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IgM-based Prevalence of Rubella virus, Cytomegalovirus, and Herpes Simplex Virus- A study from Bihar Region, India

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Abstract: **The rubella virus (RUV), cytomegalovirus (CMV), and herpes simplex virus (HSV) cause mild illness in immune-competent individuals, pregnant women, and fetus/newborns. The study estimated the prevalence of RUV, CMV, and HSV infection based on IgM antibody levels, and also investigated the association of seasonal variation. The study involved analysis of laboratory based data of blood sample over a period of 4 years with reference to RUV, CMV, and HSV. A total of 2027 patients were analyzed to determine the prevalence of IgM antibodies against the RUV, CMV, and HSV. The positive IgM antibody against RUV, CMV, and HSV was 9.08 %, 4.39%, and 8.19%, further 3.30%, 1.48%, and 1.73% were found equivocal respectively. The majority of positive cases were seen from July to September. So, based on this study, we can say that serological testing has been crucial for the control and monitoring of RUV, CMV, and HSV viruses. Additionally, during the impacted period, preventive actions should be done to reduce losses caused by these viruses.**

Index Terms: **ToRCH infection, RUV, CMV, HSV, IgM, seasonal variation.**

I. INTRODUCTION

The ToRCH stands as *Toxoplasma gondii* (TG), other infections (includes, HIV, syphilis, Hepatitis viruses, varicella virus and Parvovirus B19), rubella virus (RUV), cytomegalovirus (CMV), and herpes simplex virus type 1 and 2 (HSV 1 and 2). However, the majority of clinical laboratories provide serological testing for the TG, RUV, CMV, and HSV viruses (Batra *et al.*, 2020). These viruses are generally cause very mild illness in immune-competent adults, but during pregnancy, they can cause severe complications in the fetus and newborn. Depending on the gestational age during transplacental infection, there are several problems including intrauterine growth restriction (IUGR), congenital abnormalities (moderate to long-term sequelae), and foetal death (Stegmann and Carey, 2002;Karacan *et al.*, 2014; Prasoona *et al.*, 2015). They passed by prenatal (via transplacental transmission), perinatal (via blood and vaginal secretion during birth), and, in rare cases, postnatal (breastfeeding). Prenatal infections by these viruses cause nearly 3% of all congenital anomalies

(Stegmann and Carey, 2002; Numan *et al.*, 2015). Due to a condition of selective immunological tolerance, immunosuppression, and immunomodulation for physiologic adaptation, pregnant women are more susceptible to several viral illnesses. The fetus's weak and developing immune system is also powerless to stop the spread of infectious pathogens that get through the placental barrier (Karacan *et al.*, 2014; Şirin *et al.*, 2015; Sirin *et al.*, 2017).

These four diseases are mostly asymptomatic or have mild clinical manifestations and diagnosis during pregnancy is frequently missed (Karacan *et al.*, 2014; Chung *et al.*, 2018). Preventive antenatal screening and counseling, leading to early diagnosis, is thus the most effective way to control birth defects caused by prenatal infection by these four viruses (Chung *et al.*, 2018; Deka *et al.*, 2022).

The enzyme-linked immunosorbent assay (ELISA) has been the most common test used for detection of IgM and IgG antibodies against these viruses. Other methods for detecting virus-specific immunoglobulins (IgM and IgG) include automated chemiluminescent immunoassay (quantitative), indirect immunofluorescence assay, and lateral flow chromatographic immunoassay (both qualitative) (Li *et al.*, 2009; Numan *et al.*, 2015; Chen *et al.*, 2019; Batra *et al.*, 2020). The IgG avidity test is critical in distinguishing between patients with acute infection and those with chronic infection. Molecular assays with high sensitivity and specificity, such as polymerase chain reaction (PCR), are useful only for molecular typing and not for routine screening in resource-constrained settings.

The ToRCH infection has been less studied in this region of Bihar therefore the study was designed to estimate the IgM based prevalence of RUV, CMV, and HSV infection in different districts of Bihar among suspected patients and also to investigate the association of seasonal variation with infection of these viruses.

