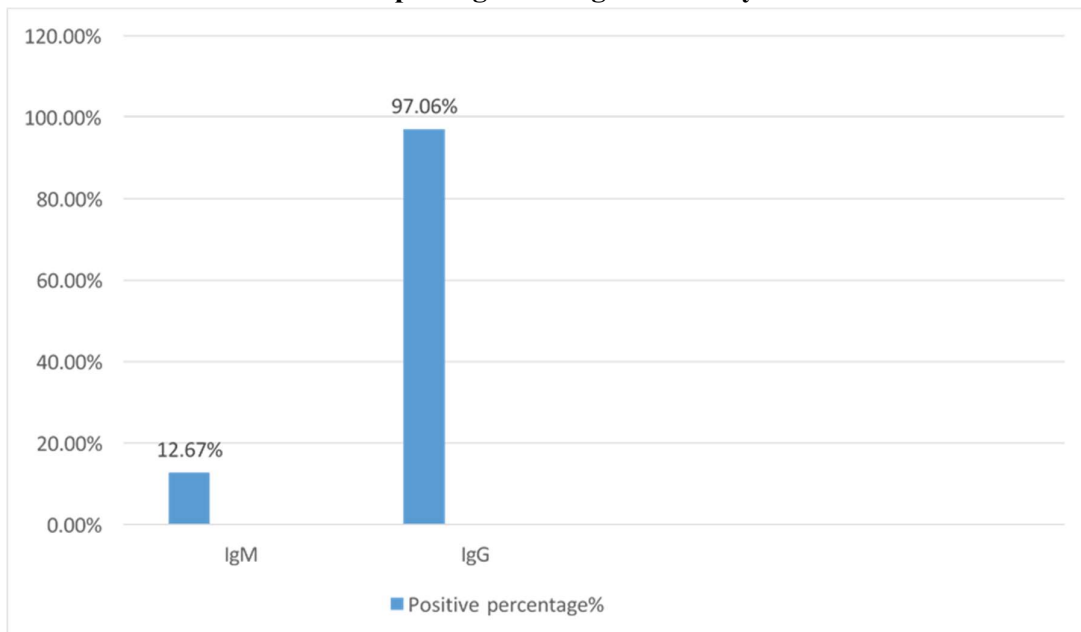
A total of 71 samples were taken for detection of IgM antibodies against CMV out of which 12 samples belonged to group N which consisted Normal ANC patients, 32 belonged to group A1 which consisted ANC patients with one abortion and 27 belonged to group A2 consisting of ANC patients with two or more abortions (BOH). Whereas a total of 63 samples were taken for detection of IgG antibodies against CMV out of which 12 samples belonged to group N, 29 samples belonged to group A1 and 22 samples belonged to group A2. Prevalence of IgM positivity was 12.67% and that of IgG was 92.06% (Table:1). The distribution of abortion has shown that the prevalence of IgM increase from 8.33% to 14.81% and that of IgG from 83.33% to 95.45% with increase in number of abortions (Table 2). Only 9 patients out of the total of 71 subjected for determination of IgM antibodies against CMV was found to be positive. Only one patient belonged to group N group, age group 36-40 years, gestational age first trimester (0-12 weeks) and parity -0. 4 patients were found to be positive in A1 group and again 4 patients were found to be positive for CMV IgM antibodies in A2 group. The age group distribution has shown high rate of IgM positivity for both A1 and A2 in age group 20-25 years (50%). (Table 3). The gestational age distribution shows that IgM was equally positive group A1 patients with gestational age 2nd and 3rd trimester (50%) but for A2 group patients, IgM was most positive for patients with gestational age 3rd trimester (50%). (Table 4)

The age group distribution has shown high rate of IgG positivity for age group 20-25years in all the 3 groups of patients that is normal patients (70%), A1group patients (40.7 %) and A2 group patients (47.6%) (Table 5). The gestational age distribution show that IgG was positive for group N patients with gestational age 3rd trimester (29-40 weeks) (40%), in group A1 patients IgG was equally positive with gestational age 2nd trimester (13-28weeks) and third trimester (37.03%). For A2 group patients again IgG was most positive with gestational age 3rd trimester (57.1%) (Table 6).

