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CASE REPORT

1 Acute lymphoblastic leukemia in a β-thalassemia intermedia child: A case report

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ABOUT COVER

Editor-in-Chief of *World Journal of Clinical Pediatrics*, Dr. Sergi obtained his MD degree with honors (1989) and Pediatric specialization (1993) at the University of Genoa, Italy, and Pathology specialization at the University of Heidelberg, Germany (2001). Dr. Sergi is currently a Full Professor of Pathology and Adjunct Professor of Pediatrics at the University of Alberta, Canada. Dr. Sergi also serves as a Consultant of Carcinogenesis in Experimental Animals at the World Health Organization/International Agency for Research on Cancer (WHO/IARC, Lyon, France) and an "ad-hoc" Peer-Referee for the National Toxicology Program of the National Institutes of Health (Research Triangle Park, NC, United States). His areas of interest are Biology and Pathology of the Cardiovascular/Gastrointestinal System with Gut/Bile Microbiome involvement as well as Bone Cell Biology. Dr. Sergi has authored over 250 peer-reviewed publications indexed in the PubMed literature database (H-index: 23, RG-score: 44.26, > 2500 citations). (L-Editor: Filipodia)

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CASE REPORT

Acute lymphoblastic leukemia in a β-thalassemia intermedia child: A case report

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Abstract

BACKGROUND

β-thalassemia intermedia (βTI) is one of the hemoglobinopathies. It constitutes 10% of β-thalassemia cases and is associated with better quality of life than β-thalassemia major (βTM).

CASE SUMMARY

We recently reported the first case of acute lymphoblastic leukemia (ALL) from Egypt in a child with βTM, and we herein report the first case of ALL from Egypt in a child with βTI. In this report, literature was reviewed for cases of malignancies associated with βTI and the possible factors underling the relationship between the two entities.

CONCLUSIO

We stress that physicians should have a high index of suspicion of malignancies in thalassemia patients if they present with any suggestive symptoms or signs.

Key Words: Acute lymphoblastic leukemia; Thalassemia intermedia; Children; Malignancies; Iron overload; Hydroxyurea; Case report

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Core Tip: Cases of malignancies in patients of β-thalassemia major have been reported. However, rare cases have been reported for malignancies in patients of β-thalassemia-intermedia as it is a non-transfusion dependent anemia. Physicians should have high index of suspicion to diagnose malignancies in patients with β-thalassemia-intermedia.

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INTRODUCTION

Thalassemia represents the most common single-gene disorder worldwide. The total annual incidence of symptomatic individuals with β-thalassemia is estimated at 1 in 100000 throughout the world, of whom nearly 10% have β-thalassemia intermedia (βTI), which is intermediate in severity between the milder thalassemia-minor and the more severe transfusion-dependent β-thalassemia-major (βTM)[1].

We herein report the first case from Egypt with βTI who developed acute lymphoblastic leukemia (ALL).

CASE PRESENTATION

Chief complaints

The reported patient is a 15-year-old girl with βTI, who presented at the age of 3 years with pallor, decreased growth rate and decreased activity. She had severe microcytic, hypochromic anemia with hemoglobin (Hb) of 7.3 g/dL.

History of present illness

Pediatric hematologist workup confirmed the diagnosis of βTI. Her Hb electrophoresis showed 69.9% HbA, 27.2% HbF and 2.9% HbA2. Genetic molecular testing revealed compound heterozygosity for cd-27 (G>T) and cd-39 (C>T) mutations. Hydroxyurea at a dose of 15 mg/kg per day was started, in addition to folic acid.

She was then followed at the pediatric hematology unit at regular intervals to monitor her tolerance to drug therapy, with special attention to hematological toxicity. There were no significant side effects during 7 years of therapy, and the patient showed good response with occasional need for blood transfusions. She underwent splenectomy during her late teens.

History of past illness

At the age of 15 years, she developed generalized bone aches, abdominal pain, persistent fever and dyspnea, and she was referred to our hospital.

Physical examination

On physical examination, there was severe pallor, tachypnea, tachycardia and hepatomegaly.

Laboratory examinations

Initial complete blood picture showed a Hb level of 3.9 g/dL, white blood cell count of 250×10^9/L and platelet count of 640×10^{-9}/L.

Serum electrolytes, cerebrospinal fluid analysis and kidney and liver function tests were normal, except for mild elevation of total serum bilirubin, which was 1.3 mg/dL.

Serum ferritin was 877 ng/dL. Serological studies including Epstein-Barr virus, cytomegalovirus, human immunodeficiency virus, hepatitis C virus and hepatitis B virus were negative. Lactate dehydrogenase was 974 U/L, and serum uric acid was 5.6 mg/dL.

Imaging examinations

Her chest X-ray was normal. Abdominal ultrasonography revealed hepatomegaly with calcular cholecystitis and bilateral diffuse renal enlargement. Echocardiography showed mitral valve prolapse with trivial mitral regurgitation.

MULTIDISCIPLINARY EXPERT CONSULTATION

The pediatric hematologist/oncologist assessment requested bone marrow biopsy, which was carried by the hematopathologist

Bone marrow examination revealed 80% blast cells in a hyper-cellular marrow with depressed erythropoiesis and granulopoiesis and normal thrombopoiesis. Immunophenotyping showed lymphoblasts that were CD10 positive, CD19 positive, CD34 positive, TDT positive, HLA-DR positive, CD13 positive and CD33 positive.

Cytogenetic examination showed a normal karyotype with a deoxyribonucleic acid (DNA) index of 1 and negative t(12,21), t(1,19), BCR-ABL and 11q23 translocations/mutations.

FINAL DIAGNOSIS

A final diagnosis of B-acute lymphoblastic leukemia (ALL) with aberrant expression of CD13 and CD33 was achieved.

TREATMENT

Induction chemotherapy of the total XV protocol with prednisone, vincristine, L-asparaginase, doxorubicin, cyclophosamide, cytarabine, 6-mercaptopurine and intrathecal chemotherapy was commenced.

She received multiple packed red cell transfusions, which eventually led to elevation of serum ferritin to 1420 ng/dL. She was then started on oral chelation therapy with deferasirox with no complications.

The patient eventually went into complete remission. She then received consolidation chemotherapy of standard risk of the total XV protocol with four times of high dose methotrexate, 6-mercaptopurine and intrathecal chemotherapy.

