Neonatal Sulfhemoglobinemia and Hemolytic Anemia Associated With Intestinal *Morganella morganii*

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abstract

Sulfhemoglobinemia is a rare disorder characterized by the presence of sulfhemoglobin in the blood. It is typically drug-induced and may cause hypoxia, end-organ damage, and death through oxygen deprivation. We present here a case of non–drug-induced sulfhemoglobinemia in a 7-day-old preterm infant complicated by hemolytic anemia. Microbiota compositional analysis of fecal samples to investigate the origin of hydrogen sulphide revealed the presence of *Morganella morganii* at a relative abundance of 38% of the total fecal microbiota at the time of diagnosis. *M morganii* was not detected in the fecal samples of 40 age-matched control preterm infants. *M morganii* is an opportunistic pathogen that can cause serious infection, particularly in immunocompromised hosts such as neonates. Strains of *M morganii* are capable of producing hydrogen sulphide, and virulence factors include the production of a diffusible α-hemolysin. The infant in this case survived intact through empirical oral and intravenous antibiotic therapy, probiotic administration, and red blood cell transfusions. This coincided with a reduction in the relative abundance of *M morganii* to 3%. Neonatologists should have a high index of suspicion for intestinal pathogens in cases of non–drug-induced sulfhemoglobinemia and consider empirical treatment of the intestinal microbiota in this potentially lethal condition.

Sulfhemoglobinemia is a relatively uncommon illness characterized by excess sulfhemoglobin (SHb) in the blood. SHb forms through the reaction of a sulfur atom with the heme moiety that has been oxidized from the normal divalent to a trivalent state, making it incapable of carrying oxygen.[1–3] Thus, formation of SHb may cause hypoxemia, resulting in end-organ damage and death though oxygen deprivation. Unlike methemoglobin (MetHb), SHb cannot be reconverted to normal hemoglobin and persists until the end of the red blood cells' life span.[1,3] The majority of cases are attributed to exposure to sulfur-containing drugs, but SHb has also been postulated to be derived from hydrogen sulphide (H_2S) produced by the intestinal microbiota.[4,5] We present a case of sulfhemoglobinemia and hemolytic anemia in a 7-day-old infant, who fortunately survived this potentially lethal condition, in addition to our efforts to identify the source of H_2S.

CASE REPORT

Our subject was a female infant born at 29 weeks' gestation by emergency cesarean delivery for placenta previa, with a birth weight of 1.49 kg to a gravida 5, para 4 mother. At day of life (DOL) 2, she was transferred to the NICU at Cork University Maternity Hospital from a peripheral hospital. Minimal enteral feeding, 30 mL/kg daily, commencing at 38 hours of life,

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was tolerated well, and the first stool passed at 54 hours appeared normal. The infant required endotracheal intubation and was given surfactant for respiratory distress syndrome but otherwise had an uneventful course at the NICU until DOL 7 when she developed hypoxemia, tachypnea, and apnea. A strong odor, similar to H_2S, was noted from her incubator and her stool, samples of which were collected.

A capillary blood gas test revealed a MetHb level of 6.1% (normal <1%), which continued to rise to 10.3%. Laboratory blood gas CO-oximeter analysis using a GEM Premier 4000 (Instrument Laboratory, Warrington, United Kingdom.) recorded the presence, but not the quantity, of SHb, which we deduced was responsible for the falsely elevated levels of MetHb. Full blood count investigations revealed raised white blood cells (27.1 g/dL) and hemoglobin (11.8 g/dL). No abnormal hemoglobin variants were detected by using high performance liquid chromatography or electrospray ionization mass spectrometry. Evaluation for sepsis was performed and all routine bacterial, viral, and fungal study results were negative, including blood, urine, and cerebrospinal fluid analysis. Throughout this time, pulse oximetry oxygen saturations were in the high 70s and low 80s and did not increase significantly with supplementary oxygen therapy or continuous positive airway pressure. Abdominal x-ray revealed nonspecific mildly dilated bowel loops without any pneumatosis intestinalis.

Despite no obvious signs of mother's breast milk being a source of toxicity, as a precautionary measure, it was discontinued and the infant was placed on donor breast milk at DOL 13. The infant was commenced on standard antibiotics for potential sepsis: 11.7 mg/kg of teicoplanin and 5.9 mg/kg of gentamicin intravenously once daily. Although

the infant passed stools normally during this period, in view of the foul odor that persisted for up to a week, 7.5 mg/kg twice daily of metronidazole nasogastrically was commenced on an empirical basis. In addition, 125 mg/kg of a commercial probiotic, Infloran, was administered nasogastrically twice daily. On DOL 12, the infant's clinical status deteriorated with the development of hemolytic anemia, when her hemoglobin dropped to 9.1 g/dL. She required 2 red blood cell transfusions, in addition to phototherapy due to her serum bilirubin level rising to 276 μmol/L. Blood cultures remained sterile throughout the infant's clinical course.