II. Methodology

A. Study Site and Design

This retrospective laboratory-based study was conducted in the Viral Research and Diagnostic Laboratory (VRDL), Department of Virology, Rajendra Memorial Research Institute of Medical Sciences (ICMR-RMRIMS), in Patna, Bihar, India. This is the only institute of Indian Council of Medical Research (ICMR), autonomous body of Ministry of Health and Family Welfare, Government of India, in Bihar. The study involved collection, classification, and analysis of blood sample data over a period of 4 years (January 2018 to December 2021) with reference to RUV, CMV and HSV.

B. Source of Samples and Inclusion Criteria

The study included blood samples of 2027 patients which attended the different hospitals of multiple districts of Bihar and referred to our VRDL for testing of RUV, CMV and HSV viruses. The samples of those patients with complete demographic data and which were tested for all the three viruses i.e. RUV, CMV and HSV were included in the study.

C. Screening of Samples

Blood samples were collected in plane vacutainer from all individuals and processed immediately after collection. The collected samples were subjected for the centrifugation at 1200 × g for 15 minutes to collect the serum from the blood and serum were aliquoted serum were stored at a -20°C for further processing. The presence of IgM level for RUV, CMV and HSV were measured by using Calbiotech IgM ELISA kits (Calbiotech R5EC 96 well ELISA, El Cajon, USA) independently for each virus. An automated Euroimmune Analyzer 1 (Walkaway automated seven plate ELISA reader; Euroimmun, Lübeck, Germany) was used to assess the optical density (OD) at 450 nm absorbance. Further results were interpreted with the antibody index (AI) calculation by dividing the OD value of every one sample by cut-off value. An AI of less than 1.1 was reported as positive, <0.9 was considered as negative and 0.9- 1.1was equivocal. In every run internal quality control were performed according to the as per kit protocol (Deka *et al.*, 2022).

D. Statistical Analysis

For statistical purposes, An Excel file (Microsoft Office 2007) was created and errors were removed before creating the graph and tables. To conduct statistical tests and descriptive statistics, the standard SPSS for Windows software programme, version 22.0 (IBM SPSS Statistics), was utilised.

Overall IgM against RUV, CMV, and HSV was subjected to descriptive statistics. The associations of ToRCH infections with the independent factors were calculated by using a chi-square test (two-tail test with a 95% confidence interval). The contingency coefficient (CC) was used to determine the relationship between dependent and independent variables. A moving average additive model was used for the trend analysis as well as seasonal variation.

III. Results

The included blood samples were the highest samples from our center (52.74%) followed by Nalanda Medical College and Hospital (NMCH) Patna (17.37%), Sri Krishna Medical College and Hospital (SKMCH) Muzaffarpur (16.38%), other private cenres from Patna (6.46%) (Figure1). Of the 2027 blood samples, IgM positive samples for RUV was found, 184 (9.08 %), for CMV 89 (4.39%) and for HSV 166 (8.19%). The equivocal results were also noted as 67 (3.30%), 30 (1.48%) and 35 (1.73%) for RUV, CMV, and HSV respectively. A detailed flowchart of summarized result has been shown in figure 2. The overall percentage of RUV, CMV, and HSV for the year January 2018 and December 2021 has been described as macro information, in which positive, negative, and equivocal results

has been shown in quarterly manner like January to March (Q1), April to June (Q2), July to September (Q3) and October to December (Q3) (Table 1). Some values are frequency based and some values are percentage form. Total and grand total mechanism was used for transparent information. Most of the samples (63%) was collected in year 2019 but gradually decrease during the period of COVID-19 situations was noted (Figure 3).