She received multiple packed red blood cell transfusions and other supportive measures during the periods of induction and consolidation. The transfusions therapy was given according to the guidelines of pediatric oncologists. They usually transfuse if Hb level is less than 8 g/dL and if associated with pulmonary or cardiac comorbidities or exposed to invasive procedure and hemorrhage, and they transfuse with Hb less than 10 g/dL. The transfusions were not associated with any complications. Deferasirox was stopped in the consolidation phase during infusion of high dose methotrexate.

The main problem observed during the periods of induction and consolidation therapy was increased requirement of blood transfusions as well as repeated infections. During this period, the child received intensive chemotherapy, which caused bone marrow suppression

OUTCOME AND FOLLOW-UP

The child is still in complete remission and is in the continuation phase for standard risk (week 40).

DISCUSSION

We recently reported the first case from Egypt with βTM who developed ALL[2]. Herein, we report similarly the first patient from Egypt with βTI who developed ALL to highlight that the coexistence of malignancy and beta thalassemia is not rare.

A thorough review of the literature for previously reported cases of malignancies in patients with βTI revealed only one report in 2014 from Turkey of a 12-year-old boy with βTI who developed ALL[3]. Other previous reports of malignancies associated with βTI described non-Hodgkin's lymphoma[4-6], chronic myeloid leukemia[6,7], Hodgkin's lymphoma[5,6,8], hepatocellular carcinoma[9-13] and thyroid malignancies[14] (Table 1). To our knowledge, our patient is the second worldwide and the first from Egypt.

Although reported cases of malignancies associated with βTI are few, physicians should have a high index of suspicion of malignancies in this group of patients when they present with unexplained new symptoms or proposed symptoms and signs of malignancy.

Table 1 Previously reported cases of thalassemia intermedia who developed malignancies

Number of patients	Type of malignancy	Ref.
1	Acute lymphoblastic leukemia	[3]
1	Non-Hodgkin lymphoma (NHL)	[4]
3	NHL, Hodgkin lymphoma (HL)	[5]
10	NHL; HL; chronic myeloid leukemia (CML)	[6]
1	CML	[7]
1	HL	[8]
12	Hepatocellular carcinoma (HCC)	[9]
2	HCC	[10]
6	HCC	[11]
3	HCC	[12]
2	HCC	[13]
2	Thyroid cancer	[14]

Some patients have thalassemia major and others have thalassemia intermedia in references 5 and 6.

There should be special concern about management plans in these patients as they usually require more frequent blood transfusions, as the chemotherapy causes suppression of the bone marrow, which adds to baseline chronic hemolysis.

Because reported cases of malignancies in βTI are scarce, attempts to find a causal relationship between βTI and cancer development are beyond the scope of our report. We tried to search the literature for possible contributing factors, but those factors are not sufficient to allow us to draw any conclusions. Possible hypotheses regarding factors need to be addressed and validated by a larger prospective cohort with large control groups and multicenter worldwide studies. Indeed, an explanation of underlying factors for the development of malignancy in TI is likely multifactorial[15].

In a large multicenter study on thalassemia patients from Iran, the proportion of patients with cancer was higher in those with βTI than those with βTM; 0.54% and 0.20%, respectively[6]. It was suggested that bone marrow in βTM patients is suppressed by the regular transfusions, while it is very active with high turnover in those with βTI[6]. This can lead to a higher rate of DNA repair faults and mutations with subsequent higher rate of hematological malignancies[6].

Another potential factor is the prolonged use of hydroxyurea, and there are conflicting data regarding its carcinogenic potential. Hydroxyurea as an antimetabolite interferes with both DNA synthesis and repair mechanisms with later accumulation of mutations and subsequent chromosomal damage. Although no studies have yet investigated the relationship between hydroxyurea and the development of cancers in thalassemia, clinical concerns have been raised regarding its potential leukemogenic potential[7,15]. Other authors were against this assumption[6,16]. The BABYHUG clinical trial, which compared hydroxyurea with placebo treated controls, refuted this assumption and did not suggest any increased risk of genotoxicity[16].

Overall, there is no evidence to suggest an increased risk of carcinogenesis in patients with thalassemia with hydroxyurea and further studies will need to be designed to establish any potential relationship[14].

One more probable factor is that patients with βTI have milder disease than those with βTM, and fewer blood transfusions might lead to delayed diagnosis. Even if diagnosed, usually there is an underestimation of their iron overload problem. Sometimes the relatively mildly elevated ferritin in βTI, as compared to βTM, is underestimated and the true iron burden in these patients usually goes unnoticed, unchelated and unmonitored. Anemia, hypoxia and ineffective erythropoiesis suppress the expression of hepcidin by increasing expression of growth differentiation factor 15 and hypoxia-inducible transcription factors with resulting increase in intestinal iron absorption and, in turn, the problem of iron overload[13].

Long-standing iron overload with its deposition in different body organs, with the well-known association between excess iron and cancer development, can be a predisposing factor for all types of malignancies; through direct and indirect effects[13].

Iron can directly damage DNA by non-transferrin-bound iron, with the consequent inactivation of tumor-suppressor genes, such as *p53*, or their products. The indirect effects include the formation of reactive oxygen species, iron-induced lipid peroxidation, altered immune system with decreased immune surveillance, suppression of tumoricidal action of macrophages and alteration of cytokine activities. βTI patients usually survive longer than βTM patients with enough time for iron overload to develop[13].

Some authors have suggested that improved management protocols of thalassemia patients have led to increased survival, with most of them reaching adult age with the consequent occurrence of diseases associated with long life span like malignancies[5,7]. This assumption can partially explain other reports in elder patients but cannot explain our patient or the Turkish one, who are teenagers.

It has been suggested that the occurrence of malignancies in thalassemia patients could be a pure coincidence or a combination of genetic and environmental factors[3,7]?

CONCLUSION

In summary, whatever the pathogenesis of malignancies in thalassemias, the most important message is to alert physicians that they should suspect malignancies if their thalassemia patients develop suggestive symptoms and signs. Worsening anemia, leukocytosis, fever, bone-aches, lymphadenopathy and splenomegaly are signs to look for leukemias and other hematological malignancies.