**Table1: IgM and IgG positivity in total number of subjects**

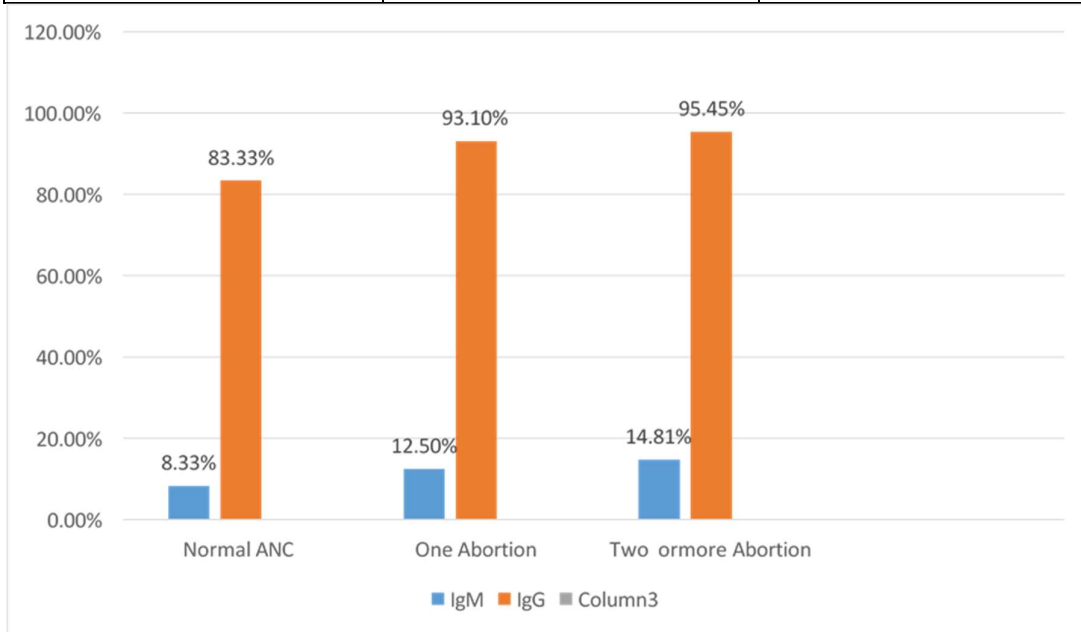
Antibody	No. of Samples	No. of Positive Samples	Positive Percentage (%)	P value
<b>IgM</b>	71	9	12.67%	0.008 Highly Significant
<b>IgG</b>	63	58	92.06%	0.0001 Highly Significant

**Graph1: IgM and IgG Positivity**



**Table2: IgM and IgG positivity as per three groups of patients**

Abortion Distribution	IgM	IgG
Normal ANC	1/12= 8.33%	10/12= 83.33%
One Abortion	4/32= 12.5%	27/29= 93.1%
Two or more Abortion	4/27=14.81%	21/22= 95.45%