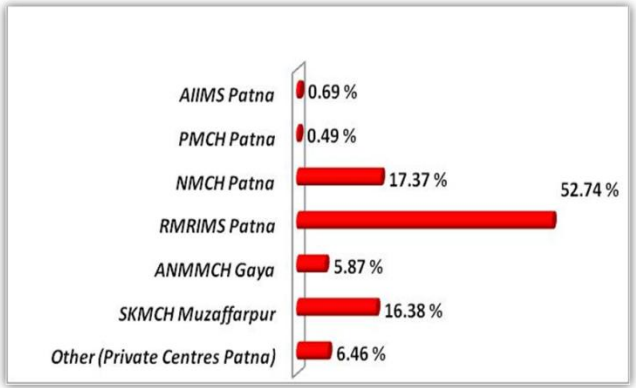

Figure 1: Graphical representation of the samples referred from different health facility to our VRDL.

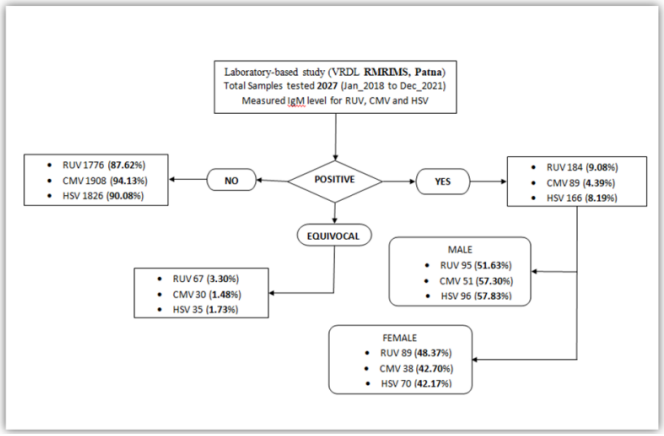

Figure 2: A flowchart with detailed summary of the study's findings.

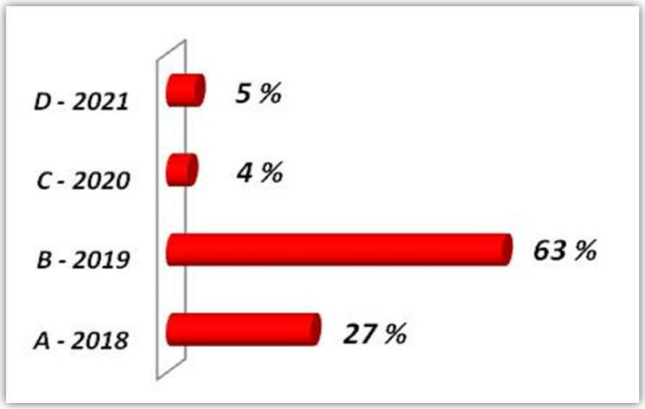

Figure 3: Graphical representation of the percentage of samples tested in different years.

The demographic of the patients showed that most of the patients with RUV, CMV and HSV were found in the age group less than 12 years. The 50% IgM antibody for RUV belonged to the age group less than 9 years only. In case of CMV and HSV, IgM antibodies were found in the patients belonging to the age group less than 15 years and 20 years respectively (Figure 4). Large variation of the age was found in positive cases for IgM CMV with standard error 2.04. The median age of IgM RUV, IgM-CMV and IgM-HSV were 9, 15 and 20 years respectively. Wide range of age was covering IgM CMV with confidence interval for mean at 95% (15.78 – 23.90) as compare to IgM RUV and IgM HSV. IgM RUV has less value of statistic like Median, Range, SD and SE was 9, 61, 13.51, and 1.63 respectively as compare to IgM CMV and IgM HSV (Table 2).

