Review

Congenital Viral Infections: Consequences on the Mother and Fetus and Management

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Congenital Viral Infections: Consequences on the Mother and Fetus and Management

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Abstract: Viruses are the most common congenital infections and an important cause of fetal malformations, morbidity, and mortality. The effects of these infections, which are transmitted in-utero (transplacentally), during childbirth or in the puerperium depend on the timing of the infections. These vary from miscarriages (usually with infections in very early pregnancy), congenital malformations (where the infections occur during organogenesis) and morbidity (with infections occurring late in pregnancy, during childbirth or after delivery). The most common of these viruses are cytomegalovirus, hepatitis, parvovirus B-19, rubella, varicella and zika. There are currently very few efficacious antiviral agents licensed for use in pregnancy and therefore for most of these infections, prevention by means of immunisation (where there is a vaccine), administration of immunoglobulins to those exposed to the virus to offer passive immunity or appropriate measures to avoid being infected would be options to minimise the infections and their consequences. In this review, we discuss the current understanding of some of the congenital infections and their consequences on both the mother and fetus and their management focusing mainly on prevention.

Keywords: congenital infections; fetal malformations; vertical transmission; cytomegalovirus; hepatitis virus; parvovirus-B19; rubella; varicella and zika

Introduction

Although infections are common in pregnancy have no maternal or fetal consequences. Some however, have serious effects not only on the mother but also on the fetus when transmitted. These infections that are transmitted to the fetus *in-utero* or at birth are collectively known as congenital infections. Micro-organisms responsible for these infections include viruses, bacteria, protozoa, and fungi with viruses being most common [1,2].

The fetal consequences of viral infections depend on the gestational age at transmission. Those transmitted early in pregnancy (especially in the early first trimester) typically cause miscarriages or act as teratogens especially during the period of organogenesis causing congenital malformations [3]. Some infections acquired *in-utero* may not cause congenital malformations but may cause significant morbidity postpartum. Most infections acquired in late pregnancy have a benign course, but some may cause significant morbidity and sometimes mortality in the neonatal and childhood periods. For those acquired intrapartum from cervico-vaginal secretions or postpartum, symptoms are related to the timing of the acquisition of the infective organism (for example the symptoms may not manifest for a few days or weeks after delivery). The spectrum of consequences of infections therefore varies from no effect through to miscarriage, congenital malformations, fetal growth restriction, autism spectrum disorders (ASDs) or postpartum sequelae including manifesting with symptoms of the infection [1,2].

Vertical transmission (from the mother to the fetus) occurs through one of three ways - transplacental, intrapartum or through breastfeeding. While the exact mechanism of transplacental transfer for most of these infections is unknown, the localisation/identification of the RNA or virion

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particles in the placenta/trophoblast from pregnant women infected with viruses indicate that these viruses do indeed get to the placenta prior to reaching the fetus [4]. With regards to mitigating the consequences of these viral infections, unfortunately anti-viral agents are not available for most of them and so too are effective vaccines. The best approach in most cases is therefore education on behavioural modifications that reduce the risk of acquiring the infection, minimisation of consequences by administering immunoglobulins (if available) or vaccinating susceptible individuals where this is available. In this review, we provide an update on some congenital viral infections (mainly cytomegalovirus, hepatitis A,B,D and E parvovirus B19, rubella and Zika virus) and their consequences on the mother and fetus and how these can be treated or minimised.

Cytomegalovirus

Cytomegalovirus (CMV) belongs to the *Herpesviridae* family of viruses. This family of viruses is ubiquitous in nature and in humans consisting of eight distinct members (Herpes Simplex viruses type 1and 2, Varicella-Zoster virus, Epstein-Barr virus, CMV, Human Herpes Viruses 6, 7 and 8) that cause diseases [5].

Morphology

Like other members of this family of viruses, CMV has a large, double-stranded DNA genome and is able to establish lifelong latent infection with periodic reactivations occurring during the lifetime of the infected individual. CMV is the largest virus species, 250nm in diameter with a DNA genome that encodes more than 150 proteins [6]. In fact, the coding sequence of CMV is thought to encompass >250kilobase pairs making it the largest human pathogen with respect to total genetic content [7,8]. Its genome is segmented into unique long and short segments, each bracketed by terminal repeats.

The replication of CMV is complex, and requires the coordinated, sequential expression of three families of viral transcripts. Interactions between both virally encoded proteins and host cellular and transcription factors regulate viral gene expression, including establishment of and reactivation from latency [9,10]. The replication cycle of CMV is divided temporally into three regulated phases namely (a) the immediate early phase (b) the early phase, and (c) the late phase. Immediate early (IE) mRNA is transcribed within the first few hours after infection of the host cell and the encoded IE proteins, which include multiple isoforms due to extensive splicing, modulate both host and viral gene expression.

Structurally there are three distinct regions of the CMV viral particle. These include (a) an icosahedral capsid, (b) the tegument layer, and (c) the outer lipid bilayer envelope. The capsid consists of 162 capsomere subunits arranged in an icosahedral symmetry. It surrounds and encloses the viral dsDNA genome (forming a nucleocapsid) and has a highly electron-dense appearance when imaged by electron microscopy [11]. In the mature virion, the nucleocapsid is surrounded by the tegument, a protein-rich layer containing several proteins that are targets of the host T lymphocyte response to infection.

Cytomegalovirus and pregnancy

CMV is the most common viral infection in pregnancy that causes a plethora of congenital malformations with resulting disabilities [12]. It has been estimated that about 0.5–1.0% of newborns are infected with CMV [13,14]. Rates of previous infection vary, for example in the USA CMV seroprevalence is ~58% overall and increases strongly with age. High CMV IgM titres are a strong predictor for low IgG avidity [15], the most reliable serologic indicator of primary infection [16,17,18].

The virus reaches the fetus by invading the utero-placental barrier. Transmission to the fetus varies with gestation age with the greatest transmission occurring in the third trimester; however, it is transmission in the first trimester that is associated with the highest risk to the fetus. Congenital malformations, however, correlate with gestational age at seroconversion and placental pathology. Reported transmission rates are 30%, 38%, and 72% in the first, second, and third trimester,

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respectively [19]. Primary maternal infection in the first trimester poses a 40% risk of virus transmission with 25% of babies having malformations. The malformations associated with CMV include cognitive dysfunction, seizures, sensorineural hearing loss, learning disorders, and microcephaly [19], with about 7% having delayed hearing loss [20]. At birth, about 10% of infected newborns will have severe manifestations and about 23% will have at least one manifestation. A proportion will be symptomatic with the symptoms (such as deafness) developing much later [21].

Unlike VZV infection which confers a lifelong immunity, previous infection with CMV does not. It does however modify the consequences of repeat infections with less severe consequences when compared to primary infections. Women for example with preconception immunity to CMV have a low risk of vertical virus transmission (0.2–2.0%) [16]. The reinfection (in a highly seropositive population tend to be with a different strain of the virus from the one that caused the primary infection leading to seropositivity) [22].

The diagnosis of CMV infection in the mother does not depend reliably on conversion from seronegative to seropositive. This is because IgG and IgM antibodies persist for months after the primary infection making it difficult to time the infection with relation to the period of organogenesis. Avidity testing is therefore used to time the infection especially if the timing is required for an infection before 20 weeks. Confirmation of intrauterine infection is made from isolation of viral DNA in amniotic fluid by PCR, performed after gestation week 21. This is best performed about 7 weeks after maternal seroconversion as testing prior to this may result in false negatives [21]. Detection of CMV DNA in urine and/or saliva at delivery is the best marker to identify newborns at risk for hearing loss [23].