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REVIEW

Pediatric surgery during the COVID-19 pandemic

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Abstract

The coronavirus disease 2019 (COVID-19) pandemic has had a major impact on pediatric surgery. The infection is often asymptomatic and atypical in children, while overlapping presentations with other infectious diseases generate additional diagnostic challenges. The high probability of missed pediatric cases and the invasive nature of surgery generate great concern for widespread transmission in this setting. Current guidelines suggest that triage of cases should be made on a case-by-case basis by a multidisciplinary team of experts. Decision-making can be assisted by classifying cases as elective, urgent, or an emergency according to the risks of delaying their surgical management. A workflow diagram should ideally guide the management of all cases from admission to discharge. When surgery is necessary, all staff should use appropriate personal protective equipment, and high-risk practices, such as aerosol-generating tools or procedures, should be avoided if possible. Furthermore, carefully designed organizational protocols should be established to minimize transmission while ensuring the uninterrupted operation of pediatric surgery units. For example, surgical teams can be divided into small weekly rotating groups, and healthcare workers should be continuously monitored for COVID-19 symptoms. Additionally, team protocols in the operating room can optimize communication and improve adherence to personal protective equipment use. Isolated operating

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rooms, pediatric intensive care units, and surgical wards should be specifically designed for suspected or confirmed COVID-19 cases. Finally, transportation of patients should be minimal and follow designated short routes. All these measures can help mitigate the effects of the COVID-19 pandemic on pediatric surgery units.

Key Words: Pediatric surgery; COVID-19; SARS-CoV-2; Coronavirus; Emergency surgery; Personal protective equipment

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Core Tip: The coronavirus disease 2019 (COVID-19) pandemic has had a major impact on pediatric surgery. The diagnostic challenges in the pediatric population and the invasive nature of surgery generate concern for widespread transmission. Each case should be assessed individually, categorized by urgency and managed according to a predesigned workflow diagram. All staff should use appropriate personal protective equipment and high-risk practices should be avoided. Protocols for organization of the surgical team and hospital infrastructure should be established to maximize safety and efficiency, while minimizing transmission. All these measures can help mitigate the effects of the COVID-19 pandemic on pediatric surgery units.

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CORONAVIRUS DISEASE 2019: A WORLDWIDE PANDEMIC

Several pneumonia cases of unknown etiology were reported on December 31, 2019 in the city of Wuhan, China. A novel coronavirus was soon identified to be the causative agent[1,2]. The virus was provisionally named 2019-nCoV and was later renamed as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses[3]. On January 30, 2020, the World Health Organization declared the outbreak a "Public Health Emergency of International Concern" following rapid transmission in multiple countries and named the disease "coronavirus disease 2019" (COVID-19) 12 d later[4,5]. By March 11, 2020, COVID-19 was officially declared a pandemic[5]. This outbreak has affected multiple aspects of healthcare and patient populations, including children requiring a surgical procedure.

DIFFERENCES IN COVID-19 BETWEEN CHILDREN AND ADULTS

Although some patients are asymptomatic[6], the presentation of COVID-19 typically includes fever, cough, myalgia and fatigue, while some patients may also experience dyspnea, productive cough, gastrointestinal symptoms, and thrombotic complications[7-10]. The disease is mild in most cases (> 80%), but severe disease develops in a considerable number of patients (approximately 14%), while nearly one-third of patients with severe disease are in a critical condition. The overall case-fatality rate was initially estimated to be 2.3% by the Chinese Center for Disease Control and Prevention, although a precise determination of the case fatality rate still cannot be made[6,11-13]; However, this rate is estimated to be approximately 50% in patients diagnosed with severe COVID-19[6]. Increasing age and underlying comorbidities are thought to be associated with inferior outcomes, including severe disease, admission to the intensive care unit (ICU), and death[7,14]. Transmission of the virus mainly occurs *via* contact of mucosal surfaces with infectious respiratory droplets, similar to other respiratory viruses[15,16]. Wölfel *et al*[17] reported that although throat and lung-derived samples showed signs of infectious SARS-CoV-2, fecal samples yielded only viral RNA, but not an infectious virus. Also, the virus was not detected in blood and urine[17]. However, the possibility for fecal-oral, parenteral, or aerosol transmission

cannot be excluded[16,18,19]. Recent data have shown that the transmission potential of the virus might be much higher than previously estimated[20].

Based on the first few pediatric case series, it was speculated early on that COVID-19 affects children differently compared to adults[21,22]. These assumptions were later confirmed by a large epidemiological study on Chinese pediatric patients; the proportion of cases in a critical condition was much lower (< 6%) compared to the general population, and only one death was documented among 2143 children[23]. In another report from China, 15.8% of pediatric patients with COVID-19 had an asymptomatic infection (27 out of 171), compared to 1% in the general population, as reported by the Chinese Center for Disease Control[6,24]. In addition, less than half of the children experienced fever at any time during the course of their illness, while 12 of 171 had imaging findings consistent with pneumonia but were asymptomatic[24]. The high number of asymptomatic cases and the frequent absence of classic symptoms indicate that COVID-19 has a predilection for atypical presentation in children. In the same study, only three children – all of whom had underlying comorbidities – were admitted to the ICU, confirming the previous reports of decreased COVID-19 severity and improved outcomes in the pediatric population[24]. The differences between pediatric and adult patients might be explained by immaturity of the immune system and differences in the expression of the viral cell receptor in children[23,25]. Despite the lack of widespread testing for COVID-19, early reports showed a disproportionately low prevalence of the disease in children compared to adults[6,7]; this could potentially be attributed to the lower overall exposure of children to infected individuals rather than to potential resistance to the virus[26]. In addition, younger children show less severe symptoms than adolescents[27]. However, this low prevalence and lack of typical clinical manifestations raise concerns about the potential role of the pediatric population in the widespread transmission of the virus[28].

A number of upper respiratory infections are prevalent among children, with symptoms resembling COVID-19. It is essential to suspect other viral or bacterial infections as well and perform tests, in order to identify possible alternative explanations for their symptoms, or even cases of coinfection[29,30]. Differential diagnosis might include viral disease from influenza virus, parainfluenza virus, adenovirus, respiratory syncytial virus, rhinovirus, other SARS viral infections, but also bacterial infections, which include *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and other bacterial-related pneumonias. In addition, the co-infection of SARS-CoV-2 and other respiratory pathogens should not be excluded[30] Therefore, a diagnostic challenge is apparent in pediatric patients. The present guidelines suggest that all children should be screened for possible symptoms of COVID-19 infection upon arrival at the hospital[31].

