



A Cross-Sectional of Serological Prevalence of Rubella, Cytomegalovirus, Herpes Simplex Virus, Treponema Pallidum and Human Immunodeficiency Virus in Antenatal Women with Poor Obstetric History.

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KEYWORDS

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ABSTRACT:

Aim: To determine the serological prevalence of Rubella, Cytomegalovirus, herpes simplex virus, treponema pallidum and human immunodeficiency virus in antenatal women with poor obstetric history.

Materials and methods: This research was a cross-sectional study done at the Department of Microbiology. A total of 200 pregnant women with a history of adverse obstetric events who presented to our Hospital were included in this research. The research was conducted after obtaining authorization from the institutional ethics committee. RV, CMV, HSV, TP, and HIV infection were examined in blood samples obtained from 200 women with a history of adverse obstetric events. A standardised case proforma was used to assess factors such as age and adverse outcomes related to the foetus, including two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early neonatal mortality, and/or congenital abnormalities.

Results: Out of the 200 cases analysed in this investigation, 190 instances tested positive for one or more of the TORCH compounds. The instances when the TORCH positive subjects tested positive for IgM, IgG, or both IgM and IgG. CMV contributed for 54.17% of abortions, HSV accounted for 71.43%, and RV accounted for 38.46%. The prevalence of congenital abnormalities was 7.69% for RV, 8.33% for CMV, and 7.14% for HSV. CMV was responsible for 8.33% of newborn deaths, whereas RV accounted for 7.69%. The occurrence of (RV) involvement in intrauterine mortality was found to be 23.08%, whereas cytomegalovirus (CMV) accounted for 8.33% of cases. Among the 22 individuals who tested positive for IgG antibodies against Rubella, 18 had evidence of protective immunity, whereas 4 did not. Among the 44 individuals who tested positive for IgG antibodies against CMV in this investigation, 36 instances indicated a previous infection lasting more than 6-8 weeks. The length of infection was not known in the other 8 cases. Out of the 26 instances of HSV infection, HSV-2 was responsible for the majority, accounting for 19 cases. The remaining cases were caused by HSV-1. All of the BOH patients in this research tested negative for syphilis in the Carbogen test. Additionally, 2% of the total BOH cases were found to be positive for HIV.



Conclusion: Our findings indicate that TORCH infections are linked to BOH and are a significant factor in repeated abortion, congenital malformations, birth complications, neonatal mortality, and intrauterine death. To minimise negative effects on the unborn baby, it is recommended to conduct serological tests for TORCH infections in pregnant women who have experienced previous pregnancy losses.

Introduction

Previous unfavourable pregnancy result refers to a history of two or more consecutive spontaneous abortions, foetal death, intrauterine growth retardation, stillbirth, early neonatal mortality, and/or congenital anomalies[1]. Human cytomegalovirus (HCMV), Rubella virus (RV), and herpes simplex type 2 virus (HSV-2) are prevalent viruses responsible for neonatal infections on a global scale. The complexity of HCMV infection during pregnancy surpasses that of other diseases owing to the virus's recurrent reactivation throughout the childbearing age and its capacity to be transferred to the foetus despite maternal immunity[2,3]. The majority of TORCH infections result in minor maternal morbidity, but often have severe repercussions for the unborn. Additionally, treating maternal illness often does not have any positive impact on the foetus. These infections are caused by viruses, bacteria, and protozoa that enter the foetal circulation via the chorionic villi during pregnancy. These maternal infections, which have negative consequences, are first not visible or symptomless, making them challenging to detect based on clinical observations. When dealing with instances of repeated pregnancy loss, it is important to take into account the serological reactivity for TORCH infections throughout the current pregnancy in order to minimise negative effects on the developing foetus. Consequently, the diagnosis of TORCH infection in pregnant women is often determined by detecting seroconversion in matched sera or by detecting antibodies[4]. The TORCH test is classified as an infectious disease antibody titre test that assesses the presence and concentration of antibodies against a particular set of infectious illnesses in the blood. A positive IgG antibody test often indicates previous exposure to the TORCH agent and does not indicate a current ongoing infection[5]. Rubella virus (RV) is the pathogen responsible for the sickness generally referred to as German measles. Congenital rubella syndrome (CRS) refers to a range of birth abnormalities that may occur in newborns when