Over the next 2 weeks, the infant improved with oxygen saturations rising to the mid-90s and hemoglobin levels normalizing. Feeding with donor milk ceased at DOL 20, and mother's breast milk was recommenced. She was discharged from the hospital at DOL 31. At her last follow-up, 10 months after her initial presentation, there was no sign of any clinical or neurologic abnormalities, nor was there any history to suggest episodic or ongoing hemolysis.

Subsequent microbial compositional analysis of DNA extracted from infant fecal samples and mother's breast milk via Illumina MiSeq sequencing identified the presence of *Morganella morganii* in the feces of the infant at DOL 7. The fecal microbiota composition of our subject was compared with the aggregate composition of 40 age-matched preterm control infants from the same NICU (Fig 1). *M morganii* represented 38% of the relative abundance in our subject, whereas it was completely absent in the control group. The relative abundance was observed to markedly reduce to 3% in the final fecal sample obtained at DOL 21 while the infant was recovering (Fig 2). *M morganii* was

not detected in either the mother's breast milk or stool.

DISCUSSION

We present a case of sulfhemoglobinemia in a preterm infant that resulted in survival after a complicated clinical course that included moderate respiratory distress, intractable hypoxemia, and hemolytic anemia. We also report the use of intestinal microbiota compositional analysis to investigate the origin of H_2S and the empirical use of intestinal antibiotics and probiotics in the management of this infant. Thus, this case is of interest from a diagnostic, microbiological, hematological, and therapeutic standpoint.

Sulfhemoglobinemia is easily misdiagnosed as methemoglobinemia; indeed, the initial capillary blood gas test in this case reported falsely elevated measures of MetHb. SHb has an absorption band similar to that of MetHb at ~620 nm (Fig 3).[2,6,7] This has been known to cause high levels of the former to be erroneously interpreted as elevated levels of the latter.[3,8] Co-oximeters can vary, and some machines are unable to distinguish between these 2 types of hemoglobin; therefore, it is important that co-oximeters with the ability to differentiate are used in cases of suspected sulfhemoglobinemia to make a correct diagnosis and administer the appropriate treatment.[9,10]

Sulfhemoglobinemia is most commonly drug induced. Sulfonamides, phenacetin, acetanilide, and phenazopyridine have been frequently implicated.[1,8,11,12] Our case was challenging because the origin of H_2S was not apparent. It has been postulated that in the absence of sulfur-containing drugs, the source may be from H_2S produced as a metabolic end product by intestinal microbiota capable of reducing sulfate or elemental sulfur.[4,13,14] Retrospectively, fecal samples were subjected to
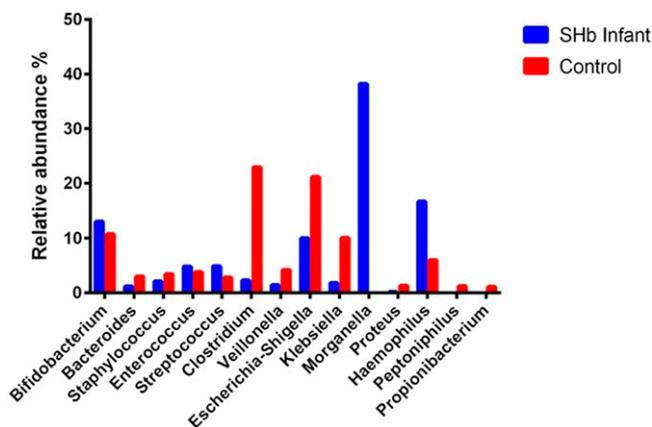
FIGURE 1
Relative abundances of the genus level microbiota composition in infant fecal samples at DOL 8. Blue bars represent the sulfhemoglobinemia infant, and red bars represent the aggregate of 40 age-matched controls.

microbiota compositional analysis via Illumina MiSeq sequencing where we noted the presence of *M morganii*. This species represented 38% of the relative abundance in the fecal microbiota of our subject, whereas it was completely absent in a control group and was observed to reduce to 3% abundance while the infant was recovering. *M morganii* was not detected in breast milk, and thus in retrospect the decision to interrupt the administration of mother's milk was not necessary.