IMPACT AND RESPONSE OF THE SURGICAL COMMUNITY

Due to their invasive nature, surgeries may facilitate widespread disease transmission between patients and healthcare workers. Super-spreading events of SARS-CoV-2 have also been described in surgical departments[32]. Apart from the high risk of transmission, it has been hypothesized that operation-associated stress may predispose COVID-19 patients to worse clinical outcomes through immune dysregulation[33]. The surgical community has promptly responded to this imminent danger by taking decisive measures. More specifically, the American College of Surgeons (ACS) has issued guidelines suggesting that all elective procedures should be postponed or performed in an ambulatory surgery center if feasible. The only exceptions are most of the oncological and high acuity surgical procedures[34]. Non-operative management should also be considered when appropriate. Decision-making during triage of elective surgeries should ideally be guided by a multidisciplinary team of experts[34,35]. When operations are deemed to be non-elective, healthcare workers should use appropriate personal protective equipment (PPE), and the number of medical professionals involved in patient care should be minimized. Additional protection should be used if the operation involves a patient suspected of having COVID-19 or if it generates an aerosol (*e.g.*, intubation)[36]. However, the liberal use of specialized PPE, such as N95 masks, may quickly lead to a shortage of supplies[37]. The Center for Disease Control has introduced a step-wise optimization strategy for the use of facemasks, aiming to counterbalance any potential shortage of supplies[38]. Various individual surgical centers have implemented additional administrative measures in an effort to further decrease transmission; these include segregating healthcare workers into working groups (*e.g.*, weekly rotating teams of attending and

resident physicians), increasing surgical turnaround times, implementing technology for communication purposes, and designing isolated operating rooms (ORs) for COVID-19 patients[29,39-41].

SPECIFIC CONSIDERATIONS FOR PEDIATRIC SURGERY

Classification of cases by surgical acuity

The pediatric surgery community has been affected to a similar degree. Apart from the previous general recommendations that apply to all surgical subspecialties, the ACS has also issued guidelines pertaining specifically to pediatric surgery[42]. Common diagnoses have been classified into three main categories according to the urgency of their surgical management. Cases are categorized as emergency, urgent, or elective according to the risk of delaying their surgical management, which is life-threatening, detrimental, or negligible, respectively. Examples from every category are presented in Table 1. As for all surgeries, the ACS emphasizes that decision-making for pediatric cases should not solely depend on this classification. Rather, it should be the result of careful clinical judgment, ideally guided by a multidisciplinary panel of experts. In general, surgeries should not be postponed if their delay may cause harm to patients, prolong their hospitalization, or predispose them to re-admission. Emergency cases and many urgent cases, according to the previous classification, are examples where postponement of surgical management would have adverse outcomes for the vast majority of patients. Aside from assessing the risks and benefits for the patients, clinicians should also consider the risk of disease transmission to themselves and the impact on hospital resources[42]. Healthcare workers, including pediatric surgeons, are considered a high-risk population for COVID-19[43,44]. An assessment on a per case basis seems to be necessary when taking into account the complex interplay between all these parameters and the unique characteristics of COVID-19 in the pediatric population.

In many cases, surgeons are called to alter their management of acute surgical cases, according to the available resources. One such case is acute uncomplicated appendicitis. A recent meta-analysis showed that appendectomy not only has a higher success rate but also a reduced length of hospital stay compared to conservative treatment with antibiotics; in fact, about 10% of the children undergoing a non-operative treatment will eventually require appendectomy before discharge[45]. Therefore, during the COVID-19 pandemic, earlier discharge achieved with surgery minimizes the risk of virus transmission in healthy children, and increases the availability of beds and healthcare personnel. In some cases, same-day discharge could even be considered postoperatively[46]. However, the non-surgical approach presents some important advantages as well, as it reduces the chance of infection in physicians and patients in the OR and during aerosol-generating procedures, such as intubation[47]. PPE and ORs are not utilized, and therefore are available for other emergent procedures[48]. In such debatable cases, each hospital should provide its own protocol based on the availability of PPE, wards, Pediatric Intensive Care Units (PICU), ORs, and specialized personnel.

Availability of personnel, facilities, and equipment

Although guidelines have been published aiming to guide planning and operational protocols in children during the COVID-19 pandemic[49,50], each hospital should establish and follow an internal contingency plan, based on variables such as the COVID-19 status of the local population at each given moment, the available infrastructure and the proximity of other pediatric hospitals that serve the same population.

Infrastructure and logistical preparedness play a pivotal role in order for a tertiary hospital to become a surgical referral center for children with suspected and confirmed COVID-19. Negative-pressure ORs[51,52] and isolated recovery rooms, PICU beds, and wards are required[49]. Furthermore, designated transportation routes are warranted from the ambulance bay or dedicated entrance to the OR, and from there to the isolated recovery room, PICU bed or ward. This planned itinerary should be isolated from high-traffic areas of the hospital and should not traverse the general, non-COVID-19 ORs[52,53].

Another important issue that challenges all healthcare operations during the pandemic is the availability of healthcare personnel. Surgical teams present at the hospital should be smaller, with only a percentage of pediatric surgeons, anesthesiologists, and specialized nurses covering the emergency shift, with a second

Table 1 Classification of common diagnoses and procedures in pediatric surgery according to their urgency by the American College of Surgeons

Emergency		Urgent	Elective	
Ischemia	Testicular/ovarian torsion	Any abscess requiring incision and drainage	Reconstruction	Anorectal anomaly after diversion
	Limb-threatening ischemia	Biliary atresia		Hirschsprung disease after diversion
Trauma	Trauma with uncontrolled bleeding	Symptomatic cholelithiasis		IBD after diversion
	Penetrating trauma	Most oncologic surgeries		Chest wall
Acute intestinal obstruction	Hypertrophic pyloric stenosis	Acute IBD exacerbation requiring resection	Enterostomy closure	
	Intussusception non-responsive to radiographic reduction	Any diagnosis requiring gastrostomy for hospital discharge	Removal of an uninfected vascular access device	
	Intestinal malrotation	Insertion of a vascular access device	Orchiopexy	
	Incarcerated inguinal hernia	Symptomatic inguinal hernia	Asymptomatic inguinal hernia	
Congenital malformations	Anorectal anomalies or Hirschsprung disease requiring intestinal diversion		Splenectomy for hematologic disease	
	Intestinal atresia		Excision of a breast lesion	
	Esophageal atresia with tracheoesophageal fistula		Fundoplication	
	Congenital diaphragmatic hernia (symptomatic)		Bariatric surgery	
Upper airway/GI foreign body ingestion			Cholecystectomy for biliary colic	
Acute appendicitis			Asymptomatic choledochal cyst repair	
Intestinal perforation			Branchial cleft cyst/sinus excision	
Any diagnosis requiring ECMO			Thyroglossal duct cyst excision	