mothers are infected with rubella during the first trimester of pregnancy[6,7]. Congenital infection occurs when the illness is transmitted from the mother to the foetus via the placenta. In the case of RV, it often causes a long-lasting infection in the foetus without causing cell destruction, and it has the ability to infect every organ in the body. According to the World Health Organisation (WHO), the number of infants born with Congenital Rubella Syndrome (CRS) each year is estimated to be over 100,000 globally, with the majority of cases occurring in poor nations. Rubella diagnosis is often overlooked due to the mild nature of the illness and the temporary presence of rash and lymphadenopathy. Infection with a virus during pregnancy might have harmful consequences on the developing baby. The incidence of infection is greatest during the first trimester of pregnancy (40-60%) and gradually declines during the fourth and fifth months (10-20%). In order to reliably establish a recent rubella infection, which is of utmost importance in a pregnant woman, it is necessary to either show an increase in the concentration of antibodies between two blood samples obtained at least 10 days apart, or identify rubella specific IgM antibodies in a single sample using the ELISA method. Due to the successful implementation of well-designed programmes, Rubella and Congenital Rubella Syndrome (CRS) have been almost eradicated in several countries[8]. Human cytomegalovirus belongs to the viral family Herpesviridae and is classified as a species under the virus genus Cytomegalovirus[9]. Cytomegalovirus (CMV) is the prevailing congenital infection, with a prevalence ranging from 0.2% to 2.2% in various regions worldwide. CMV infection is often asymptomatic in persons with good health, but may pose a significant risk to those with weakened immune systems and newborn babies. The global incidence of CMV infection is roughly 40% - 80%, with estimates ranging from around 45% in wealthy nations to 100% in impoverished areas[10]. CMV infection during pregnancy is particularly intricate compared to other infections, since the virus has the capability to



reactivate repeatedly throughout the reproductive years and may be transferred to the foetus despite the presence of maternal immunity[11]. The incidence of primary CMV infection in pregnancies ranges from 0.15% to 2.0%, with transmission to the foetus occurring in up to 40% of instances. Approximately 15% of intrauterine CMV infections lead to symptomatic congenital illness at birth, and 10 to 15% of individuals born with asymptomatic congenital CMV will have major clinical complications throughout infancy. CMV diagnosis may be achieved by human fibroblast culture obtained from urine, saliva, or other bodily fluids. A serological test may identify the presence of CMV IgG antibodies, which indicate a previous infection. The presence of viral IgM antibodies indicates an ongoing infection. The development of a highly efficient vaccination to prevent CMV infection would be of significant significance. At now, there is no commercially available CMV vaccine. It is currently undergoing research and development. Herpes simplex virus (HSV) is a widespread virus that has an envelope and contains double stranded DNA. It belongs to the Herpesviridae family and is transmitted via mucosal membranes and damaged skin. After infection, the virus moves to nerve tissues and remains in a dormant condition. The primary cause of genital herpes, which is mostly spread via sexual contact, is the Herpes simplex virus type 2 (HSV-2). Contracting genital herpes while pregnant has been linked to miscarriage, stunted foetal development, premature birth, and the transmission of herpes to the baby during childbirth. The incidence of neonatal infection ranges from 30% to 50% for HSV infections that occur in the late stages of pregnancy (third trimester), whereas infection in early pregnancy carries a risk of around 1%. When primary HSV infection develops in the latter stages of pregnancy, there is insufficient time for the development of the necessary antibodies to limit viral replication before childbirth. Syphilis is a complex illness caused by the bacterium *Treponema pallidum* (TP) and is often spread by contact with the open sores of an infected sexual partner or from an infected pregnant mother to her unborn child[12]. In 1999, the World Health Organisation estimated that there were 12 million fresh instances of syphilis, with over 90% of these infections happening in underdeveloped nations. Congenital syphilis is a prominent factor contributing to stillbirth and perinatal death rates in several nations.