Fetal growth restriction (FGR) is a consequence of congenital CMV. The precise mechanism of this is uncertain but it is likely to include invasion (with resultant pathological changes) and infection of the placenta (placentitis) by the virus as evidenced from histopathological examination. Precisely how CMV reaches the decidua has not been clearly demonstrated but it is known that latently infected decidual leukocytes likely reactivate the virus triggered by cytotrophoblast invasion and inflammation. The virus then establishes latency in $CD14^+$ monocytes [24] and reactivates as differentiation occurs [25,26]. These monocytes which are infected by CMV then make latency-specific transcripts and maintain the viral genome that can re-enter the lytic cycle [27]. The implication is that even a few latently infected $CD14^+$ monocytes in the decidual leukocyte population could reactivate CMV and infect decidual stromal cells [28,29,30]. In cytotrophoblasts and in the chorion, and epithelial cells in the amnion, CMV replicates [31,32]. The cytotrophoblast cells infected with CMV produce cmv IL-10 which reduces metalloproteinase-9 (MMP-9) levels and activity, thereby impairing invasion [33,34]. The infected trophoblast progenitor cells (TBPC) in the chorion in turn interfere with the growth of STBs and chorionic villi in CMV infected placentas [35]. A consequence of the loss of the TBPC reserve is a reduction in the responsiveness of the placenta to hypoxia and thus limiting the development of syncytiotrophoblasts (STB)s and chorionic villi [36]. These changes affect placental development and predispose the fetus to growth restriction of early onset.

It would seem that pathological findings in the placenta vary with the gestational age at the infection reflecting the greater risk of fetal growth restriction (FGR). with earlier infections. Placental pathology associated with congenital CMV infection diagnosed at mid gestation include pronounced villous maldevelopment, diffuse villitis, cytomegalic cells, and areas of necrosis and calcification [36]. Examination of placentas from women with CMV infection in late gestation showed infected blood vessels (BVs) in the villus core and villus necrosis and infected endothelial cells in the villus stroma. Interestingly these findings were without pathologic changes [37]. Cytotrophoblast, endothelial cells, and stromal fibroblasts in chorionic villi from placentas show viral proteins as evidence of CMV placentitis [38]. These findings suggest that CMV infection contributes to the FGR. In a study of paired maternal and cord sera and placentas from 19 pregnancies in which 5 out of 7 cases were FGR due to primary or recurrent CMV infection, Pereira et al showed large fibrinoids with avascular villi, oedema, and inflammation CMV proteins in epithelial cells of amniotic membranes [35]. These

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findings provide evidence that congenital CMV infection should be considered as an underlying cause of FGR especially the early onset type [35,39].

The factors responsible for transmission and severity of congenital CMV infection are not well understood [40]. Previous infection does not confer lifelong immunity, unlike that for rubella and varicella zoster (VZV). Thus, while congenital CMV infections have severe consequences in cases of primary infection, these have also been shown (though to much less extent and severity) in children born to mothers who have had CMV infection before pregnancy (nonprimary infection) [41,42,43,44]. It would seem that previous infection provides significant protection against intrauterine transmission; however, this protection is incomplete [45]. The prevalence of congenital CMV infection at birth is directly related to maternal seroprevalence rates; these are highest in populations with higher CMV sero-negative prevalence in women of reproductive age [46]. The rate of transplacental transmission of CMV decreases from 25% to 40% in mothers with primary infection during pregnancy to < 2% in those with pre-existing seroimmunity [19]. Why maternal immunity does not provide complete protection against vertical transmission is not well understood, but it has been suggested that reinfection with a different strain of CMV can lead to intrauterine transmission and symptomatic congenital infection [47]. Furthermore, accumulated data, especially from studies in highly seropositive populations, suggest that once intrauterine transmission occurs, pre-existing maternal immunity may not modify the severity of fetal infection and the frequency of long-term sequelae [41,42,43,48,49].

Most transmission occurs at birth with approximately 50% of infants born to mothers shedding CMV from the cervix or vagina at the time of delivery being infected [50]. Factors that increase lower genital tract shedding and therefore a greater risk of vertical transmission at birth include concomitant infection with other STIs, number of sexual partners and younger age are [51].

Following maternal infection, the virus is shed in bodily fluids including breastmilk, saliva, and tears. The virus has been detected in breastmilk in 13% to 50% of lactating women tested with conventional virus isolation techniques [52]. With the more sensitive polymerase chain reaction (PCR) technology, the presence of CMV DNA in breastmilk has been demonstrated from more than 90% of seropositive women [53]. Risk factors for postnatal transmission from milk have been shown to include an early appearance of viral DNA in milk whey, and a higher viral load in breastmilk [53]. Interestingly, freeze-storing or pasteurization of maternal breastmilk has been shown to reduce the viral load; however, transmission of CMV to infants that have received treated breastmilk has not been documented [54].[24]

Prevention and treatment

Antiviral drugs licensed against CMV infection in immunocompromised (non-pregnant) women include ganciclovir, valganciclovir, cidofovir, foscarnet and valaciclovir. Except for valaciclovir, the teratogenic and toxic effects of the others preclude their use in pregnancy. Jacquemard et al. [55] and Lereuz-Ville et al. [56] investigated the use of valaciclovir in pregnancies with CMV-infected symptomatic fetuses. Valaciclovir a prodrug that is converted *in-vivo* by esterases into the active drug aciclovir in the liver during first pass metabolism was preferred because it has greater oral bioavailability than aciclovir (55% versus 10–20%) [57,58]. Aciclovir, however, has an excellent safety profile in pregnancy as it is not genotoxic *in-vitro*, and there is considerable evidence that its use in the first trimester in humans is not associated with any increase in the rate of congenital malformations [59,60]. Both agents (aciclovir and valaciclovir) though have limited antiviral activity against CMV. In a series of 20 cases with CMV in pregnancy, Jacquemard et al offered oral valaciclovir for 7 weeks (range 1-12 weeks) at a dose of 8mg/day for 7 weeks starting at 22-34 weeks (average 28 weeks). Out of the 20 cases 7 were terminations, 6 of which had evidence of progressive disease, and one termination was performed on parental request; 13 were live births (10 with normal clinical examination at 6 months - follow-up was for 6–39 months), 2 had isolated unilateral sensorineural hearing loss (SNHL) and one had hearing loss, microcephaly and incontinentia pigmenti). When these outcomes were compared to those of 24 untreated symptomatic CMV-infected fetuses, 14 (58%) in the untreated group were either terminated (n=), intrauterine fetal death (n=) or severe neonatal

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infection (n=). Ten (41%) infants were healthy compared to 71% in the treated group that did not undergo a termination. Further evidence of benefit for valaciclovir was generated from the trial "In Utero Treatment of Cytomegalovirus Congenital Infection with Valaciclovir (CYMEVAL)" a phase II open label study [56]. Oral valaciclovir 8g/ was given for a median of 89 days to women with a moderately-infected fetus, presenting with non-severe ultrasound features which included extracerebral ultrasound abnormalities and/or mild ultrasound brain abnormalities. Treatment resulted in a significantly greater proportion of neonates born asymptomatic in the treatment group (82% versus 43%) and there were no maternal or neonatal adverse effects reported. It does therefore seem that valaciclovir may be the treatment option for confirmed CMV infection during pregnancy but more robust evidence from randomised trials would be much welcomed.

As there is currently no licenced vaccine for CMV, the best way to reduce the risk of infection during pregnancy is through behaviour modification. Simple hygiene-based measures that have been shown to reduce the risk of CMV acquisition include handwashing after contact with urine or saliva, and avoiding sharing utensils, drinks, or food with young children. Such educational interventions have been shown to be more effective in pregnancy. For example, in a study by Alder et al. [61] of seronegative women with a child younger than 36 months who received preventative information in pregnancy, the seroconversion rate was 1.2% compared to 7.6% in a group of women who did not receive such advice ($P < 0.001$), providing evidence that risk reduction is possible.

Intrauterine infection (congenital CMV) should be confirmed at birth by viral PCR from either urine or oral swabs obtained within 3 weeks of birth. For symptomatic neonates with congenital CMV infection, postnatal valganciclovir/ganciclovir treatment should be considered and commenced within the first 4 weeks of life. There is evidence this treatment can reduce or prevent progression of SNHL and improve long-term neurodevelopmental outcomes in some infants [62,63,64].