*M. morganii* is an opportunistic pathogen which has been implicated in diarrhea and in infections of blood, respiratory tract, urinary tract, and wounds.[15–19] *M morganii* is known to cause disease in immunocompromised patients; prematurity was likely a risk factor in this case with these infants being more susceptible to infections because of their immature immune systems.[20–23] The genus *Morganella* currently consists of 1 species, *M morganii*, which has been separated into 2 subspecies, *morganii* and *sibonii*, on the basis of the ability to ferment trehalose.[24] *M morganii* subsp *morganii* contains 4 biogroups A, B, C, and D, and it does not ferment trehalose, whereas *M morganii* subsp. *sibonii* contains biogroups E, F,

and G and does ferment trehalose. Of particular relevance here is the ability of biogroups B, C, and G to produce H_2S and its production of a diffusible α-hemolysin.[16] Like many other members of the *Enterobacteriaceae* family, *Morganella* are capable of producing β-lactamases and have an intrinsic resistance to a number of antibiotics. Therefore, third-generation cephalosporins are the recommended treatment of *M. morganii* infections.[25]

We could not find any previous reports of *M morganii* being associated with sulfhemoglobinemia, but there has been a recent case study of SHb induced cyanosis in a neonate involving a hemolytic *Escherichia coli*.[26,27] Unlike our subject, the previous patient did not survive and died of multiple organ failure with an additional underlying diagnosis of meconium ileus due to cystic fibrosis. In addition, *E coli* and hemolysis were detected in nearly all organs, strongly suggesting an extraintestinal role of the *E coli* hemolysin. Interestingly, its hemolysin is 1 of a close family of membrane targeted toxins, which includes the hemolysin of *M morganaii*.[28,29]

CONCLUSIONS

Members of the *Enterobacteriaceae* family such as *M morganii* are emerging as significant pathogens but are usually missed by the routine identification methods used in most hospital laboratories. Although non–drug-induced sulfhemoglobinemia is rare, neonatologists should consider the intestinal microbiota in cases with unexplained source of H_2S and request fecal culture examination. Antimicrobial therapy with broad-spectrum antibiotics should be administered until culture results are obtained, and treatment should be reassessed according to the culture results. We believe prompt administration of intestinal
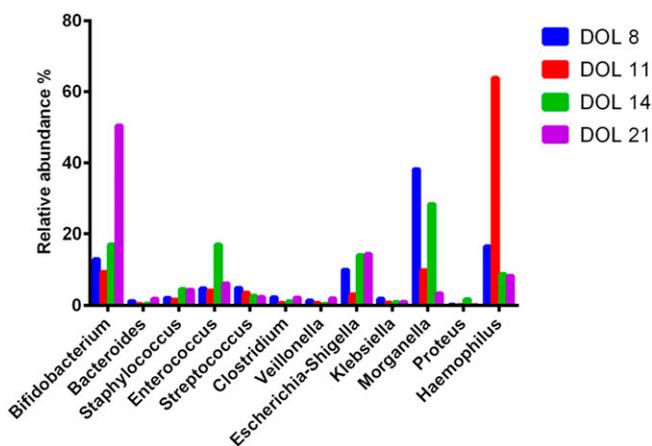
FIGURE 2
Progression of the genus-level fecal microbiota composition from the onset of symptoms at DOL 8 to recovery at DOL 21.
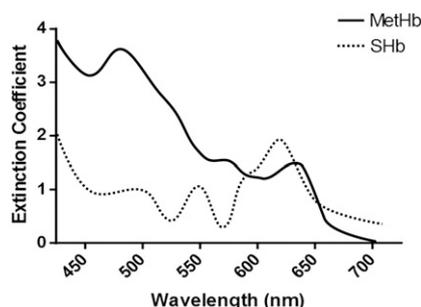
FIGURE 3
Extinction curves for methemoglobin and sulf-hemoglobin. Methemoglobin and sulfhemoglo-bin have similar peak spectral absorbance at ~620 nm. The extinction coefficient is expressed in cm-1/(mol/L) $\times$ 10^4.

antimicrobial and probiotic therapy in addition to systemic antibiotics may have contributed to the survival of this infant. In conclusion, we present a case that illustrates the potential of H_2S-producing bacteria, namely *M morganii* in this instance, in the development of sulfhemoglobinemia and the importance of maintaining a high level of clinical suspicion for the intestinal microbiota in non–drug-induced sulfhemoglobinemia.

ABBREVIATIONS

DOL: day of life
H_2S: hydrogen sulphide
MetHb: methhemoglobin
SHb: sulfhemoglobin

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