Congenital Syphilis is typically categorised as early congenital syphilis and late congenital syphilis. indicators of early congenital syphilis manifest during the first two years of life, while indicators of late congenital syphilis emerge within the first two decades. Syphilis has a wide range of clinical presentations and presents similar clinical characteristics to both treponemal and non-treponemal disorders. Syphilis has resurfaced in some affluent nations, despite the presence of improved diagnostic tests and antibiotic therapy[13]. HIV/AIDS infection significantly contributes to maternal and perinatal morbidity and death. HIV in pregnancy is linked with many problems, including anaemia, pre-term labour, intrauterine growth restriction, foetal deaths, still births, and low birth weight [14,15]. During pregnancy, the immune system is weakened in women, regardless of whether they are infected with HIV or not. Vertical transmission, which refers to the spread of a pathogen from an infected mother to her kid, poses a unique challenge. Infection may happen either during pregnancy or during childbirth. Postpartum infection may occur when a nursing newborn consumes breast milk from a mother who is infected[16]. The limited number of research examining the relationship between maternal infection and the development of BOH is likely owing to the absence of proper facilities for isolating the specific agents responsible for BOH and the high cost of commercial diagnostic kits. The current investigation was conducted on pregnant women with BOH. The research aimed to investigate the serological prevalence of RV, CMV, HSV, TP, and HIV in pregnant women with BOH.

Materials and methods

This research was a cross-sectional study done at the Department of Microbiology. A total of 200 pregnant women with a history of adverse obstetric events who presented to our Hospital were included in this research. The research was conducted after obtaining authorization from the institutional ethics committee. RV, CMV, HSV, TP, and HIV infection were examined in blood samples obtained from 200 women with a history of adverse obstetric events. A standardised case proforma was used to assess factors such as age and adverse outcomes related to the foetus, including two or more consecutive spontaneous abortions, history of intrauterine foetal death, intrauterine growth retardation, stillbirth, early



neonatal mortality, and/or congenital abnormalities. This research included pregnant women with benign ovarian hyperstimulation. The research excluded women who were not pregnant and prenatal women who did not have a history of adverse obstetric events.

Methodology

3 millilitres of whole blood samples were taken from 100 expectant women having a history of poor obstetric outcomes. The collection was done utilising aseptic precautions by venipuncture, using sterile disposable syringes. Following centrifugation, the purified serum was put into vials and kept at a temperature range of 4-8°C. The serum samples underwent Line immunoassay to detect IgG and IgM antibodies for RV, CMV, and HSV infections. VDRL positive cases were verified using the Carbogen test for syphilis and the Comb aids test for HIV. If the results were positive, they were further validated using the Triline test and Trispot test, following the recommendations set by NACO.

TORCH test protocol: The reagents were prepared according to the manufacturer's instructions and allowed to reach room temperature for a minimum of 30 minutes prior to commencing the test. The test strips were immersed in a 2ml solution of pre-prepared wash buffer A. For each incubation combination, 20µl of undiluted serum samples were pipetted onto the test strip. The sample was subjected to incubation for a duration of 1 hour, with moderate agitation. Individual wells were used to extract diluted serum, and each well was then filled with 2ml of wash buffer-A, which was ready for use. The sample was subjected to a 5-minute washing step with moderate agitation, followed by removal of wash buffer-A. The washing procedure was performed three times. Next, a 2ml pre-prepared conjugate solution was introduced and left to incubate for 45 minutes with mild agitation. The washing process was performed three times. Substrate reaction refers to the chemical process in which a substrate undergoes a transformation or reaction. A volume of 1.5 millilitres of substrate solution that was already prepared and ready to use was added to the mixture. The mixture was then incubated for a duration of 8 minutes, during which it was gently shaken. The substrate solution was extracted and rinsed three times briefly with deionized water. Prior to examination, the strips were subjected to a 2-hour drying process,

