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Stillbirth: the role of viral infections

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INTRODUCTION

The definition of stillbirth (SB) is the demise of a fetus at or beyond 22 weeks (154 days) of gestation, or with a birthweight of at least 500 grams in cases where the gestational age was indeterminate. Although several conditions and specific risk factors have been associated with SB, in most of the cases it is difficult to identify the definitive pathway causing death and a significant number of cases remain unexplained. The aim of our study is to assess the role of viral infections in the cases of SB that occurred between 2014 and 2022 in the four birth centers of the Bologna wide area.

METHODS

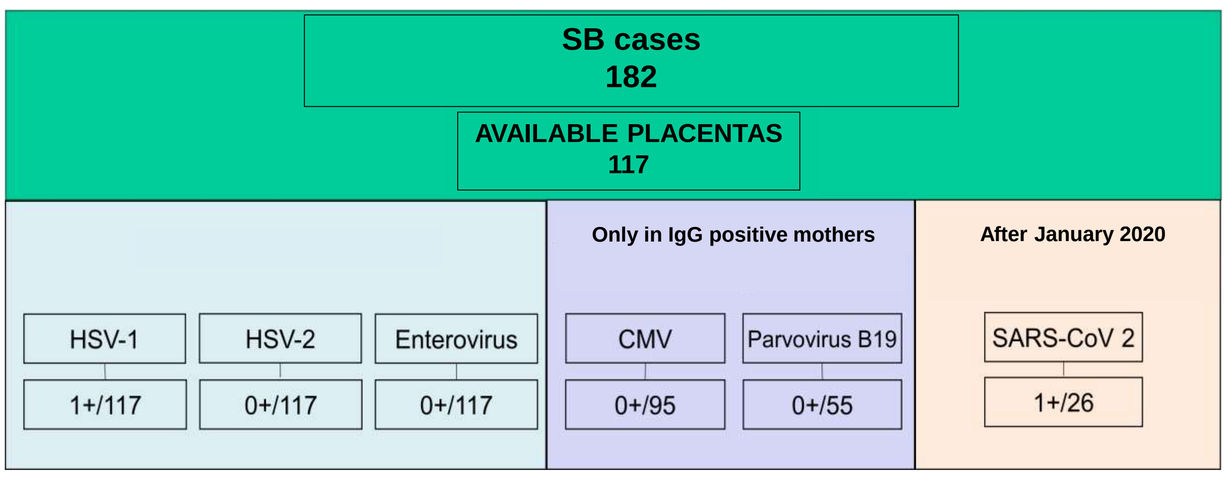
The genome of enterovirus (EV), herpes simplex virus-1 (HSV-1), herpes simplex virus-2 (HSV-2), parvovirus B19 and cytomegalovirus (CMV) was tested on the placentas of SB cases at the Operative Unit of Microbiology, IRCCS Azienda Ospedaliero-Universitaria di Bologna. In addition, the genome of SARS-CoV-2 was also investigated after January 2020. In case of a positive result on the placenta, further molecular investigations were performed on paraffin-embedded foetal tissues. Specifically, 10µm of paraffin-embedded sections were pre-treated using 160µL of deparaffination solution, 180 µL of tissue lysis buffer and 20 µL of proteinase K. Viral genome extraction and amplification were performed using ELITe MGB kits on the ELITeInGenius automated instrument (ELITechGroup, Italy).

RESULTS

A total of 182 cases of SB occurred in the period January 2014-December 2022, out of 68741 live birth, with an overall rate of 2.65‰.
Maternal blood was analysed for specific IgG and IgM for Parvovirus B19 and CMV with a seroprevalence of 53.8% (78/153) and 88.8% (135/162), respectively.
Only 117 placentas were available for further molecular investigations. All placentas were negative for EV and HSV-2 and only 1 case (0.9%) was positive for HSV-1 with a viral load of 100 copies/µg DNA. HSV-1 DNA was investigated also on paraffin-embedded tissues of fetal lung and brain with a negative result. Histological examination of placenta and fetal organs was not suggestive of infection. CMV and Parvovirus B19 PCR was performed only in placentas of women with a previous immunity. CMV and Parvovirus B19 were negative in all the cases.
The detection of the SARS-CoV 2 genome was carried out on the 26 placentas collected after January 2020. Only one case of a mother with clinical symptoms of SARS-CoV 2 infection (fever, non-productive cough, vomiting, and bilateral low back pain) and a positive swab just before delivery tested positive, with a placental viral load of 4.640.000 copies/µg of RNA, UK variant.

The cause of fetal death defined by the multidisciplinary team was indicated as placental abruption. This was confirmed by post-mortem placental examination as adherent blood clots were observed in the decidua.
On the fetal paraffin-embedded tissues SARS-CoV 2 RT PCR was negative in the stomach, pancreas, liver, thymus, lungs, heart, spleen, intestine and kidneys while the brain was positive with a viral load of 30 copies/20ng of RNA.

Figure 1: Molecular investigations on placenta



CONCLUSIONS

In our study we defined a SB-related infection only in those cases where positive microbiological findings are correlated with the simultaneous histological evidence of fetal organ damage.
Regarding viruses, in one placenta the low HSV-1 load, the absence of an inflammatory infiltrate and the lack of infection in fetal organs, ruled out an SB infection-related, assuming that the viral contamination might have occurred during the passage through the birth canal. Regarding SARS-CoV 2 infection, in our case, the high viral load in the placenta and the detection of a placental histological damage, suggest that there may be a causal relationship between the placental abruption and SARS-CoV 2 infection.
Some authors suggested that EV infection may be a common cause of unexplained SB, but these findings are debated in literature. Our in-depth investigations excluded EV placental infection suggesting that EV is not a major cause of SB, but further studies are needed.