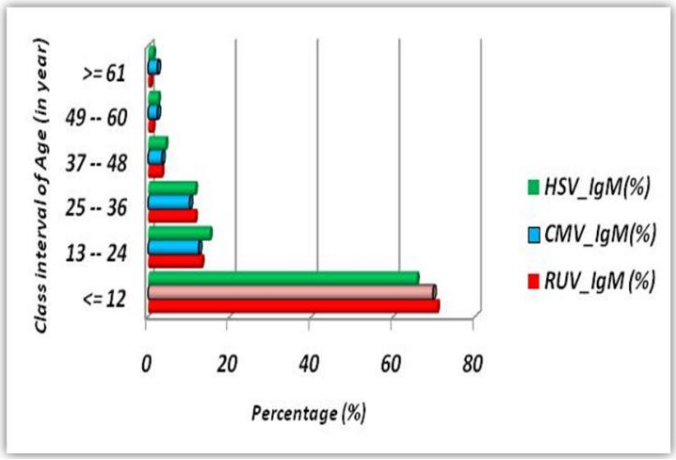

Figure 4: Graphical representation of the age distribution of the positive cases for RUV, CMV and HSV infection.

Generally RUV, CMV and HSV infection was found peak in the month of July to September (Q3) which slowly increase from month of April to June (Q2) and decrease from peak for each year from 2018 to 2021 (Figure 5). RUV trend was more up and down as compare to others. Further in the month of July to September the seasonal variations for RUV, CMV and HSV was 183.13, 210.64 and 142.90 respectively (Figure 6). Minimum seasonal variation of RUV and HSV cases were detected of at quartile Q1 (January to March) but CMV in second quarter Q2 (April to June) as shown in the figure 7 & 8. The characteristics of seasonal variation of CMV were different as compare to other.

Testing of hypothesis was done with help of Non-Parametric test Chi-square at 95% confidence interval which was not statistical significant between gender, age against outcomes (IgM RUV, IgM CMV, IgM HSV). Further contingency coefficient (C.C.) test was also done for estimating the association, and found no appreciable between dependent variables gender and age against outcomes (IgM RUV, IgM CMV, IgM HSV) as shown in table 3.

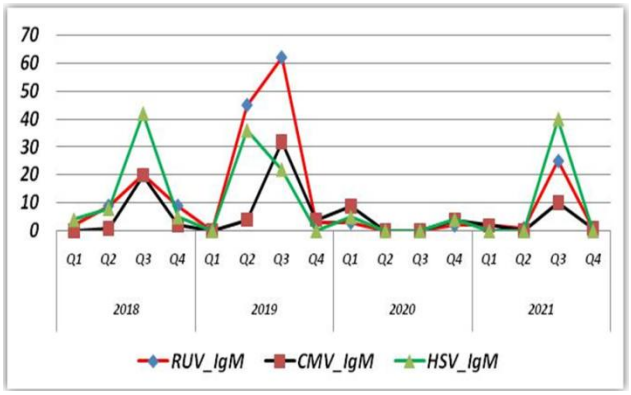

Figure 5:- Graphical representation of the seasonal trends of positive cases in different years and in quarterly manner.

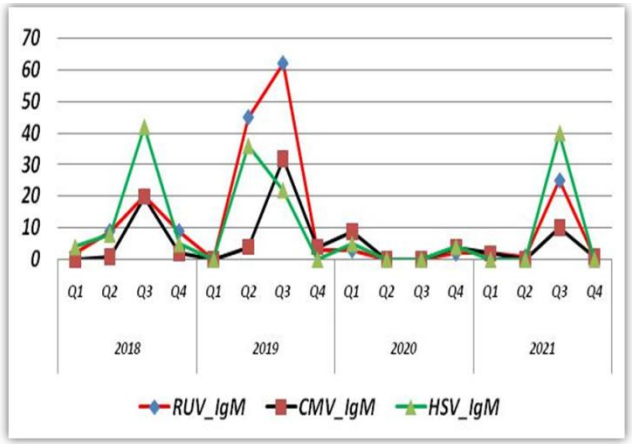

Figure 6:- Graph showing the Additive model of seasonal variation for RUV, CMV and HSV in different years and in quarterly manner.