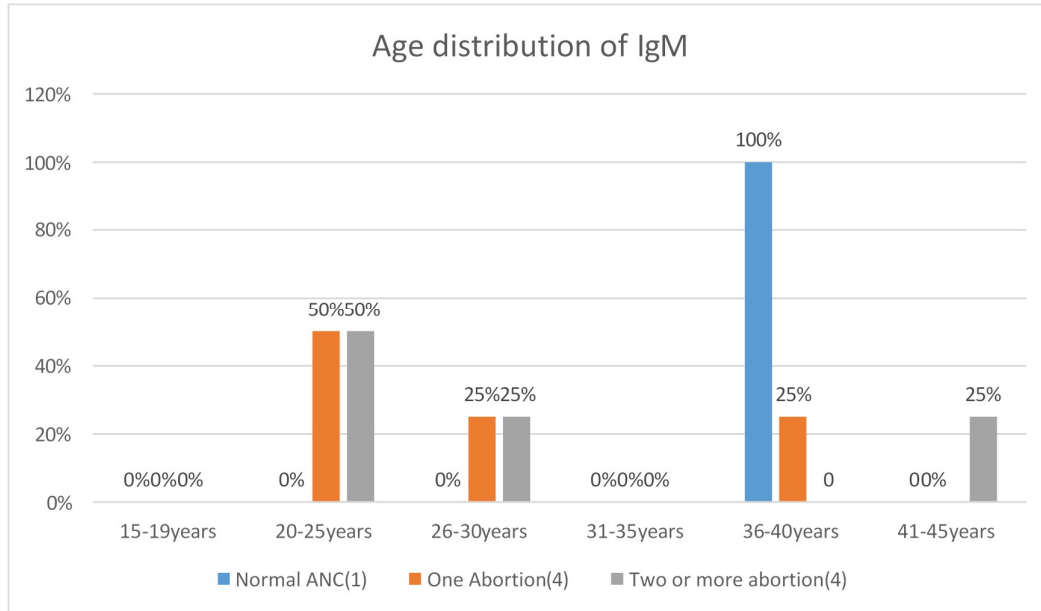
Graph2: IgM and IgG Positivity increase with increase in number of abortions

**Table3: Age distribution of subjects (IgM)**

Age Distribution	Normal ANC(1)	One Abortion(4)	Two or more abortion (4)
15-19years	0 (0%)	0 (0%)	0 (0%)
20-25years	0 (0%)	2 (50%)	2 (50%)
26-30years	0 (0%)	1 (25%)	1(25%)
31-35years	0 (0%)	0 (0%)	0 (0%)

<b>36-40years</b>	1 (100%)	1 (25%)	0 (0%)
<b>41-45years</b>	0 (0%)	0 (0%)	1 (25%)

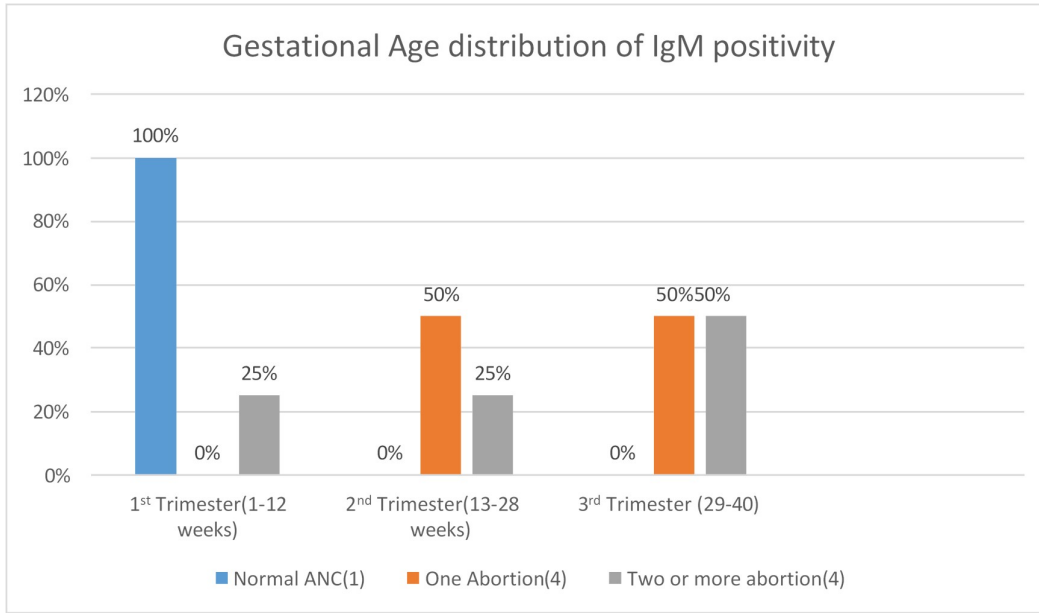
**Table 4: Gestational Age distribution of IgM positivity**