Viral hepatitis in pregnancy

Acute viral hepatitis is caused by hepatitis A, B, C, D, E viruses. The mode of transmission of these viruses varies so too are the clinical consequences both on the mother and the fetus/neonate.

Hepatitis A virus (HAV)

Morphology: Hepatitis A virus (HAV) is a 27nm non-enveloped, single-strand positive sense RNA virus belonging to the *Hepatovirus* genus and the *Picornaviridae* family [65]. There are 6 genotypes but only genotypes I to III infect humans [66,67]. Two infectious forms exist in the host - namely the naked virions which are shed in the faeces (hence transmitted faeco-orally) and the quasi-enveloped virions which circulate in blood. It is important to note that the synthetic genome length RNA of the virus is also infectious [66,67]. The viral genome consists of about 7,500 nucleotides of positive sense RNA, which is polyadenylated at the 3′ end and have a polypeptide attached to the 5′ end. Most of the genome is occupied by a single, large open reading frame (ORF) which encodes a polyprotein with a theoretical molecular mass of M_r 252,000. Hepatitis A viral polyprotein is processed to yield structural (located at the amino-terminal end) and non-structural viral polypeptides [68].

Epidemiology Clinical course and transmission

Hepatitis A virus (HAV) is endemic in countries with poor hygiene and sanitation systems - mainly in the Middle East, North Africa, Sub-Saharan Africa, South and Central Asia and Latin America [65]. It is a common cause of mainly self-limiting acute viral hepatitis with reported mortality rates of 0.3% to 0.6% [66,69]. World-wide, it is estimated that about 1.5 million new cases are reported annually. This is likely to represent under reporting as the true incidence may, however, be much high since milder cases are likely to go unrecognised in view of its self-limiting nature [66,70,71].

HAV is transmitted via the faecal-oral route either by direct contact with an infected person or indirectly by ingestion of contaminated water and food, especially raw and undercooked shellfish

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[72,73]. It has an incubation period of 28 days (range 15–50 days) [74] . The virus can survive for >1 week in ambient conditions in a dried state and for up to 1 year in fresh or salty water [75,76]. Its prevalence is categorised as low, intermediate, or high based on the levels of HAV-IgG in serum [66]. This categorisation is important as it provides guidance on the route of transmission and prevalence of HAV-IgG. Transmission in high-endemic areas is mainly through contaminated water and therefore, more than 90% of the populations would have anti-HAV IgG by the age of 10 years. Consequently, large epidemics are infrequent, as most people are immune from asymptomatic/mild HAV infection or acute hepatitis A during childhood [66,71,77]. In intermediate-endemic areas on the other hand, most transmission is through ingestion of contaminated food and water and the prevalence of anti-HAV IgG is equal to or > 50% by age 30 but < 50% at the age of 15 years. Because of the wide distribution of HAV-IgG, the virus causes large-scale cyclic outbreaks [78,79,80]. In low-endemic areas, transmission occurs mainly through food handlers, travel to high-endemic areas or with oral-anal sex. The infection rates in these settings are very low, and < 50% of people > 30 years have immunity against HAV [78].

Hepatitis A virus enters the body by ingestion and then causes intestinal infection. From there it spreads, probably by the bloodstream, to the liver, a target organ. Large viral particles are detectable in faeces from as early as 10–14 days after exposure and persist (in general), until peak elevation of serum aminotransferases and early in the acute phase of illness, but relatively infrequently after the onset of clinical jaundice. Clinical features vary from mild pyrexia, upper abdominal pain, and jaundice. The antibody to HAV that persists interestingly is also detectable late in the incubation period, coinciding approximately with the onset of biochemical evidence of liver damage. The virus produces pathological changes exclusively in the liver where it causes cytologic necrosis, conspicuous focal activation of sinusoidal lining cells; accumulations of lymphocytes and more histiocytes in the parenchyma, often replacing hepatocytes lost by cytolytic necrosis predominantly in the periportal areas; occasional coagulative necrosis in the form of acidophilic bodies; and focal degeneration [81]. The virus itself is not directly cytopathic to hepatocytes, but the liver injuries are mainly secondary to the host immune response. Viral clearance after the primary infection is achieved by cellular immunity, whereas humoral immune response is responsible for protection and prevention of infection. Individuals with defects in cellular immune response, such as those with human immunodeficiency virus (HIV) infection, or on immunosuppressants following transplant can produce longer viral shedding with high infectivity, but without an apparent increase in the severity of symptoms [66].

HAV and Pregnancy

HAV is an uncommon infection in pregnancy and when it occurs, vertical transmission is uncommon. However, there are numerous reports of vertical transmission with meconium peritonitis and perforation of the distal ileum, a very rare fetal complications of vertical transmission requiring surgery [82,84,84]. Although no serious outcomes have overall been reported to be associated with HAV infection during pregnancy [4,85], there are associations between acute infections and preterm labour, placental abruption and premature rupture of fetal membranes especially if the infection occurred in the second or third trimesters [82]. Maternal pyrexia is likely to be the mechanism through which these complications are precipitated. It is extremely rare for infants born to mothers with HAV infection to be affected and indeed many tend to have normal antibody and transaminase levels. In the rare cases of mother-to-child HAV infection, complications of fetal ascites, meconium peritonitis, neonatal icterus and distal ileum perforation have been reported [86]. While it is a rare but accepted cause of mortality especially in those >50 years, there are no documented reports of mortality in pregnant women and infants exposed to HAV infection and in these cases, there is usually full resolution of the infection [87].

There is no evidence the virus is transmitted through breastfeeding even though mothers infected with HAV have anti-HAV antibodies and HAV RNA in their breast milk. Breastfeeding is therefore not contraindicated in these women [88]. Administering immunoglobulin or the inactivated vaccine to children of infected mothers is likely to offer protection from HAV infection [88]. There is

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also evidence that administering HAV vaccine to children <2 years of age, induces seropositivity that could persist for at least 10 years regardless of presence of maternal anti-HAV [89].

Since maternal anti-HAV IgG antibodies cross the placenta to the baby and may persist well till the second year of life, depending on the level of HAV endemicity and the average anti-HAV antibody levels in a given maternal population,[90,91,92] the timing of vaccination is critical for efficient HAV vaccination in high-endemic areas as high levels of maternal anti-HAV IgG antibodies present in the first year of life may impede the vaccine response. On this basis, it has been recommended that in high endemic areas children <1 year should not be vaccinated [88,93,94].

Treatment and Prevention

There is no specific antiviral therapy for hepatitis A [67], however pre-exposure (by vaccination) and post-exposure prophylaxis (with immunoglobulin) are recommended to provide protection for unvaccinated pregnant or potentially pregnant individuals, especially those working or travelling to countries with high or intermediate HAV endemicity [95]. When administered within 2 weeks of exposure, post-exposure prophylaxis (PEP) with HAV vaccine or IG, prevents infection with HAV [96,97] Advantages of the vaccine over the immunoglobulin as PEP include ease of administration, greater acceptability and availability, induction of active immunity and longer duration of protection [98]. Counselling and educating pregnant women or those who could become pregnant travelling to highly HAV endemic areas will undoubtedly reduce the risk of HAV infection in pregnancy. Pregnant women and women of reproductive age need protection against HAV before visiting HAV-endemic countries or low-income countries with poor sanitation and hygienic standards [66].

Vaccination is with an inactivated virus which is considered safe during pregnancy. It is available both in the movalent and in combination with hepatitic B virus immunisation. Two doses are usually administered. After 2 weeks of the first dose, about 70% of individuals develop protective levels of antibodies [96]. Therefore, giving HAV vaccine immediately before travel will ensure adequate protection in most individuals, because the incubation period for HAV is 15 to 50 days. The levels of antibodies are likely to persist for at least 10 to 29 years or perhaps for life after receiving the second dose of HAV vaccine [97].