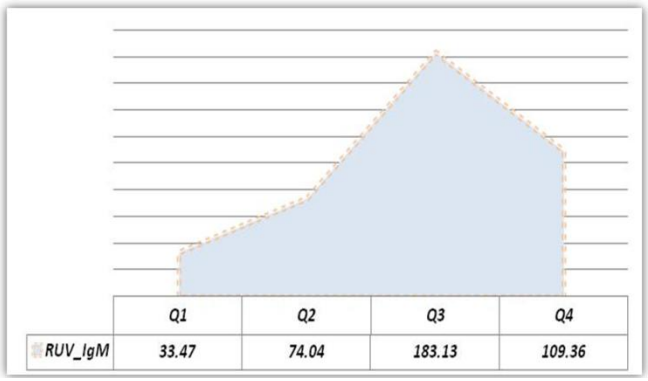

Figure 7:- The graphical representation of the Minimum seasonal variation of RUV and HSV positive cases at Q1 (January to March) quartile.

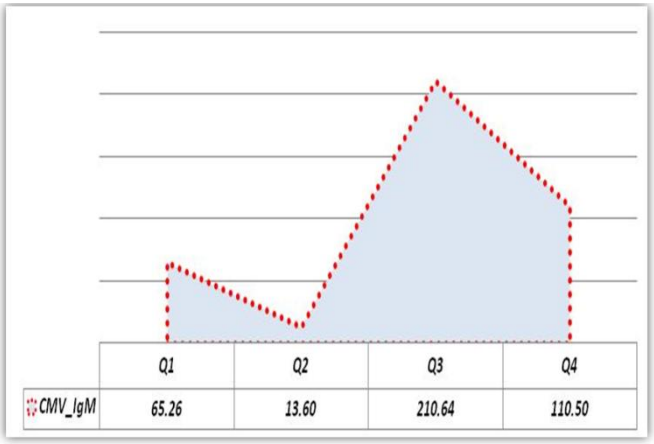

Figure 8:- The graphical representation of the Minimum seasonal variation of CMV positive cases at Q2 (April to June) quartile.

IV. Discussion

The study highlights the IgM based prevalence of RUV, CMV and HSV viruses in different districts of Bihar with seasonal variation as the major factor for the increase of infection with these viruses. According to various epidemiological studies from all over the world the TORCH infection appears to affect a wide range of racial groups. The exact IgM prevalence of such infections is still unknown from Patna, Bihar. According to many factors including climatic circumstances, socio-economic status, including private sanitation, cultural viewpoint, nutritional lifestyle, and other factors, the prevalence of ToRCH infections varies greatly from region to region (Sirin *et al.*, 2017; Deka *et al.*, 2022). Occasionally estimating the area seroprevalence of ToRCH agents is quite helpful in developing prenatal screening programmes and assisting doctors in selecting screening options. In nations like India, where there are no national screening programmes for ToRCH infections in pregnant women, it is even more crucial. Thus this study helps us to know the IgM prevalence of three important ToRCH infection viruses in the Patna, Bihar. Of the 2027 samples in the present study rubella infection was detected in 184 (9.08 %), CMV infection in 89 (4.39%) and HSV infection in 166 (8.19%) which demonstrating virus specific IgM antibodies. Earlier research on congenital rubella infection from India relied on the existence of greater titres of rubella antibodies in newborns compared to healthy controls or on the finding of higher titres of rubella antibodies in children with congenital abnormalities along with their moms (Broor *et al.*, 1991). According to S. Broor *et al.,* 1991, 12% infants were positive for rubella IgM antibody and 20% had CMV specific IgM antibody (Broor *et al.*, 1991). According to M.R. Sen *et al.,* 2012, 74 (19.4%) toxoplasmosis, 126 (30.4%) RUV, 130 (34.7%) CMV, and 151 (33.5%) HSV-2 infections were found to be positive for specific IgM antibodies (Sen *et al.*, 2012).