sandwiched between two layers of absorbent paper. The recom Line TORCH Screening IgG/IgM test was interpreted using a two-band method, as outlined below. The presence or absence of particular antibodies to an infectious pathogen is shown by a prominent band (lysate antigen). The accessory band indicates the length of prior infection of Toxoplasma and CMV, the type specificity of HSV, and the protective immunity of Rubella. The recommended test is the TORCH Screening IgM test. The analysis of this test was conducted using a single band approach. The test strips consist of individual bands for each infectious agent, allowing for the detection of specific IgM antibodies to Toxoplasma, Rubella, CMV, and HSV type 1 and 2. The recom Line TORCH Screening IgG assay is complemented by the recomLine TORCH Screening IgM assay, hence both tests are performed simultaneously. The presence of recombinant antigen p30 for Toxoplasma suggests a previous infection that occurred more than three months ago. The presence of recombinant antigen gG2 for CMV suggests a previous infection that occurred during the recent 6-8 weeks. The existence of a lysate band with greater intensity compared to the cut off band of Rubella vaccine indicates a better level of protective immunity against Rubella. The recombinant antigen gG2 for HSV is indicative of infections induced by HSV-2. Evaluation of TORCH test The assessment of the test's quality control was based on the existence of three specific bands: the Reaction control band, the Antibody class band, and the Cut-off control band. The same process was also replicated for IgG antibodies.

Carbogen® test: A Carbogen test, specifically a Rapid Plasma Reagin card test, was conducted to diagnose syphilis. 50 microliters of the test specimen, positive control, and negative control were pipetted onto individual reaction circles of a disposable slide using a sample dispensing pipette. A single droplet of carbogen reagent was introduced to the test specimen, positive control, and negative controls using the reagent dropper included with the kit. The test specimen and the carbogen reagent were thoroughly combined using separate mixing sticks, ensuring even distribution around the whole reaction circle. The slide underwent continuous rotation on a mechanical rotator at a speed of 180 rotations per minute. Microscopic flocculation was found after 8 minutes. The test was classified as reactive when there were big and medium-sized black



clumps against a white backdrop, mildly reactive when there were little black clumps against a white background, and non-reactive when there were no clumps present.

Combaids – Hiv 1+2 Immunodot Test: The test reagents were equilibrated at room temperature and the necessary combs were marked. The washing buffer was diluted. 0.1ml of both the sample and control were introduced to each micro test well, which already contained 0.1ml of sample diluents. The comb was inserted into its designated well and left to incubate at room temperature for 10 minutes. Following incubation, 0.2ml of the Colloidal gold signal reagent was introduced into the micro test wells. The combs were cleansed for a duration of 10 minutes by oscillating them back and forth in a wash pan. Combs were introduced into small test wells that contained Colloidal gold signal reagent and left to incubate at room temperature for a duration of ten minutes. The washing process was performed once more. The comb was let to dry naturally, and the colour change on the spotted region at the tip of the comb's teeth was observed to assess its responsiveness, as well as to compare it with the look of the control dot. The reference colour index was used to analyse and contrast the findings.

Pareekshak® Hiv- 1/2 Triline Card Test: Kit and samples were brought to the room temperature. Test device removed from the pouch just prior to testing. Device was placed on a flat surface. 10µl of serum or plasma was added into the sample window and allowed to soak in. 20µl of diluents was added into the same sample window. Positive result was read within 10min and Negative result in 20min.

Aidsan® Hiv-1/2 Trispot Test Kit Procedure: The reagents, equipment, and specimens were equilibrated to room temperature. Subsequently, a volume of 20µl of buffer solution was introduced into the test device. Subsequently, 20µl of either serum or plasma was introduced, and then 40µl of buffer solution was added. 20µl of gold conjugate was introduced into the mixture, followed by the addition of an additional 40µl of buffer solution. The results were interpreted as follows: A negative result is shown when just one red spot, known as the control spot, appears in the control area "C". This means that the material does not contain