Hepatitis B (HBV)

Morphology: Hepatitis B virus (HBV) is a 42-nm particle member of the *hepadenoviridae* family[36]. [99] It comprises a 27 nm diameter electron-dense core (nucleocapsid) surrounded by an outer envelope of the surface protein antigen (HBsAg) embedded in membranous lipid derived from the host cell. Infected hepatocytes produce the surface antigen which is secreted as 22nm particles and tubular structures of the same size. These particles are composed of the major surface protein in both non-glycosylated (p 24) and glycosylated (gp 27) form in approximately equimolar amounts, together with a minority component of middle proteins (gp 33 and gp 36) which contain the pre-S2 domain, a glycosylated 55 amino acid N-terminal extension. [100] The virion nucleocapsid consists of the viral genome surrounded by the core antigen (HBcAg). The genome, which is approximately 3.2 kilobases in length, is composed of two linear strands of DNA held in a circular configuration by base-pairing at the 5′ ends.

Epidemiology, Clinical course and Transmission

The WHO estimates that there are over 257 million people living with chronic HBV worldwide, with the highest prevalence in Sub-Saharan Africa, Southeast Asia, and the Eastern Mediterranean regions. The disease is maintained in these regions by either maternal-fetal transmission or child-to-child spread [81]. It is estimated that chronic HBV disease is responsible for 900,000 deaths per year secondary to either liver cirrhosis or hepatocellular carcinoma [81]. Rates of chronic HBV are related to the age at acquisition, approaching 100% following HBV infection in the neonatal period, exceeding 70% in early childhood and <1% after acute infection in post-pubertal immunocompromised individuals [101,102] . The prevalence of maternal HBV infection varies, with

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the overall prevalence being highest in regions identified as high risk. In the USA, the prevalence in pregnant women was 8.5/100000 deliveries between 1998 and 2011 [103].

Acute HBV hepatitis presents with a variety of symptoms which include jaundice, right upper quadrant pain, nausea, vomiting, anorexia, low-grade fever, and fatigue. It is characterised by the presence of HBV surface antigen (HBsAg) in blood. Biochemically, serum transaminases are significantly raised and may peak in their thousands. About 1-2% of adults with the acute infection will progress to acute liver failure. HBV has a prolonged incubation period that varies from 4 weeks to 5 months. Symptoms and changes in liver biochemistry typically appear after this period. In general, resolution occurs within 2 months of the acute infection (evidenced by the clearance of HBsAg), but the development of chronicity may result in persistent liver injury. Neonates who acquire the virus either at the time of birth or shortly after, have a high rate of chronicity and may indeed enter an immunotolerant phase with high levels of HBV DNA in serum which may persist for decades. Preventing vertical transmission is therefore of great importance.

HBV infection and pregnancy

Symptoms of acute HBV infection in pregnancy are not different from those outside pregnancy. In the first trimester, the symptoms of nausea and vomiting may be confused with those of pregnancy and hyperemesis gravidarum. Because of the altered immune status induced by pregnancy, there is an increased risk of flare in women with chronic inactive and non-replicative HBV. When this happens, there is typically an increase in HBV DNA and increased levels of transaminases. Acute/chronic HBV does not increase fetal complications, however, where there is associated liver cirrhosis or failure, maternal morbidity and mortality may be increased. The risk to the fetus is mainly at the time of birth where transmission is high. It is not known to be transmitted through breastfeeding; however, infected mothers should be educated on preventative measures.

Treatment and prevention

There are two aims of instituting treatment of HBV in pregnancy. The first is a reduction of liver injury (in those with high HBV DNA and elevated liver enzymes) in those with acute hepatitis and the second is to reduce the risk of vertical transmission. In the first category the treatment will be long-term. It is important to note that the risk of transmission to the infant is linked to the maternal blood HBV DNA level - higher levels are associated with increased risk. For example, it has been shown that up to 25% of newborns will acquire HBV if the mothers' HBV viral titre exceeds 200,000 IU/mL [104]. Treatment with nucleoside/ nucleotide-based medications given in the third trimester has been shown to significantly reduce vertical transmission [105]. Guidelines from the American Association for the Study of Liver Disease (AASLD) and the European Association for the Study of Liver (EASL) recommend administration of anti-viral therapy to woman with HBV DNA levels of >200,000 IU/ml [106,107]. The antiviral agents, lamivudine and tenofovir have been used in various studies and shown to be safe and effective in reducing vertical transmission. Most of the data on these drugs come from treatment of HIV positive women [108]. The risk of vertical transmission can also be reduced by HBV vaccination combined with hepatitis B immune globulin (HBIG).

Vaccination against HBV is with a vaccine containing a recombinant hepatitis B surface antigen hence safe in pregnancy. This vaccine offers a high protective efficacy especially in those who are immunocompetent where a three dose vaccine regimen has been reported to achieve 90–95% protective efficacy, defined as a serum titre of anti-HBs antibody of 10 mIU/ml or greater. Universal screening for HBV in pregnancy is advocated and vaccination of non-immune women during pregnancy, recommended for those at higher risk of HBV exposure.

Hepatitis E (HEV)

Morphology: Hepatitis E virus (HEV) is a single-stranded positive-sense RNA virus of approximately 7.2 kb in length in the family of *Hepeviridae*. The viral genome contains three open reading frames (ORFs) (a) ORF1 which encodes a non-structural polyprotein for viral replication and

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transcription, (b) ORF2 which encodes the capsid protein that elicits neutralizing antibodies, and (c) ORF3, which partially overlaps ORF2, and encodes a multifunctional protein involved in virion morphogenesis and pathogenesis [81]. HEV virions are non-enveloped spherical particles found in faeces but exist as quasi-enveloped particles in circulating blood. There are 2 types of HEV virus-like particles (VLPs) that have been reported. These are, small $T = 1$ (270 Å) and native virion-sized $T = 3$ (320–340 Å). Two distinct forms of HEV capsid protein exist - the secreted form ($ORF2^s$) which inhibits antibody neutralization, and the capsid-associated form ($ORF2^C$) which self-assembles to VLPs. At least 8 major genotypes of HEV have been identified from geographically distinct locations. These may differ in up to 20-50% at the nucleotide level [109]. They are diverse in all three ORF regions but are serologically indistinguishable and are also cross-reactive [110-114].

Epidemiology clinical course and transmission

HEV is the most common cause of acute viral hepatitis especially in young adults in many low and middle income countries [115-118]. It is transmitted mainly by the faeco-oral route with an average incubation period of six weeks (range 2-6 weeks). The highest attack rates are found in young adults, and high mortality rates of up to 20% have been reported in women during pregnancy. Clinically, acute cases are indistinguishable from other causes of acute viral hepatitis. There are approximately 20 million HEV infections world-wide with an estimated 3.3 million symptomatic cases [119,120]. The mortality from HEV is estimated to be about 56,600 annually [120]. The sero-prevalence of anti-HEV IgG ranges from 10% to 47%, in countries with poor hygienic conditions.

Most patients with acute viral hepatitis from HEV recover completely but mortality rates vary from 1-4% [121-127]. In pregnant women, it has been associated with spontaneous miscarriages, stillbirths, and a maternal mortality rate of 20-40% in some parts of the world especially in the Indian Subcontinent [121-127].· HEV infection can progress to fulminant hepatic failure, chronic hepatitis, and extrahepatic neurological and renal diseases [128]. The transmission and distribution of HEV varies with the different genotypes. For example, genotypes 1 and 2 are transmitted faeco-orally (through ingestion of contamination food and water), genotypes 3 and 4 (endemic in Europe and North America and indeed other high income countries) are transmitted by eating undercooked pork, deer, and wild boar [129-134] with a primary zoonotic reservoir in these animals. Genotypes 5 and 6 are mainly present in Japan and infect boars and potential humans while genotypes 7 and 8 are predominantly in the Middle East and China infecting camels and humans [135-137]. Contamination of water supplies and/or food with faecal material especially after natural disasters, heavy rains or drought is responsible for HEV outbreaks in about 90–95% of cases [122]. Vertical transmission occurs antenatally and intrapartum with a reported rate of 30-100% [138-140]. Although anti-HEV antibody and HEV-RNA have been shown to be present in the colostrum of HEV-infected mothers, breastfeeding is not thought to be associated with transmission [141]. Postpartum transmission, however, may occur through close contact of mothers and their infants, especially when the mother has HEV- caused acute viral hepatitis [141].