Similarly a study from, National Centre for Disease Control (NCDC), India where they found overall IgM antibody positivity for CMV, Rubella, and HSV in children was 20.7%, 5.4%, and 2.3%, respectively (Shweta *et al.*, 2015). Recently a study from Indore, Madhya Pradesh, India, where ToRCH ELISA was used to test 64 individuals out of which, 35 (54.68%) tested positive for IgG in a single infection (Rubella 15, toxoplasma 6, CMV 10, and HSV 4) and 28 (43.75%) tested positive for IgM (Rubella 4, toxoplasma 19, and CMV 5). The majority of cases had 82.8% coinfection by TORCH agents (Shrivastava *et al.*, 2014). One another study from Vellore, Tamil Nadu, India where they compare the Chemiluminescence immunoassay (CLIA) and Enzyme linked immunosorbent assay (ELISA) for the detection of IgM antibodies against measles (0.86), mumps (0.92), and rubella (0.52), CMV (0.57), EBV (0.50), and HHV-1 and -2 (0.47) (Steve *et al.*, 2022).

Rubella, which is also known as German measles, is a viral infection spread through the air that causes various symptoms. Congenital rubella syndrome can cause unprompted abortion, stillbirth, IUGR, hepato-splenomegaly, thrombo-cytopenia, and a purple rash (Stegmann and Carey, 2002). Infected newborns have impaired vision, hearing and heart defects, further in brain calcium deposits. The WHO, South-East Asia Region had set a target of eliminating rubella and mumps by 2023 (up from earlier in 2020). Reduce the prevalence of CRS by incorporating rubella-containing vaccines into public-sector childhood immunization programmes (World Health Organization). Cytomegalovirus is one of the utmost common viral marketers that cause uterine contamination. It is expected to occur in 0.3 - 2.4% of all thriving deliveries. CMV contamination has been linked to severe headaches, which can lead to increased retardation, hepato-splenomegaly, and also intra-cerebral damage (Karacan *et al.*, 2014; Prasoona *et al.*, 2015). HSV-1 and HSV-2-caused neonatal herpes is typically acquired during birth from infected mothers who already have genital lesions. It can include symptoms of the central nervous system, hepatitis, conjunctivitis, and skin blisters.

Though the study predicts the prevalence and included large number of samples from different districts of Bihar still the study has few limitations. The study did not tested infection against *Toxoplasma gondii* which is the important member of TORCH. The seasonal variation and infection with RUV, CMV and HSV could not be established for the year 2020 due to less collection of samples as a result of COVID 19 situation.

V. Conclusion

So from present study we can conclude that presumptive detection of congenital infections by TORCH agent has been indissoluble by serological test for the prevention and monitoring the prevalence of RUV, CMV and HSV viruses. Preventive measures should be taken during July to September period of the year to minimize any loss by these viruses in this region of India.

VI. Acknowledgement

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VII. Abbreviations

ICMR- Indian Council of Medical Research, RMRIMS- Rajendra Memorial Research Institute of Medical Sciences, TG- *Toxoplasma gondii*, RUV- Rubella virus, CMV- cytomegalovirus, HSV 1 and 2- Herpes simplex virus type 1 and 2, TORCH- *Toxoplasma gondii*, other infections (includes, HIV, syphilis, Hepatitis viruses, varicella virus and Parvovirus B19), rubella virus, cytomegalovirus, and herpes simplex virus type 1 and 2, VRDL- Viral Research and Diagnostic Laboratory, IUGR- intrauterine growth restriction, ELISA- Enzyme-linked immunosorbent assay, PCR- Polymerase chain reaction, OD- Optical density, AI- Antibody index, CC- Contingency coefficient, NMCH- Nalanda Medical College and Hospital, SKMCH- Sri Krishna Medical College and Hospital, NCDC- National Centre for Disease Control, CLIA- Chemiluminescence immunoassay.

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Table 1: Showing the summarized detail of overall results for RUV, CMV, and HSV for the year 2018-2021 in quarterly manner.