**Graph4: Gestational Age distribution of IgM positivity**

**Table 4: Gestational Age distribution of IgM positivity**

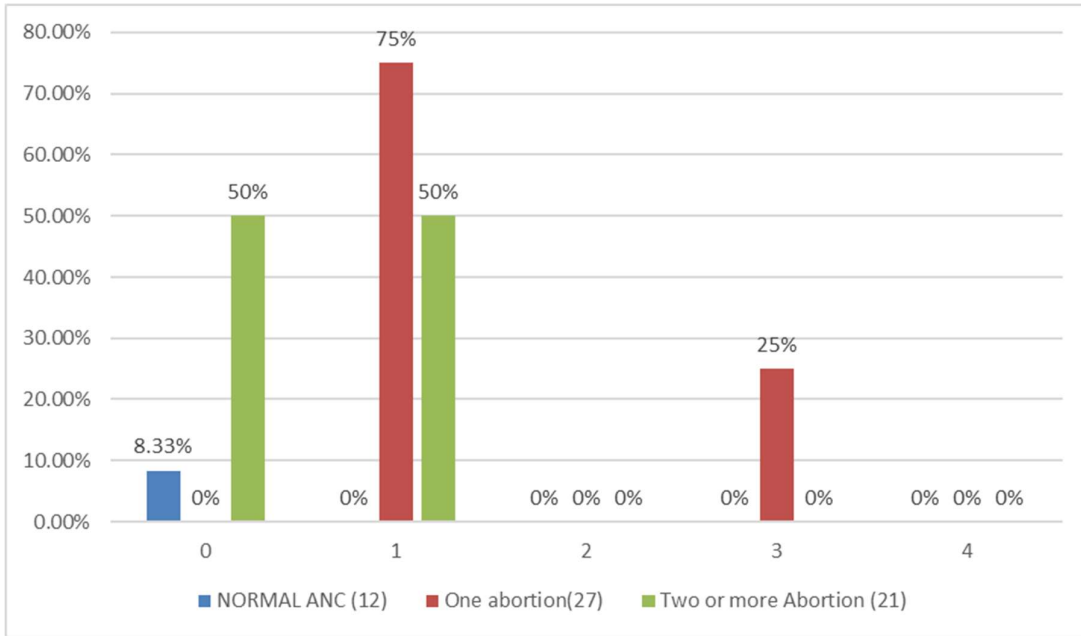
<b>GestationalAge</b>	<b>NormalANC(1)</b>	<b>OneAbortion(4)</b>	<b>Two or more abortion (4)</b>
<b>1<sup>st</sup> Trimester(1-12 weeks)</b>	1 (100%)	0 (0%)	1 (25%)
<b>2<sup>nd</sup> Trimester(13-28 weeks)</b>	0 (0%)	2 (50%)	1 (25%)
<b>3<sup>rd</sup> Trimester(29-40)</b>	0 (0%)	2 (50%)	2 (50%)



Graph4: Gestational Age distribution of IgM positivity

Table 5: Parity Distribution of IgM

PARITY	NORMAL ANC (12)	One abortion(27)	Two or more Abortion (21)
0	8.33%	0%	50%
1	0%	75%	50%
2	0%	0%	0%
3	0%	25%	0%
4	0%	0%	0%