Most patients are asymptomatic. When symptoms develop, they include low-grade fever, nausea, vomiting and anorexia. About 40% of patients develop hepatitis-like symptoms such as jaundice, pruritis, dark urine and pale stools. Those who are immunosuppressed either from infections such as HIV or on immunosuppressive drugs for solid organ transplant, may fail to clear the virus after primary infection, leading to chronic HEV infection (i.e. lasting >6 months) [142]. The laboratory diagnosis of HEV infection is based on the detection of HEV antigen, HEV RNA and antibodies against HEV [143]. Anti-HEV IgM antibodies which represent recent exposure and last for 4-5 months can be detected during the acute phase of the illness. Anti-HEV IgG antibodies on the other hand represent remote exposure and can last more than 10 years.

Hepatitis E and pregnancy

Unlike HAV and HBV, HEV infection in pregnancy is associated with a high mortality although this is mainly in low- and middle-income countries. This is more so for infections in the third

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trimester. In a review of 930 cases from Asia and Africa, a mortality of 15.9% (range 6.9-31%) was reported [144]. The difference in mortality between the high-income countries and the less resource countries may be secondary to better medical care and that these infections in high-income countries are with the much lower pathogenic HEV-3 and -4 genotypes, which have also not been reported to lead to congenital infection. The vertical transmission rate for HEV is reported to be 23–50% [144,145]. Complications of intrauterine infections included premature delivery, low birth weight, stillbirth, and death [144]. It has been estimated that HEV may be responsible for up 2,400–3,000 stillbirths annually (188). Vertical transmission in HEV IgM-positive mothers appears to be significantly correlated with viraemia and a viral load of 13,300 copies/ml or higher [145].

The rate of vertical transmission in pregnant women infected with HEV is high. The consequences of intrauterine infection include stillbirth, spontaneous miscarriages and a higher perinatal morbidity and mortality and preterm birth. In a study from the United Arab Emirates, it was reported that vertical transmission from HEV RNA-positive mothers to their infants occurred in 100% resulting in significant perinatal morbidity and mortality [125].

The mode of maternal-fetal vertical transmission remains unclear, but it has been suggested that immune complexes with non-neutralized HEV particles may facilitate FcRn-mediated transport. HEV replicates in the placenta, and the virion structural protein ORF3 has been detected in decidual cells, STBs, and stromal cells of the villus core, suggesting a high viral load and extrahepatic sites of viral replication [146,147].

Treatment and Prevention

There is currently no effective anti-viral treatment for HEV. There is however a vaccine that was licensed (by China) in 2011 with the trade name of Hecolin®. This vaccine contains an antigen that is a truncated version of the capsid protein encoded by ORF2 [148]. Although the safety and efficacy of this vaccine have been demonstrated in a large-scale phase III clinical trial [149], this was not in pregnancy, however, since it is not a live attenuated vaccine it is likely to be safe in pregnancy. However, it must be used in pregnancy with caution although preliminary data suggest it to be safe for both mother and fetus [150]. This vaccine has not yet had regulatory approval in the USA and other high-income countries.

Parvovirus B19 (PB19V)

Parvovirus B19 was first discovered serendipitously as a new antigen in the sera of asymptomatic patients during screening for hepatitis B in 1975 [151-153]. Later, it was shown to cause erythema infectiosum (also called fifth disease or slapped cheek disease) [154]. This infection is common in children. It was several years later (1984) that parvovirus B19 was linked with hydrops fetalis [155]. The virus has a world-wide prevalence that increases gradually with age from 2–20% at <5 years to 40–80% at >18 years [4].

Morphology: Parvovirus B19 (B19V) is a small icosahedral particle (~25-nm diameter) virus with a non-enveloped single-stranded linear DNA (ssDNA) of 5–6 kb. It belongs to the family *Parvoviridae*, the subfamily *Parvovirinae*, the genus *Erythrovirus* and Human parvovirus B19 type species. There are three genotypes (genotypes 1–3)- type 1 being the most prevalent. These differ by >11% in their DNA sequence. The genome of the virus contains two main open reading frames encoding the (i) non-structural protein NS1 (which is involved in viral replication) and (ii) two structural viral proteins VP1 and VP2. Additional open reading frames encode two small proteins of 7.5 kD and 11 kD whose functions are unknown [4]. The virus is highly species and cell-type specific and infects only human erythroid progenitor cells of the bone marrow, the main site of its replication. Cell lines of the megakaryocyte-erythroid lineage are also susceptible to this infection [156].

Epidemiology, clinical course, and transmission

Transmission of B19V is mainly by aerosols during the incubation phase which is 3-21 days. Within a week, very high titres of $>10^{10}$ genomes/ml blood are reached without apparent symptoms.

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This is followed by the onset of nonspecific symptoms, which include fever, malaise, headache, and myalgia. Viral DNA levels decrease with the onset of these non-specific symptoms and at the same time there is development of protective antibodies. In some cases, the infection usually resolves without symptoms, irrespective of the peak level of viraemia. The viral DNA gradually disappears from the blood within months although it may persist in tissues at low levels ($<10^4$ genomes/ml) for longer. In some cases, a rash appears 2 weeks after the infection. Antibody titres peak during the development of the rash. IgM antibody disappears within several months, whereas IgG antibody which is mainly against the conformational VP2 epitope and VP1u, may persist for life. Although quantification of IgM antibodies or IgG avidity against VP1u may distinguish recent from previous infection, this is rarely indicated in clinical obstetrics.

B19V and pregnancy

Susceptibility to Parvovirus B19 varies world-wide in women of childbearing age. For example it has been reported that 26–44% of women of childbearing age in Europe and Japan are not immune [4]. Transmission is usually from contact with infected young children [157]. A seroconversion rate of ~1% has in general been reported but this may rise to 13% during epidemics which are thought to occur every 4-5 years. Viral PCR for DNA is the gold standard diagnostic test for maternal infection, and this is usually performed in combination with assays of either IgM antibody or low avidity IgG antibodies [158]. These tests can be performed soon after exposure [159]. The rate of vertical transmission transmission rate is about in the first trimester in those in the acute phase of the infection, where the viral load is high, is about 24-39%. The strongly expressed globoside receptor on differentiating cytotrophoblasts cells support the fact that the virus invades the placenta. The virus targets progenitor erythroblasts and because erythropoiesis in the fetus is significantly greater, this results in a viral load in the fetus much higher than that in the mother [160].

The consequences of fetal infection vary depending on gestation. When transmission occurs before 20 weeks of gestation, the risk of fetal hydrops and death is about 4-10% [161]. The hydrops results from severe anaemia and hyperdynamic cardiac failure with resulting cardiac deficiency, oedema, and fluid in various compartments - notably the chest and peritoneal cavity [158]. Fetal hydrops may resolve spontaneously when the fetal immune system develops, but untreated severe hydrops is always fatal. Interestingly, as the levels of IgG antibodies increase in the fetal circulation, viral DNA levels decrease, and fetal anaemia becomes less severe, suggesting that transcytosed IgG reduces infection [162]. Parvovirus B19 is the only treatable non-immune cause of hydrops fetalis. Treatment aims to correct the resulting anaemia by intrauterine blood transfusion. This allows time for the fetal immune system and/or maternal antibodies to control the infection [163]. Babies infected by parvovirus B19 are viral DNA positive at birth and develop an active antibody response to the infection [158]

Prevention and therapy

Like most viruses, there is no known effective anti-viral treatment for parvovorus B-19 infection. Hydroxyurea and acidofovir are however active against the virus *in-vitro* but there are no clinical studies to confirm benefit. Intravenous IgG can, however, suppress viral replication in cases of persistent infection [4]. There is no available vaccine against this infection and screening of women before or during pregnancy is not routinely performed.