IBD: Inflammatory bowel disease; GI: Gastrointestinal; ECMO: Extracorporeal membrane oxygenation.

backup team on stand-by at home[54]. In areas with high COVID-19 prevalence, two separate surgical teams can be formed, one for children with COVID-19 and a second one for healthy children requiring surgery. In that instance, each team should be isolated as much as possible from the other to minimize cross-transmission and maximize operational continuity[53]. Reasonable shift implementation is also required to ensure the safety of healthcare workers and prevent burnout[55]. All healthcare workers should be monitored daily prior to their shift, and are encouraged to monitor themselves throughout the day for the presence of fever or other COVID-19-related symptoms, such as cough or fatigue[49,53]. An organized "sick leave" policy should also be established in case a health care worker presents with symptoms suggestive of COVID-19[31].

Because children commonly present with mild or no symptoms – as mentioned above[53] – SARS-CoV-2 transmission from children to healthcare workers has been reported[21]. Therefore, PPE is suggested in all cases of healthcare workers interacting with children. In cases of a potential or confirmed COVID-19 patient needing surgery, the responsible surgical team should use disposable N95/FFP2 respirators, double gloves, goggles or visors, surgical caps, shoes, and full-body gowns[48]. Additional Powered Air-Purifying Respirators are required for the anesthesia team to minimize aerosol exposure during intubation and extubation[48,53]. Healthcare workers are explicitly required to use N95 respirators according to the United States[38], Chinese[56], and Spanish guidelines[57].

To ensure effectiveness in prevention, training of healthcare workers is of the utmost importance and mask fittings and PPE training should be arranged on a regular basis for all personnel. In addition, simulations of surgical scenarios while wearing all PPE equipment ("dress rehearsals") should be applied to familiarize the teams with appropriate PPE protocols and troubleshooting in advance[47,52]. An issue that presented in a simulation scenario was the noise reduction by Powered Air-Purifying Respirators worn by the anesthesia team, which impaired effective oral

communication. This was circumvented by the use of a whiteboard for communicating essential information during the operation[53]. It was shown that physicians were more likely to become infected during the donning and doffing procedures than when actually taking care of infected patients[58]; Thus, the designation of a colleague as a "provider" who can help and oversee the donning and doffing procedures not only reduces the likelihood of contamination of healthcare workers[50,51] but also reduces the anxiety around a possible infection[48].

Workflow organization for suspected and confirmed cases

Standardized protocols[53] have been published for adult operations and should be adapted to pediatric surgery as well. If possible, all pediatric patients with respiratory symptoms or those undergoing high-risk procedures should be assessed for their COVID-19 status prior to the operation[50]. Each patient can be tested for the SARS-CoV-2 RNA *via* rapid reverse transcription-polymerase chain reaction (RT-PCR) of an oropharyngeal swab or sputum sample, with a turnover time of 2 to 4 h[47]. However, early intervention is crucial in urgent or emergency cases for the survival of the child. If the RT-PCR results are not accessible soon enough, the surgery team should proceed as if the child has COVID-19[52]. In such cases, an early assessment for COVID-19 can be made based on contact history, clinical symptoms, or findings on chest imaging[47].

The equipment and surgical personnel inside the OR should be kept to a minimum to reduce potential exposure to and transmission of SARS-CoV-2[53]. The use of electrocautery and high-speed tools, such as ultrasonic scalpels, should be limited as much as possible because there are reports of aerosol viral spread[31,59] from viral particles present in the smoke produced by electrical cauterization[21,60,61]. On the other hand, the extensive use of a suction machine is advised to limit smoke and aerosol production[49,61]. These practices have already been reported in case series, where laparotomy was preferred over laparoscopy for the management of acute abdomen to minimize operating time and decrease the risk of contamination through airborne aerosol transmission, by relieving the pneumoperitoneum[52]. Despite concerns, minimally invasive procedures are considered safe, as the risk of transmission from intraoperative aerosolization is minimal, and the pneumoperitoneum could be evacuated through a protective filtration system[62,63]. Nevertheless, the safest approach in terms of aerosol spread is the one that the surgeon is most comfortable with, and can operate for a shorter period and in the safest possible way[48,49]. At the end of the operation, any unused drugs and consumables should be considered as "potentially contaminated" and thus should be discarded[49]. If a negative-pressure OR is not available, COVID-19 suspected or positive cases should be planned as the last cases of the day if possible, whereas in emergent situations, an adequate period of time (approximately 30 min) should be set for air exchange after the procedure[48].

After the operation, children with confirmed COVID-19 should be transferred to an isolated ICU or ward designated for COVID-19 cases, where a dedicated COVID-19 team takes care of infected patients[31,52]. The designated team should not travel to other in-hospital places to minimize the possible spread of the disease and should perform close follow-up of the pediatric patients, considering that children positive for SARS-CoV-2 have a higher postoperative mortality rate[52]. If transportation of a patient is required, it is advisable that the patient is accompanied by security personnel to ensure that the route is clear of other patients, visitors, or personnel[53].

In patients suspected of having COVID-19, the patient should not be transported to the designated COVID-19 ward or ICU immediately during the postoperative period but should remain in an isolated recovery room, while awaiting the final RT-PCR results for COVID-19. After a positive or negative result, the pediatric patient will then be transported to COVID-19 or non-COVID-19 units accordingly.

The hospital should limit visitors to only one person at a time, essential for the pediatric patient's physical or emotional well-being and care, such as one parent, guardian, or primary caregiver[31,64]. In some tertiary hospitals, a "parent pass" is provided to one parent at a time, to accompany their inpatient child[64]. All visitors should actively be assessed prior to their entrance to the hospital for fever and other COVID-19 signs and symptoms[31,53], and should be advised to wear a protective face mask while in the hospital[31]. It should be noted that in order for the visitor to be allowed into the hospital, they must not show any COVID-19 symptoms[64].