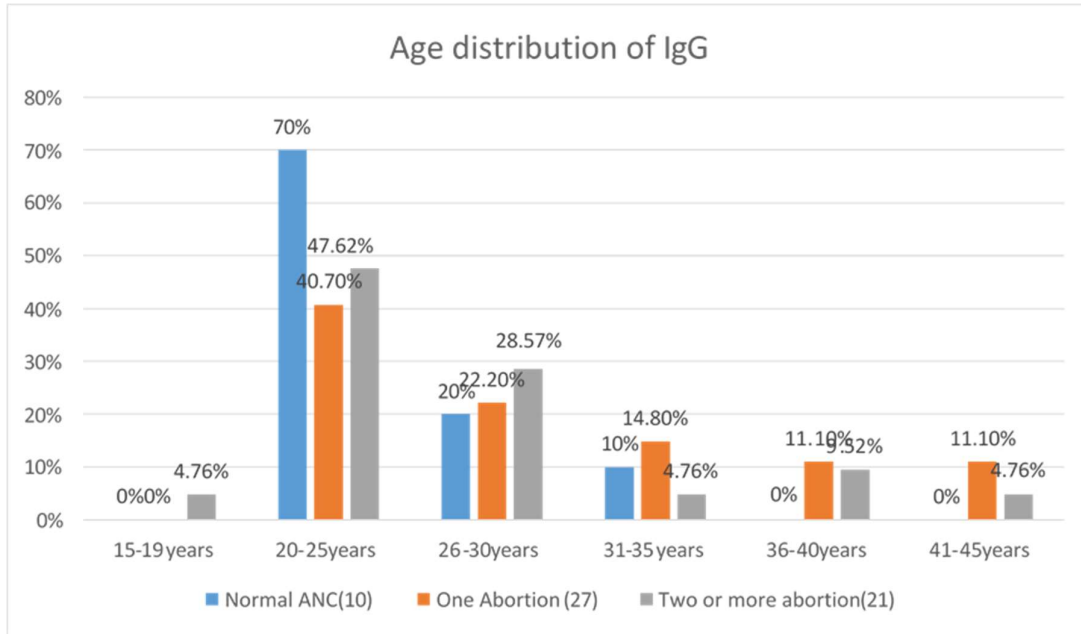


**Graph 5 Parity Distribution of IgM**

**Table 6: Age distribution of subjects (IgG)**

Age Distribution	Normal ANC(10)	One Abortion(27)	Two or more abortion (21)
15-19years	0 (0%)	0 (0%)	1 (4.76%)
20-25years	7 (70%)	11 (40.7%)	10 (47.62%)
26-30years	2 (20%)	6 (22.2%)	6 (28.57%)
31-35years	1 (10%)	4 (14.8%)	1(4.76%)
36-40years	0 (0%)	3 (11.1%)	2 (9.52%)
41-45years	0 (0%)	3 (11.1%)	1 (4.76%)