antibodies for either HIV-1 or HIV-2. A positive result is shown by the presence of two red dots, one at the control area "C" and the other at the test region for HIV-1 and/or HIV-2. This indicates that the material contains antibodies for HIV-1 and/or HIV-2. b) When three red spots (Control, HIV-1, and HIV-2 Spot) are seen in the control area "C" and the test regions for HIV-1 and HIV-2, it indicates that the material has antibodies for both HIV-1 and HIV-2, therefore suggesting a reactive result. recomLine TORCH Screening IgG kit and recomLine TORCH Screening IgM kit, Carbogen® Rapid Plasma Reagin card test for testing syphilis, Comb Aids RS Advantage HIV 1+2 Immunodot Test Kit, PAREEKSHAK® HIV- 1/2 TRILINE CARD TEST, AIDSCAN® HIV-1/2 TRISPOT TEST KIT were used for diagnosis.

Statistical methods

The statistical tests used were rate, proportion, nonparametric test. Chi-square test was applied for testing hypothesis regarding qualitative variable.

Results

Out of the 200 cases analysed in this investigation, 190 instances tested positive for one or more of the TORCH compounds. The presence of TORCH agents in the blood is detailed in Table 1. Table 2 displays the instances when the TORCH positive subjects tested positive for IgM, IgG, or both IgM and IgG. CMV contributed for 54.17% of abortions, HSV accounted for 71.43%, and RV accounted for 38.46%. The prevalence of congenital abnormalities was 7.69% for RV, 8.33% for CMV, and 7.14% for HSV. CMV was responsible for 8.33% of newborn deaths, whereas RV accounted for 7.69%. The occurrence of (RV) involvement in intrauterine mortality was found to be 23.08%, whereas cytomegalovirus (CMV) accounted for 8.33% of cases. All 200 instances of BOH in this investigation tested negative for syphilis, as shown in Table 4. Among the 22 individuals who tested positive for IgG antibodies against Rubella, 18 had evidence of protective immunity, whereas 4 did not (as shown in Table 5). Among the 44 individuals who tested positive for IgG antibodies against CMV in this investigation, 36 instances indicated a previous infection lasting more than 6-8 weeks. The length of infection was not known in the other 8 cases (Table 6). Out of the 26 instances of HSV infection, HSV-2 was responsible for the majority, accounting for 19 cases.



The remaining cases were caused by HSV-1 (Table 7). All of the BOH patients in this research tested negative for syphilis in the Carbogen test. Additionally, 2% of

the total BOH cases were found to be positive for HIV (Table 8).

Table 1: Seropositivity of TORCH agents

Seropositivity of TORCH agents	Number =200	Percentage
TORCH Positive	190	95
TORCH Negative	10	5

Table 2: Seropositivity of IgM and IgG antibodies among TORCH positive cases

Total TORCH Positive cases=190		
	Number	Percentage
IgM Positive	20	10.53
IgG Positive	159	83.68
IgM and IgG Positive	11	5.79

Table 3: The seropositivity of IgM, IgG and both IgM, IgG among the various TORCH positive cases

Agent	IgM Positive=20		IgG Positive=159		IgM and IgG Positive=11		Total =190	
RV	4	20	20	12.58	2	18.18	26	13.68
CMV	4	20	44	27.67	0	0	48	24.49
HSV	2	10	26	16.35	0	0	28	14.74
RV+CMV	2	10	2	1.26	0	0	4	2.04

Table :4 shows distribution of TORCH infection in various BOH patients with different presentations. In this study CMV was the second most common agent responsible for BOH. Abortion was the commonest mode of presentation of BOH, followed by still birth and IUD.