After departure from the hospital, postoperative follow-up should preferably be in the form of a video-call – provided that the postoperative course is normal – to minimize unnecessary exposure, or the follow-up can be rescheduled to a future date. Such options have been previously suggested in the follow-up of surgical and pediatric patients[29,65,66]. A specialized team could visit patients at home for suture removal and wound treatment. Postoperative wound management can also be

performed by parents in some cases, following an adequate "home skills program"[67]. In surgical cases, the use of absorbable sutures could even be considered to avoid the pediatric patient returning to the hospital for their removal. Nevertheless, the final decision on the follow-up lies with the pediatric surgeon and is decided on a case-by-case basis. In case any atypical symptoms or complications occur, the child's family should contact the surgeon without hesitation.

CONCLUSION

In conclusion, COVID-19 has had a major impact on pediatric surgery. The diagnostic challenges of COVID-19 in children have generated great concern for widespread transmission. The surgical community has responded by establishing guidelines to ensure the continued operation of pediatric surgery units and minimize transmission in this setting. All cases should be assessed individually and managed according to pre-established protocols. Management can be assisted by classifying cases as elective, urgent, or an emergency based on the risks associated with delaying surgical treatment. Surgical teams should be organized in a way that maximizes safety, and hospital infrastructure should be appropriately modified to accommodate the needs of COVID-19 patients. These measures can mitigate the effects of this pandemic by minimizing transmission and adverse outcomes, while also safeguarding the appropriate management of pediatric surgical cases.

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ORIGINAL ARTICLE

Basic Study

Does carrier fluid reduce low flow drug infusion error from syringe size?

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Author contributions: Madson ZC and Lin JA conceived and designed and personally conducted all the experiments, made observations, analyzed results, and wrote the initial draft and revisions of the manuscript; Sund GT helped to conceive and design and interpret the experiments; Vangala S provided statistical analysis, analyzed results, and helped draft and revise portions of the manuscript. All authors reviewed and approved of the manuscript.

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Abstract

BACKGROUND

Critically ill neonates and pediatric patients commonly require multiple low flow infusions. Volume limitations are imposed by small body habitus and co-morbidities like cardiopulmonary disease, renal failure, or fluid overload. Vascular access is limited by diminutive veins. Maintenance fluids or parenteral nutrition in conjunction with actively titrated infusions such as insulin, fentanyl, prostaglandins, inotropes and vasopressors may necessitate simultaneous infusions using a single lumen to maintain vascular catheter patency. This requirement for multiple titratable infusions requires concentrated medications at low flows, rather than more dilute drugs at higher flows that in combination may volume overload small infants.

AIM

To determine whether carrier fluid reduces variability that variability of low flow drug infusions is proportional to syringe size in pediatric critical care.

METHODS

We assessed concentrations of orange "drug" in a 0.2 mL/h low flow clinical model with blue dyed carrier fluid at 5 mL/h, using 3-, 10-, or 60-mL syringes. A graduated volumetric pipette was used to measure total flow. Mean time to target concentration was 30, 21, and 46 min in 3-, 10-, and 60-mL syringes, respectively (

additional data are available.

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$P = 0.42$). After achieving target concentration, more dilute drug was delivered by 60-mL ($P < 0.001$) and 10-mL syringes ($P = 0.04$) compared to 3-mL syringes. Drug overdoses were observed during the initial 45 min of infusion in 10-and 60-mL syringes. Total volumes infused after target concentration were less in the 60-mL condition compared to 3-mL ($P < 0.01$) and 10-mL ($P < 0.001$) syringes.

RESULTS
Linear mixed effects models demonstrated lesser delivered drug concentrations in the initial 30 min by 3-mL compared to 10-and 60-mL syringes ($P = 0.005$ and $P < 0.001$, respectively) but greater drug concentrations and total infused drug in the subsequent 30-60 and 60-90 min intervals with the 3- and 10-mL compared to 60-mL syringes.

CONCLUSION
With carrier fluid, larger syringes were associated with significantly less drug delivery, less total volume delivered, and other flow problems in our low flow drug model. Carrier fluid should not be used to compensate for inappropriately large syringes in critical low flow drug infusions.

Key Words: Infusion pumps; Intensive care; Neonatal; Nursing research; Patient safety; Spectrophotometry; Syringes

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Core Tip: Infusions of critical drugs in infants frequently require low flow rates. We previously observed errors in low flow infusions that were directly proportional to syringe size. Because low flow infusions in clinical practice are essentially always co-infused with a primary carrier fluid, we now use a similar model to test whether carrier fluid improves accuracy and flow continuity of low flow drug from large compared to smaller syringes. We report that despite carrier fluid, larger syringes were associated with less overall drug and fluid volumes delivered, worse flow continuity, and other flow problems in low flow infusions compared to smaller syringe sizes. Carrier fluid should not be used to compensate for errors introduced by syringe size in critical low flow drug infusions. Syringe size should be matched to the rate of infusion.

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INTRODUCTION

Critically ill neonates and pediatric patients commonly require multiple low flow infusions. Volume limitations are imposed by small body habitus and co-morbidities like cardiopulmonary disease, renal failure, or fluid overload. Vascular access is limited by diminutive veins. Maintenance fluids or parenteral nutrition in conjunction with actively titrated infusions such as insulin, fentanyl, prostaglandins, inotropes and vasopressors may necessitate simultaneous infusions using a single lumen to maintain vascular catheter patency. This requirement for multiple titratable infusions requires concentrated medications at low flows, rather than more dilute drugs at higher flows that in combination may volume overload small infants.

Drug flow rates may commonly reach as low as 0.1-0.2 mL/h (100-200 microliters/h) in infants and small pediatric patients[1-3]. At low rates, flow variability is proportional to syringe size[1,3-5]. Larger syringes exhibit increased friction and variable compliance of the syringe plunger tip, hindering the necessary precision to displace their plungers in short increments[1,3-5]. However, competing safety considerations encourage pharmacy equipment standardization to the largest common syringe size within a hospital. Unit dosing, prefilled syringes, pre-programmed drug libraries, and pharmacy standardization[6-12] seek to minimize equipment options and, thus, avoid errors in calculations, drug preparation and dispensing, pump

programming, and drug administration.