Year	**RUV IgM**					**CMV IgM**			**HSV IgM**		
		Total sample tested	**Positive (%)**	**Negative (%)**	**Equivocal (%)**	**Positive (%)**	**Negative (%)**	**Equivocal (%)**	**Positive (%)**	**Negative (%)**	**Equivocal (%)**
2018	Jan-Mar (Q1)	36	2	33	1	0	35	1	4	31	1
	April-Jun (Q2)	84	9	74	1	1	83	0	8	75	1
	July-Sep (Q3)	280	20	257	3	20	256	4	42	235	3
	Oct-Dec (Q4)	152	9	140	3	2	147	3	5	147	0
	Total	552	40 (7.25)	504 (91.30)	8 (1.45)	23 (4.17)	521 (94.38)	8 (1.45)	59 (10.69)	488 (88.41)	5 (0.90)
2019	Jan-Mar (Q1)	2	0	2	0	0	2	0	0	2	0
	April-Jun (Q2)	547	45	478	24	4	537	6	36	500	11
	July-Sep (Q3)	692	62	601	29	32	647	13	22	654	16
	Oct-Dec (Q4)	42	3	38	1	4	35	3	0	41	1
	Total	1283	110 (8.57)	1119 (87.22)	54 (4.21)	40 (3.12)	1221 (95.17)	22 (1.71)	58 (4.53)	1197 (93.29)	28 (2.18)
2020	Jan-Mar (Q1)	70	3	65	2	9	61	0	5	63	2
	April-Jun (Q2)	0	0	0	0	0	0	0	0	0	0
	July-Sep (Q3)	0	0	0	0	0	0	0	0	0	0
	Oct-Dec (Q4)	12	2	10	0	4	8	0	4	8	0
	Total	82	5 (6.1)	75 (91.46)	2 (2.44)	13 (15.85)	69 (84.15)	0	9 (10.98)	71 (86.58)	2 (2.44)
2021	Jan-Mar (Q1)	27	2	25	0	2	25	0	0	27	0
	April-Jun (Q2)	11	1	10	0	0	11	0	0	11	0
	July-Sep (Q3)	68	25	40	3	10	58	0	40	28	0
	Oct-Dec (Q4)	4	1	3	0	1	3	0	0	4	0
	Total	110	29 (26.36)	78 (70.91)	3 (2.73)	13 (11.82)	97 (88.18)	0	40 (36.36)	70 (63.64)	0
Total		**2027**	**184 (9.08)**	**1776 (87.62)**	**67 (3.30)**	**89 (4.39)**	**1908 (94.13)**	**30 (1.48)**	**166 (8.19)**	**1826 (90.08)**	**35 (1.73)**

Table 2: Table showing age descriptive statistics for RUV, CMV, and HSV positive patients (in years).

Statistic	Age_RUV_IgM	Age_CMV_IgM	Age_HSV_IgM
Median	9	15	20
Range	61	68	59
Standard Deviation	13.51	16.92	15.39
Standard Error	1.63	2.04	1.85
CI for Mean at 95%	11.27 to 17.76	15.78 to 23.90	19.67 to 27.07
Note: - Those patients were excluded who has less than one year			

Table 3: Table showing the association between patient demographics and RUV, CMV, and HSV infection

Gender & Age vs. Cases		Outcomes			Row_Total	Chi-square test at 95% CI	
		RUV_IgM (Positive)	CMV_IgM (Positive)	HSV_IgM (Positive)	Total	P-Value	Contingency Coefficient (CC)
Gender	Male	95 (51.63%)	51 (57.30%)	96 (57.83%)	242	P > 0.05	CC = 0.04 (4%)
	Female	89 (48.37%)	38 (42.70%)	70 (42.17%)	197		
	Total	184	89	166	N = 439		
Age Groups	< = 24	154 (83.70%)	73 (82.02%)	134 (80.72%)	361	P > 0.05	CC = 0.06 (6%)
	> = 25	30 (16.30%)	16 (17.98%)	32 (19.27%)	78		
Column_Total	Total	184	89	166	N = 439		
Note :- Test is not significant at 95% CI and Contingency Coefficient is also not appreciable							