Table 4: Distribution of TORCH infection in various BOH cases

	RV=26		CMV=48		HSV=28		RV+CMV=4	
Abortion	10	38.46	26	54.17	20	71.43	0	0
Still birth	6	23.08	10	20.83	4	14.28	0	0
IUD	6	23.08	4	8.33	2	7.14	4	100
Congenital anomaly	2	7.69	4	8.33	2	7.14	0	0
Neonatal death	2	7.69	4	8.33	0	0	0	0

Table 5: Rubella IgG positive patients with protective immunity

Total Rubella IgG Positive cases=22		
	Number	Percentage



With protective immunity	18	81.82
Without protective immunity	4	18.18

Table 6: Time duration of past infection of cytomegalovirus

Total CMV IgG positive		
	Number	Percentage
Past Infection of > 6- 8 weeks	36	81.82
Past infection without knowing duration	8	18.18

Table 7: Type specific distribution of herpes infection

Total HSV Positive cases=26		
	Number	Percentage
HSV-1	7	26.92
HSV-2	19	73.08

Table. 8: Seropositivity of HIV infections

	Number =200	Percentage
HIV Reactive cases	5	2.5
HIV Non-reactive cases	195	97.5

DISCUSSION

Human cytomegalovirus (HCMV), Rubella virus (RV), and Herpes simplex type 2 (HSV2) are often responsible for adverse pregnancy outcomes, particularly in low- and middle-income countries (LMICs).

Prenatal screening for antibodies against Toxoplasma, Rubella, CMV, Herpes simplex virus, and other pathogens like as Treponema pallidum and HIV is a standard procedure in many regions worldwide, generally known as TORCH. The primary purpose of this screening routine is to identify pregnant moms who are at a high risk of transmitting viral or protozoan infections to their unborn babies. It is also used to examine newborns who have vague and unexplained symptoms that are suspected to be caused by an infection[17]. All TORCH infections are classified together due to their shared characteristic of causing severe congenital abnormalities when passed from an infected woman to her foetus during pregnancy.

TORCH infections result in minor illness in pregnant women, but they may have severe effects on the developing foetus. Diagnosing TORCH infections in pregnant women is challenging due to the fact that most cases are asymptomatic. To confirm the diagnosis of TORCH infection in pregnant women, it is necessary to demonstrate seroconversion in matched sera or the presence of antibodies. The study was carried out at the Department of Microbiology, utilising the recomLine TORCH Screening IgG/IgM kit to detect TORCH agents, the Carbogen® test kit for syphilis testing, the Comb Aids RS Advantage HIV 1+2 Immunodot Test Kit for HIV testing, and the PAREEKSHAK® HIV-1/2 TRILINE CARD TEST and AIDSCAN® HIV-1/2 TRISPOT TEST KIT for confirming HIV infection. Out of a total of 200 instances of BOH, 95% tested positive for TORCH infections in this investigation. A research conducted by Padmavathy M et al found that 98% of the patients of BOH tested positive for TORCH infections[18]. Out



of all the patients that tested positive for TORCH, 83.68% were only positive for IgG, while 5.79% were positive for both IgG and IgM. In a research conducted by Padmavathy M et al[18], it was shown that 85% of patients tested positive for IgG alone, whereas 13% tested positive for both IgG and IgM. According to the research by AL-Taie AA[19], RV accounts for 13.68% of cases, CMV accounts for 24.49% of cases, and HSV accounts for 17.74% of all the TORCH positive patients. CMV contributed for 54.17% of abortions, HSV accounted for 71.43%, and RV accounted for 38.46%. The prevalence of congenital abnormalities was 7.69% for RV, 8.33% for CMV, and 7.14% for HSV. CMV was responsible for 8.33% of newborn deaths, whereas RV accounted for 7.69%. Among cases of foetal demise, RV abnormalities were responsible for 23.08% of the cases, while cytomegalovirus (CMV) accounted for 8.33%. All 200 instances of BOH in this research tested negative for syphilis. This finding aligns with a research conducted by Padmavathy M et al, where all participants tested negative for syphilis[18]. In this research, the prevalence of HIV positive patients was 2.5% among the total cases of BOH.

Conclusion

Our findings indicate that TORCH infections are linked to BOH and are a significant factor in repeated abortion, congenital malformations, birth complications, neonatal mortality, and intrauterine death. To minimise negative effects on the unborn baby, it is recommended to conduct serological tests for TORCH infections in pregnant women who have experienced previous pregnancy losses. If the tests show positive results, appropriate treatment should be administered to reduce the transmission of the infection to the foetus.

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