We previously demonstrated that syringe size is directly proportional to variability of low flow infusions[1]. However, as low flow drug infusions are generally found in clinical practice only with a primary infusion fluid, it is necessary to investigate the possible benefits or harms introduced by primary fluid combined with low flow infusions. To our knowledge, the influence of carrier fluid on low flow variability associated with syringe size has not been previously investigated. One might hypothesize that carrier fluid improves syringe-associated low flow drug variability by flushing drug from tubing dead space during start-up or drug interruptions[13] and diluting concentrated drug in dead space[14]. If verified, then use of carrier fluid would allow streamlining of options using larger common syringe sizes and simplified infusion pump libraries within institutions. In contrast, we hypothesized that carrier fluid might exacerbate low flow errors *via* perturbations attributable to the carrier fluid delivery system. We report here the results of our study of an *in vitro* low flow drug and carrier fluid model.

MATERIALS AND METHODS

As this study did not require patient participation or patient data, the study was granted exemption from review by the Institutional Review Board of the University of California, Los Angeles.

Equipment

All medical devices and equipment used in this study were standard equipment in our pediatrics care units. All infusions were performed with a Medfusion 4000 smart pump (Baxter; SIGMA, Medina, NY, United States). Disposable sterile BD syringes (Becton-Dickinson, Franklin Lakes, NJ, United States) sized 3-, 10-, and 60-mL were used. Blue (BL) food coloring (McCormick Culinary, Santa Rosa, CA, United States) in 0.9% normal saline was used as carrier fluid and diluent for orange (OR) (Chefmaster Liqua-Gel, Fullerton, CA, United States) low flow drug. For real time spectrophotometry, absorbances of colored fluids were measured directly through clear intravenous tubing (Intensive Care Unit Medical Extension Set 60 Inch Tubing 0.4 mL Priming Volume B2020) using a Public Lab Desktop Spectrometry Kit 3.0 (Publiclab.org).

Infusion simulation:

Carrier fluid was infused from a smart infusion pump (Baxter Sigma Spectrum Infusion Pump 35700BAX) *via* a valveless burette (Baxter Buretrol Clearlink System 2H8865) connected to an infusion tubing set with 2 Luer activated valves and a backcheck valve above the upper Y-site (Baxter Clearlink Continu-Flo UC8519). Per Food and Drug Administration recommendations[3], the lowest Y-site closest to the "patient" (in this case, the spectrometer) was used to connect a smart syringe pump (Medfusion Syringe Infusion Pump Model 4000) for simultaneous infusion using extension tubing. The length of IV tubing from the drug infusion pump to the spectrometer (Public Lab Desktop Spectrometry Kit 3.0) was set at 18.5 cm to allow complete mixing of drug and carrier fluids. Spectrometry was measured inline through the tubing. To simulate patient-side intravenous catheter resistance and perform volume measurements, the end of the tubing after the spectrometer was connected *via* 5 cm of extension tubing (cut from a Smiths Medical MX451FL extension set) to drain into the narrow end of a 1 mL glass pipette with 0.02 mL volumetric gradations.

Flow rate for the carrier fluid pump was set at 5 mL/h. This rate is commonly used in the neonatal clinical setting[5] and was used in our previous study[1]. Flow rate for the low flow drug was set at 0.2 mL/h for the same reasons. This rate was programmed into the smart pump as the drug model for all infusions: Epinephrine with standard neonatal drug concentration of 40 mcg/mL (our institutional practice) at a dose of 0.027 mcg/kg/min in a 5 kg infant, which yields a flow rate of 0.2 mL/h. For purposes of our experiments, we used OR food dye for "drug" diluted in BL dyed carrier fluid as our drug model. Changes in 433 nm BL λ peak transmittance were used to determine drug concentrations. Concentration curves were established in calibration studies by assessing 433 nm transmittance for 10 replicates at each concentration, averaging the results, and fitting to a power-law function. For each trial of 3-, 10-, and 60-mL syringe sizes (Becton-Dickinson Luer Lok), 5 spectrometry measurements per time point were recorded and the replicate determinations

averaged. Spectrometer readings were recorded in 5 min intervals until either target concentration (determined by concentration curve studies of OR drug in BL carrier fluid) was reached and maintained or more than 90 min had elapsed. Total volume infused was measured using a 1-mL volumetric pipette with 0.02 mL gradations, which was connected directly to the end of the IV tubing for all experiments. A schematic of the experimental apparatus is diagrammed in Figure 1.

The same normal saline calibration was used for all calibrations and spectral analyses. Multiple factors that could affect spectral analysis, such as light source, ambient lighting, distance from the spectrometer, and alignment, were kept constant. Heights of the infusion pumps, spectrometer, infusion tubing, and volumetric collection pipette were kept constant.

Statistical analysis

Single factor ANOVA and Student's *t*-test were used to compare continuous data where appropriate. In our initial analyses, start-up effects were censored by analyzing data after target concentration was achieved. Subsequently, we included start-up data in our linear mixed effects analysis of different time intervals during the infusions. To account for effects of different syringe sizes and time, we used linear mixed effects models of log-transformed dilution and amount of drug delivered to estimate percentage differences between syringe sizes at three 30 min time intervals, with the lesser dilution value as denominator. For infusion trials that reached and maintained target concentration before 90 min had elapsed, a series of the last recorded dilution values recorded at target concentration were repeated forward in order to make statistical comparisons with the infusions that required the full 90 min to reach target concentration. Models were also used to estimate within-infusion variances and compare these between syringe sizes. To account for excessive drug concentrations caused by flow variability of our syringe pump and carrier infusion pumps resulting in oversaturation of the spectrometer, we replaced oversaturated values with the highest detectable concentration of OR drug, which was 20-fold dilution based on calibration experiments. P values less than 0.05 were considered statistically significant.

RESULTS

Concentration curve calibrations

Absorbance at 433 nm was recorded at 12 different OR drug dilutions of 40-, 50-, 80-, 100-, 160-, 200-, 250-, 320-, 400-, 500-, 640-, and 800-fold. The curve was fitted to the power-law function, $\text{Absorbance}_{433\text{nm}} = 87.732\ (\text{fold-dilution})^{-1.059}$ and is demonstrated in Figure 2 ($R^2 = 0.9378$). Based on this concentration curve and with a goal to maximize the range of measured absorbance *vs* fold-dilution in our experimental set-up, we set the target OR drug dilution at 100-fold in steady state flow. This translated to a flow of OR drug at 0.2 mL/h diluted by carrier flow at 5 mL/h.