Rubella Virus (RV)

Rubella virus (RV) infection was the first to be associated with teratogenicity. This followed the observation that ocular malformations, notably congenital cataracts were common in babies delivered to women who had been infected with the virus in 1941 [164]. The virus causes a mild exanthematic measles-like disease that was first described by German physicians, leading to the term “German measles” [165]. The full pathogenic spectrum of congenital rubella syndrome (CRS) develops with infection in the first 2 months of gestation. Most frequently observed are sensorineural

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deafness (~80%), cataracts (~50%), and congenital heart disease (>50%). Other common defects include glaucoma, retinopathy, mental retardation, thrombocytopenia, hepatosplenomegaly, and fetal growth restriction (FGR) [165,166]. Although the Centers for Disease Control and Prevention declared that RV was eliminated in the United States in 2005 due mainly to childhood vaccination programs with the attenuated RV strains, [165], most of the world population has not comprehensively (now compounded more than ever with vaccine hesitancy fuelled by social medial) received the RV vaccine; hence, CRS remains a major public health problem in Africa and large parts *of Asia.*

Morphology: RV is a member of the *Togaviridae*, family. There are two genera in this famly (*a*) alphavirus, with various arbovirus species, and (*b*) rubivirus, with RV as its only species [165,166]. The virus which is spherical and measures approximately 60nm in diameter is able to replicate only in humans and experimentally in infected primates. It has spikes which are formed by the envelope proteins E1 and E2. The genome of rubella virus is a 9.8-kb plus strand RNA with a $5'$ cap and poly-A tail. This RNA which encodes for structural and the non-structural proteins required for replication, functions as a messenger RNA that is infectious without other viral components. When RV infects cells, the envelope protein E1 binds to the cell membrane component myelin oligodendrocyte glycoprotein, a host receptor for RV [165] most prevalent in the nervous system, as well as other receptors [167]. In these cells it does not cause any obvious cytopathology [4]. Once RV infects a cell, it induces interferon but can replicate in its presence. Its capsid protein by virtue of its antiapoptotic activity can prevent elimination of the virus ([4[.

Epidemiology, clinical course and transmission

The virus is spread through aerosols generated from infected persons with whom contacts are made. It attacks the upper respiratory tract and titres from pharyngeal swabs at this stage have been reported to reach 10^5 which is 50% infectious doses per 0.1-ml secretion [165]. The infection is then spread by infected lymphocytes and alveolar macrophages from the respiratory tract to the regional lymph nodes where it causes lymphadenopathy. From here, it spreads throughout the body with symptoms manifesting in the joints and the skin. Symptoms vary from those of mild disease which may not be recognised as the symptoms are mild to fever, malaise, and a rash. By the time the rash appears, the virus is secreted in lacrimal fluid, cervical secretions, and synovial fluid. The rash which is characteristically erythematous is distributed over the body and usually disappears within a few days. Accompanying lymphadenopathy and joint symptoms may however last for weeks. The most severe forms present with encephalitis which has been shown to complicate about 1:6000 cases and importantly 1:5 (20%) of these cases is fatal [4]. Neutralising antibodies produced in response to this infection are directed mostly against the E1 spike and to a lesser extend to the E2 spike [166].

Rubella virus and pregnancy

Vertical transmission is a common occurrence in those infected with RV. Since this is associated with congenital malformations, the transplacental route is most definitely one through which the virus reaches the fetus. It has been suggested that prolonged viraemia or infected monocytes in maternal circulation disseminate the virus to the intervillous space or lymphovascular channels in basal decidua. On entering the placenta, the virus replicates and causes placentitis. The virus has been demonstrated in the cytotrophoblast, endothelial cells of the placentas, the amniotic epithelium, and cells in basal decidua [168]. When it reaches the fetus, it infects various cells causing significant cell damage with resultant consequences. These consequences of RV infection in pregnancy depend largely on the gestational age. In the first trimester, especially in the first 8 weeks about 75% of fetuses are infected [165]. It causes spontaneous miscarriages in up to 20% of these cases, and most will have congenital rubella syndrome (CRS) - some of whom would be miscarried [165,166]. Fetal growth restriction results in a significant number of cases secondary to infection of endothelial cells, leading to a necrosis of BVs and avascular villi which affects transport functions, and an overall reduction in cell numbers following their infection with the virus [4]. The proportion of infants infected with

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consequences drops in the second trimester and in the third trimester, there are virtually no consequences of the infection although some of the neonates may develop late onset disease. The spectrum of late onset diseases that has been reported include cardiac abnormalities (58%), psychomotor retardation (62%), and mental retardation (42%) [165]. A study by Toizubmi et al. [169] of children with congenital rubella syndrome found late-onset sequelae (60%), including sensory defects, developmental delay, and autism spectrum disorder in a significant number of cases (45).

When the infection occurs in a susceptible pregnant woman (i.e. someone with absent rubella antibodies - or not previously vaccinated or infected) the fetal consequences will usually point to the likely timing of the infection. However, with later infections, it may be difficult. A low avidity test would help in the postnatal diagnosis of congenital rubella virus infection [170] especially as months after transplacental maternal antibodies have waned.

Prevention and therapy

Immunisation against rubella virus is a very effective means of preventing infections during pregnancy. Vaccination is recommended in childhood (in the form of the MMR vaccine). It is a live attenuated vaccine. Women who are susceptible (seronegative) and planning pregnancy should be vaccinated and counselled to avoid pregnancy for at least 4 weeks. Although this vaccine has not been reported to cause congenital malformations, a study showed that amongst 661 neonates born to mothers who erroneously received the vaccine, 3% had signs of RV infection, but none had CRS [171]. The proportion of seronegative women varies from country to country depending on the policy on childhood vaccination. In the USA for example nearly all women of childbearing age have been vaccinated whereas in China, which does not have universal childhood vaccination, 40% of women of childbearing age are seronegative [172]. Globally, an estimated 105,000 cases of CRS occurred in 2010, virtually unchanged from 1996 [173]. Although the WHO recommended RV vaccination worldwide in 2011, [172], this has not been universally implemented and vaccine hesitancy which unfortunately is increasing is also affecting uptake by parents. The nonimmune pregnant women who have recently been exposed to RV may achieve passive immunization with normal immune globulin if administered soon after exposure [174]. There is no current known effective antiviral therapy for RV.

Varicella-Zoster Virus (VZV)

Varicella zoster virus (VZV) causes chickenpox (varicella) and shingles (zoster) - the former predominantly in children and young adults and the latter predominantly in adults and rarely in children. [175,176]. It is a human alpha herpesvirus 3(HHV3) and one of nine herpes viruses that can infect humans. It is also able to survive in the external environment for hours [177].

Morphology: Structurally VZV is closely related to the herpes simplex viruses (HSV). It shares much of its genome homology. Its envelope glycoproteins for example correspond to those in HSV (gB, gC, gE, gH, gI, gK, gL) except that there is no equivalent of the HSV gD protein. Additionally, VZV does not produce the latency-associated transcripts (LAT) that are essential in establishing HSV latency [178]. The virions of VZV are spherical and measure 180-200nm in diameter. The DNA of VZV is a single, linear, double-stranded molecule. The virion capsid is surrounded by loosely associated proteins known collectively as the tegument; many of which play critical roles in initiating the process of virus reproduction in the infected cell [179].

VZV enters the host through the upper respiratory tract where it replicates in the mucosa. It then spreads to the tonsils and lymphoid tissues from where infected T cells transport the virus in the blood stream to the skin and peripheral nerves. It has an incubation period of 10–21 days (average of 14 days). The vesicular rash which typifies chickenpox, appears 10-21 days after the infection [180] and takes about 4 days to crust. This rash is widely distributed (on the face, the trunk, and shoulders) reflecting the spread of the virus. The rash is typically vesicular and fluid filled, but may also be filled with pus (if superinfected with bacteria) and/or rupture with scab formation. Symptoms include, fever, body aches and muscle pains, headaches, and pharyngitis from tonsillar infection.