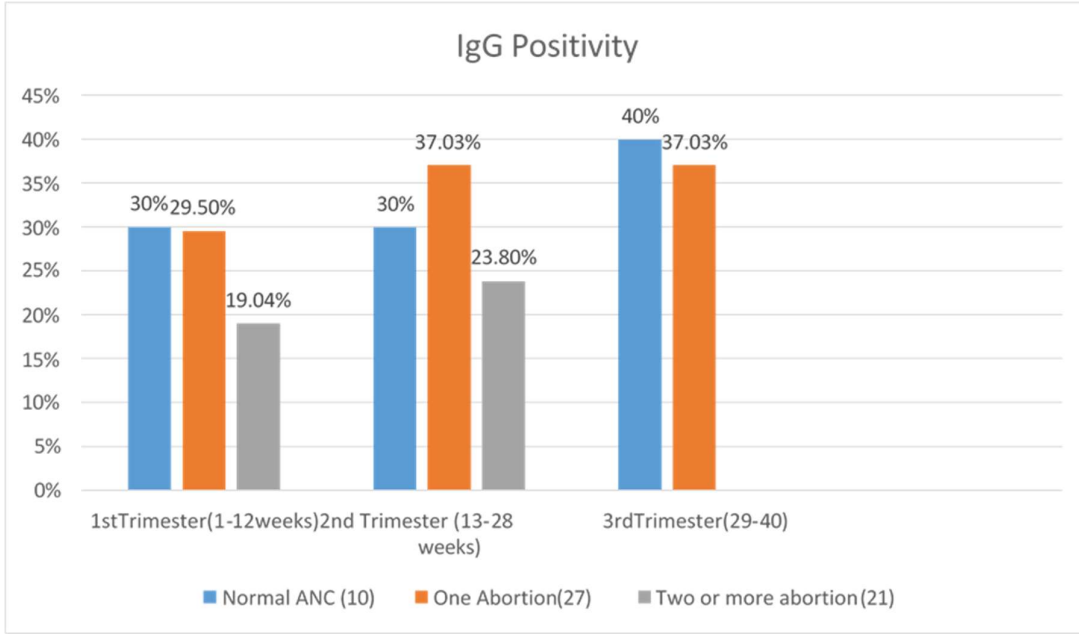




**Graph6: Age distribution of subjects (IgG)**

**Table7: Gestational Age distribution of IgG positivity**

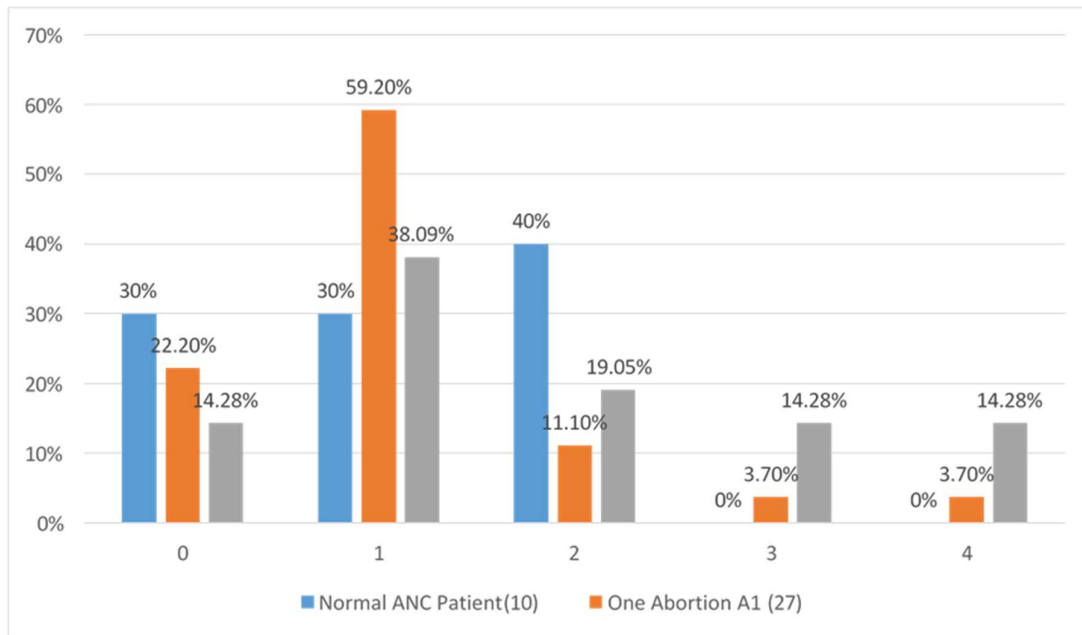
Gestational Age	Normal ANC(10)	One Abortion(27)	Two or more abortion (21)
<b>1<sup>st</sup> Trimester(1-12 weeks)</b>	3 (30%)	7 (25.9%)	4 (19.04%)
<b>2<sup>nd</sup> Trimester(13-28 weeks)</b>	3 (30%)	10 (37.03%)	5 (23.8%)
<b>3<sup>rd</sup> Trimester(29-40)</b>	4 (40%)	10 (37.03%)	12 (57.14%)



**Graph7: Gestational age distribution showing IgG Positivity**

**Table 8: Parity Distribution of IgG**

Parity(IgG)	Normal ANC Patient (10)	One Abortion A1 (27)	Two or More Abortion A2(21)
0	3 (30%)	6 (22.2%)	3 (14.28%)
1	3 (30%)	16 (59.2%)	8 (38.09%)
2	4 (40%)	3 (11.1%)	4 (19.05%)
3	0 (0%)	1 (3.7%)	3 (14.28%)
4	0 (0%)	1 (3.7%)	3 (14.28%)



Graph 8 :Parity Distribution of IgG

## DISCUSSION

The CMV IgG and IgM antibodies were tested by ELISA method. In this study, out of 63 cases subjected to CMV IgG ELISA, 58 cases (92.06%) are positive for CMV specific IgG antibodies. (p value:0.0001 Significant) Out of 63 cases, 12 cases are of normal pregnancy (NP) without abortion. The seroprevalence of IgG in this group is 83.33% (10/12). Similarly, out of 63 cases 29 cases are patients with history of one abortion. The seroprevalence of IgG in the group 93.1% (27/29). Out of 63 cases, 22 patients are with a history of 2 or more abortions (A2) (BOH) with an IgG seroprevalence rate of 95.45% (21/22). The present study shows that seroprevalence for IgG increases with number of abortions (Table 2). Presence of IgG antibodies indicates prior exposure, infection or vaccination IgG seropositivity also increases with gestational age (Table 7).