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During the primary phase of the VZV infection, the virions gain access to the sensory nerve cell bodies in ganglia by retrograde axonal transport, and latent infection is established [4]. When replication of these latent infection is reactivated, the virus then spreads by anterograde axonal transport to reach the skin and manifest as shingles (zoster). This is typically a vesicular rash in the dermatome innervated by the affected ganglion. It may also occur in the eyes [181]. All the skin lesions (in cases of primary infection or reactivation in the form of shingles) contain high concentrations of infectious virus which can be transmitted to susceptible individuals. Spread of the virus is mainly through significant contact with an infectious individual. The period of infectivity is typically 24-48 hours before the rash appears and until it crusts [181].

Varicella-zoster virus and pregnancy

Although the mechanism by which VZV transmission from mother to fetus *in-utero* is unknown, vertical transmission occurs during pregnancy, delivery and postpartum. Evidence for intrauterine infection comes from the isolation of viral VZV DNA in placentas and amniotic fluid, detection of viral replication proteins in infected cytotrophoblasts and fetal tissues [182,183] and the presence of VZV-specific IgM and IgG at birth in infants with clinical symptoms [184]. The mechanisms of virus dissemination identified for primary infection suggest VZV-infected T cells traffic to basal decidua, where the virus replicates and spreads to the adjacent placenta in the intervillous blood space [4].

VZV is teratogenic and the gestational age at the time of infection has a significant bearing on the spectrum of malformations that result. These malformations are collectively referred as the "congenital varicella syndrome" (CVS) [185] (see Table 1 for details). Infection in the first trimester results in VZV transfer across the placenta in 24% of cases, resulting in symptomatic infections in 50% of infected babies, whereas second- and third-trimester infections are rarely associated with congenital malformations [182]. Pregnant women with zoster (shingles) have not been shown to have fetuses with CVS or indeed other abnormalities. This is thought to be because these women already have IgG antibodies which cross the placenta and provide passive immunity to the fetus. VZV infection is not considered life threatening in most cases, but during pregnancy, because of the relative immune suppression and the impact of the gravid uterus on the respiratory system, VZV infection maybe life-threatening. It may also result in spontaneous miscarriage, preterm delivery, or stillbirth.

Table 1. Viral infections that are associated with congenital infections, their diagnosis, fetal consequences and treatment where available.

Virus	**Incubation period (IP)**	**Diagnosis**	**Treatment**	**Risk of Vertical transmission**	**Fetal & Neonatal effects**	**Diagnosis of IU infection**
CMV	3-12 weeks	IgM and IgG combined with avidity test to hep time infection	No licensed anti-viral agent but valaciclovir shown to be beneficial	25-40% with primary infection & <2% with secondary infection	Microcephaly, fetal growth restriction, low birth weight, hepatosplenomegaly, sensorineural deafness, retinitis, thrombocytopenia, visual impairment	Amniocentesis for viral PCR - best done 7 weeks after infection
HAV	28 (15-50) days	*Maternal anti-HAV IgM*	Post exposure prophylaxis with	Very rare (few reported cases)	Ascites, Meconium peritonitis, perforation terminal ileum, Jaundice	Not usually performed

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			immunoglobulin and Vaccination			
HBV	90 (160-150) days	HBsAg and if positive obtain HBeAg and quantify HBV DNA	Nucleoside or nucleotide analogues (lamivudine, telbivudine, or tenofovir) during the last trimester in highly viraemic mothers HBIG to mothers and neonate	70-90% for hepatitis e antigen positive mothers and 20-40% for hepatitis e antigen negative	Persistent chronic hepatitis	Not usually performed
HEV	6 (2-9) weeks	Maternal anti-HEV IgM	No recommended treatment Chinese vaccine available	23-50%	Miscarriage, stillbirth, and neonatal hepatitis E infection	Not usually performed
PB19V	3-21 days	Maternal IgM antibodies or seroconversion. IgG and IgM may persist for some time after acute infection	No anti-viral agent Intrauterine transfusion corrects hydrops	Up to 33%	Hydrops fetalis, myocarditis	Amniocentesis for viral PCR
RBV	4-14 days	Positive maternal IgM antibody or antibody, IgG seroconversion, or a ≥ 4-fold rise between acute and	No recommended anti-viral treatment. Vaccination available	80% in the 1st trimester with up to 90% of fetuses affected. 25-30% affected >16 weeks with minimal effect of the fetus	Microcephaly, cataract, congenital glaucoma, congenital heart disease, hearing impairment, hepatosplenomegaly, purpura, jaundice, radiolucent bone	Amniocentesis for viral PCR; IgM in fetal blood (unreliable); RT-PCR on fetal blood or chorionic

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		convalescent IgG titres.			disease developmental delay, pigmentary retinopathy	villus biopsy specimens.
VZV	10-21 days	Characteristic rash and positive IgM antibodies	Aciclovir started within 24 hours of the rash	24% in 1st trimester	Affect **skin, eyes and CNS and limbs** **Eyes** - chorioretinitis, cataract, nystagmus, cortical atrophy **Limbs** - atrophy, malformed digits, hypoplasia **CNS** - microcephaly, atrophy of the brain **Autonomic nervous dysfunction** - neurogenic bladder, hydronephrosis, oesophageal dilatation gastrointestinal reflex) Neonatal disease - pneumonia, meningoencephalitis, severe coagulopathy	Amniocentesis for viral PCR, Fetal IgM in blood not reliable
ZIKV	3-14 days	Maternal IgM - detected from 4 days after infection (note may persist for 12 weeks after acute infection) Avidity test will help time infection	No recommended anti-viral agent available	47% (26-76%)	Microcephaly, brain atrophy, cerebral and ocular calcifications, ventriculomegaly, periventricular cysts, callosal abnormalities, vermes agenesis, cerebellar atrophy, cortical atrophy	Amniocentesis for viral PCR. Fetal blood IgM maybe present >3. days but unreliable in utero

CMV - Cytomegalovirus; HAV - Hepatitis V virus, HBV - Hepatitis B virus, HEV - Hepatitis E virus; PB19V - Parvovirus -B19; RBV - Rubella virus; ZIKV - Zika virus, VZV - Varicella virus.

Prevention and therapy

Treatment with varicella-zoster immune globulin along with antiviral drugs (e.g. aciclovir) has been recommended for women exposed to or infected with the virus, irrespective of gestational age. Although aciclovir is not licensed for use in pregnancy, there are no reported teratogenic effects from

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a record of treatment in a Danish Registry of >100,000 cases treated with famciclovir and aciclovir [181]. Aciclovir is recommended if the infected pregnant woman presents within 24 hours of developing the rash. This is given orally but if there is evidence of severe systemic disease, parenteral treatment would be recommended. Susceptible women who have had significant contact but have not developed the symptoms should be given the VZV immunoglobulin. Infants of mothers who develop the rash a week before delivery should also be given the immunoglobulin. VZV vaccine is available and has been shown to reduce the frequency of infections. It is a live attenuated vaccine hence should be avoided in pregnancy although no congenital malformations have been reported in women who have been accidentally vaccinated.

Zika Virus (ZIKV)

Zika virus (ZIKV) belongs to the family, *Flaviviridae*. There are approximately 70 viruses in this family, transmitted by the *Aedes Egypti* mosquito. Members of this genus include dengue virus (DENV), yellow fever virus (YFV), West Nile virus (WNV), Japanese encephalitis virus (JEV), and tick-borne encephalitis virus. It was first isolated in the Zika forest of Uganda in 1947. A few reported cases of human infection with ZIKV in Africa and analysis of seroprevalence indicates that the virus had circulated silently for two decades [186]. The first large outbreak outside of Africa and Asia was reported on the island of Yap in Micronesia [187], and in 2013–2014, a second outbreak occurred in French Polynesia [188]. Thereafter it gradually spread to New Caledonia, the Cook Islands, and Easter Island in the South Pacific [189]. A phylogenetic analysis of nucleotide sequences of isolates collected between 1947 and 2010 to determine the viral lineages revealed two main types-an African and an Asian, type. The strain responsible for the Micronesia epidemic and Cambodian cases originated in Southeast Asia [190]. The virus was first reported in Brazil in 2014 from where it spread rapidly within South America. [191,192]. It was not previously considered a major teratogen until a series of severe congenital malformations were reported in the Americas between 2015 and 2016 that were attributed to ZIKV [193,194].

Morphology: Zika virus is a positive, single-strand positive-sense ribonucleic acid (RNA) virus with a genome size of approximately 10.8 kilobases. The RNA is translated into a single polyprotein (3423 amino acids in length) encoding 3 structural proteins—capsid (C); membrane (M), which is generated from its precursor pre-membrane (prM); and envelope (E)—as well as 7 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [195]

Of the 7 non-structural proteins, NS1 is secreted, and just like the NS1 of dengue virus (DENV) and West Nile virus, it is considered a pathogenesis factor that degrades the glycocalyx on endothelial cells to trigger antiviral responses [196-198]. ZIKV NS1 has been shown from a recent analysis of the crystal structure and sequence of full-length to be an elongated hydrophobic surface for membrane association and a polar surface that varies substantially among flaviviruses [199]. When the structure of this non-structural protein is compared between ZIKV, West Nile virus, and DENV, NS1 structures is shown to have conserved features, but diverse electrostatic characteristics at host-interaction interfaces [200,201]. It has been reported that enhancement of NS1 antigenaemia in infected hosts promotes ZIKV infectivity and prevalence in mosquitoes and could have facilitated dissemination in recent epidemics. [202]

Zika virus and pregnancy

The epidemic in the Americas responsible for the outbreak of the various congenital malformations has been attributed to the Asian-lineage ZIKV strains. This epidemic was first reported in northern Brazil causing microcephaly *in-utero* and Guillain-Barre´ syndrome in adults [203.204]. Subsequently, prenatal maternal infection with ZIKV strains of the Asian lineage was associated with a plethora of malformations that collectively have been labelled congenital Zika virus syndrome (ZVS). These include microcephaly, neurological impairment, cerebral calcifications, and retinal damage [205,206] (see Table 1 for more details). While most transmissions are from the bite of the *Aedes Egypti* mosquito, sexual transmission of ZIKV has also been reported [207]. The magnitude of the intrauterine consequence of ZIKV infection stems from its link to more than 2,700 cases of

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microcephaly among confirmed maternal infections in the Americas during recent epidemics [208]. RNA of the virus has been found in neonatal brain, the placenta, and amniotic fluid of affected babies, confirming transplacental transmission and therefore regarding ZIKV as aetiological in these malformations [209-201]. The reported rates of congenital malformations vary with gestational age at intrauterine infection. In a series of 2,549 pregnancies from USA territories, with laboratory evidence of recent maternal infection from January 2016 to April 2017, in the first, second, and third trimesters, reported rates of congenital malformations rates were 8%, 5%, and 4%, respectively [212]. The rate of microcephaly reported in a series from Brazil varied from 0.03-17.1% depending on the geographical area [213]. It has been suggested that the wide variation in rates of congenital malformations could be secondary to possible modifiers such as undernutrition, dense population in urban slums, and lack of information on measures to prevent infection during pregnancy [214,215].

That the virus is responsible for a series of congenital malformations most of which occur at the time of organogenesis indicates that transplacental vertical transmission must occur early in pregnancy. How does the virus affect target cells to cause these malformations? The virus is known to target cells of the developing placenta and contribute to pathology. ZIKV has been shown to replicate in primary cells isolated from chorionic villi and amniochorionic membranes, including CTBs, Hofbauer cells, umbilical cord endothelial cells, TBPCs, and amniotic epithelial cells [216-218]. The virus also infects numerous cell types of basal decidual explants, also targeting proliferating CTBs in cell columns and villus sprouts and Hofbauer cells in stromal villus cores anchoring villi, but not STBs, possibly because of the antiviral effects of IFN-λ [216, 219-221].

Several studies - mainly on first trimester villous explants have been undertaken to explain why American strains disseminate to the pregnant uterus and replicate in the placenta while the African strains do not. Four possible explanations have been suggested from these studies. Firstly, the main targets of ZIKV infection in humans, $CD14^{+}CD16^{+}$ monocytes strongly produce the chemokine CXCL12/SDF-1 and the cytokine IL-6 [222]. These infected monocytes could cross uterine BVs into basal decidua (176) where they differentiate into $CD14^{+}$ macrophages that inhibit dNK cell functions [223]. Furthermore, ZIKV infection in endometrial glands, macrophage/dendritic cells, and decidual cells could increase virus titres in the intervillous blood space [217,219,224,]. Secondly the fact that , the African prototype strain displayed more rapid replication kinetics and produced higher virus titres in human dendritic and placental cells compared with the American (Nicaraguan) strains, may imply it stimulates a strong cellular immune response that may indeed suppress infection [216,224,225] and thus inhibit vertical transmission. The third explanation comes from analysis of villus explants infected with either the American or the African strains which showed differences in functions of infected CTBs [224]. Explants infected with the American strains replicated in proliferating cell columns and differentiated into infected CTBs that could invade basal decidua while explants infected with the African strains were severely impaired and thus had limited ability to invade the basal decidua. The fourth possible explanation is the ability of the American (Nicaraguan) strains to replicate in Hofbauer cells more frequently and cell proliferation suggested a source of infection that spreads to fetal BVs in villus cores compared to the African strain [209,225].

Treatment and prevention

There are currently no antiviral agents or vaccines approved for use against Zika viruses. Developing a safe and immunogenic vaccine against Zika virus remains an unmet medical need. One of the platforms that have reached clinical trial is the mRNA vaccine. Two randomised, placebo-controlled, dose-ranging, multicentre, phase 1 trials, one of mRNA-1325 (mRNA-1325 trial) and the other of mRNA-1893 (mRNA-1893 trial), have been performed. Three dose levels of mRNA-1325 (10, 25, and 100 μg) were used and these were generally well tolerated, but the vaccine elicited poor Zika virus-specific nAb responses after one dose. On day 57, all evaluated mRNA-1893 dose levels induced robust Zika virus-specific nAb responses, independent of flavivirus serostatus, that persisted until month 13. The conclusion from these findings was that the development of mRNA-1893 against Zika virus, which was well tolerated at all evaluated dose levels and induced strong Zika virus-specific

serum nAb responses after two doses, regardless of baseline flavivirus serostatus should be continued [226].

Summary and Conclusions

Viral infections in pregnancy are common and the relatively immunocompromised status of the women, in some cases increases the severity of these infections. Some of these viruses cross the placenta, and/or are secreted in various bodily fluids including vaginal, cervical secretions and breastmilk and infect the fetus/newborn. The consequences depend on the virulence of the virus and the gestational age at the infection. These effects include miscarriages, congenital malformations, preterm birth, stillbirth, and neonatal morbidity. Precisely how some of these viruses reach the fetus is uncertain, however the basal decidua has been shown to be invaded by most of the viruses that reach the fetus. Protection against these infections may be from maternally acquired antibodies (IgG from previous infections) or from immunisation. There are currently very few approved anti-viral agents for use in pregnancy and for most of these infections, prevention is the best approach to avoiding them. The Table summarises the viral infections that have been discussed, their incubation periods, diagnostic approaches, consequences on the fetus and/or neonate and treatment/prevention. Once diagnosed, a multidisciplinary approach to management must be adopted. This would include a fetal medicine physician, a virologist and neonatologist where appropriate.

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