A total of 71 cases were subjected to CMV IgM ELISA, 9 cases 12.67% were seropositive for IgM antibody (p-value: 0.008 significant). 12 cases out of 71 are of normal pregnancy (NP) without abortion, the IgM seropositivity in this group is 8.33% (1 out of 12 cases), 32 cases are with history of one abortion (A1) an IgM seropositivity rate 12.5%(4 out of 32) and 27 cases are BOH with history of two or more abortions (A2) with an IgM seropositivity of 14.81% (4 out of 27 cases).

The geographical distribution of prevalence of CMV in pregnant women has been evaluated by different authors in India and abroad. The CMV IgG prevalence in normal pregnancy in our study was 83.33% which is closer to IgG prevalence of 96.8% as reported by Denoj Sebastianetal(6)from kerala and Hala Mohammed et al(7) from Iraq (90%). The CMV IgM antibody prevalence in normal pregnancy in our study was 8.33%which is closer to the results of Mini.P.Singh et al(3) from New Delhi (7.8%) and Hala Mohammed et al(7) (9.3%).

The CMV IgM prevalence in BOH group in our study is 95.45% which is closer to the findings reported by D.Turbadkar et al (8) from Mumbai (91.05%) Padmavathy et al (2) from Bangalore (95.4%) and Hala Mohammad et al(7) (94.2%). Lower prevalence was reported by

Kh.Sulochana Devi et al(9) Imphal (Manipur) (72.09%) and M.J.Golalipour et al(10)from Iran (58.7%). The CMV IgM prevalence in BOH patients in our study was 14.81%. This finding was closer to a 10.8% IgM prevalence reported by Mini P.Singh et al (3) Higher values were reported by Shashi Chopra et al (2) (24%), and Denoj Sebastian et al(6) (28.2%).

In our study the CMV IgG antibody prevalence in normal pregnancy NP was maximum in the age group 20-25 years (70%), in gestational age 3rd Trimester (40%) and with parity 2 (40%). The CMV IgM prevalence in NP was 8.33% (1/12). This patient belonged to age group 36-40 years, 3rd trimester and parity-0.

The prevalence of CMV IgG in BOH group (A2) (95.45%) was maximum in the age group 20-25 years (47.6%), 3rd trimester (57.1%) and with parity-1 (38.09%). The CMV IgM antibody prevalence in BOH group (14.81%) was maximum in the age group 20-25 years (50%),3rd trimester (50%) and with parity-1&2 (50%).

## CONCLUSION

Our study on CMV seroprevalence indicates that the prevalence of CMV IgG antibodies increases with the age, gestational age & the number of abortions.(NP=20-35years,A1=20-40 years, A2=15-45 years). The CMV IgG antibodies were found in the all 3 trimesters and most of it in the 3rd trimester of pregnancy. Prevalence of CMV IgG antibodies also increased with the parity of the patient (NP=0-2,A1=0-4,A2=0-4). The prevalence of CMV IgG antibodies increased from normal pregnancy group NP to patients with one abortion (A1) and two or more abortion (A2).

The CMV prevalence IgM antibodies increased with gestational age and number of abortions from 8.33% in NP to 12.5% in A1 and 14.81% in A2. The CMV IgM antibodies were found in the all 3 trimesters and most of it in the 3rd trimester of pregnancy. The presence of CMV IgM antibody in these two patient with one and two abortions, indicates a close association with CMV infection. Early detection in pregnant women can reduce severity of the disease in the new-born.

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