Flow characteristics after target concentration achieved

OR drug concentrations during 0.2 mL/h low flow infusions with different syringe sizes and a 5 mL/h carrier fluid are demonstrated in Figure 3A-C. One infusion failed to reach 1:100 target concentration by 90-min in the 60-mL syringe size (Figure 3C). No differences were observed in time to reach target drug concentration (Figure 3D). Times to target concentration were 30 ± 7, 21 ± 19, and 46 ± 55 min (mean ± SD, $P = 0.42$) for 3-, 10-, and 60-mL syringes, respectively. Only 3-mL syringes maintained target concentration after start-up (Figure 3A). Both the 10- and 60-mL syringe sizes (Figure 3B-C) were associated with under- and over-dosing after rapid achievement of 1:100 target concentration. In the 10-mL syringes, average OR drug dilutions after reaching target concentration were 73 ± 25, 137 ± 209, and 176 ± 146 for 3-, 10-, and 60-mL syringes, respectively ($P < 0.001$ for 3- *vs* 60-mL and $P = 0.04$ for 3- *vs* 10-mL syringes, Figure 3E).

Observed infusion inaccuracies

By design, a leading edge of visible color change in the infusion tubing was observed as OR drug traversed the tubing. OR drug mixed with BL carrier fluid into a green color (Figure 4A). This drug-containing green column ceased advancing shortly after achieving target concentration despite ongoing pump operation in three out of seven 10-mL syringes, three out of four 60-mL syringes, and none of the 3-mL syringes

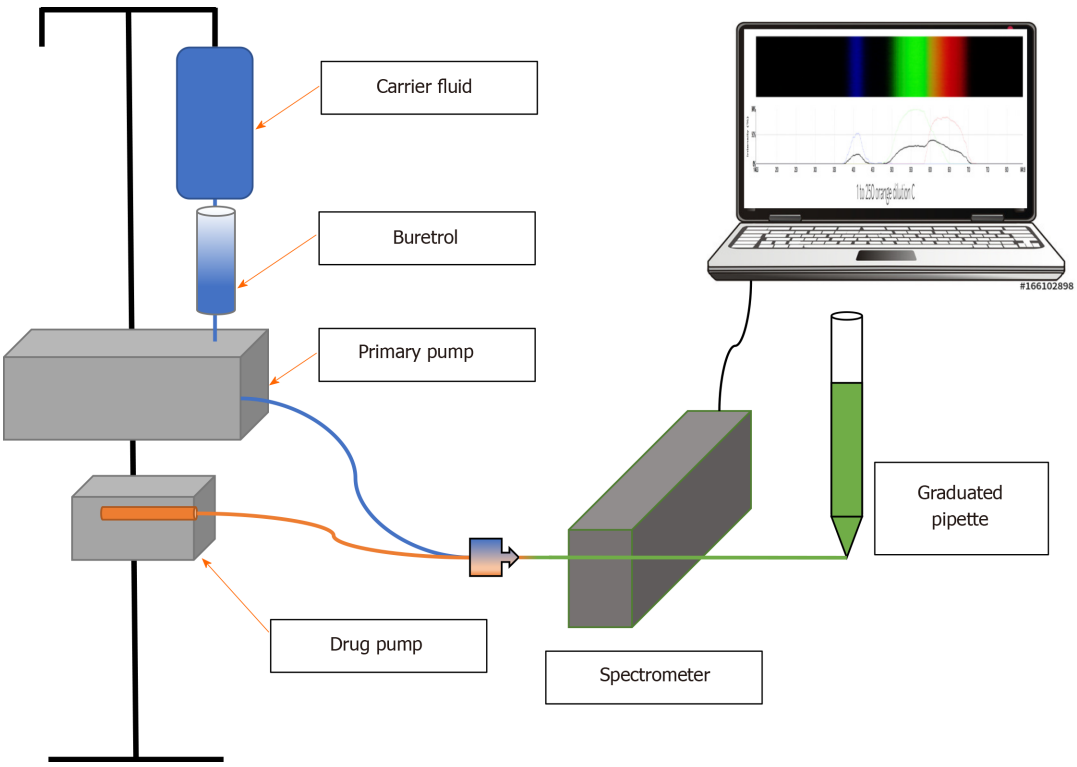


Figure 1 Experimental set-up.

(Figure 4B). This interruption of steady state drug flow ranged from 15 to 40 min for trials of affected 10-mL syringes and at least 90 min for 60-mL syringe trials (Figure 4B and C). Despite careful and repeated priming of the drug infusion and carrier fluid lines using standard nursing practices, air bubbles were frequently observed entering the infusion system from the buretrol of the carrier infusion set (Figure 4B-D). Backward flow of OR drug into the proximal BL carrier fluid tubing was seen mainly in 60-mL syringes and to lesser degrees in smaller 3- and 10-mL syringes. In contrast to the green forward column of mixed OR and BL dyes, the fluid columns of backward flows maintained an OR color and persisted for unpredictable periods of time (Figure 4D).

Based on carrier flow of 5 mL/h and drug flow of 0.2 mL/h, expected volume of fluid per 5 min interval was 0.43 mL. To avoid errors introduced from start-up effects, we measured volumes after achieving 1:100 target concentration. Total infused volumes per 5 min period were 0.44 ± 0.02 mL, 0.44 ± 0.01 mL, and 0.43 ± 0.02 mL for 3, 10, and 60-mL syringes, respectively ($P < 0.01$ for 60- *vs* 10-mL and 60- *vs* 3-mL syringes).

Mixed model analysis

Based on the experimental run curves in Figure 3A-C, we observed that most infusions reached 1:100 target concentration within 30 min and all the 10-mL syringes maintained 1:100 concentration by 60 min. Therefore, we analyzed the infusions in 30 min intervals up to 90 min in linear mixed effects models to compare overall drug delivery and variance, including the start-up periods. For total infused volumes, no mean differences were observed over each 30 min period.

Significant differences by syringe size were observed in OR drug concentration over time. In the initial 0-30 min period, 3-mL syringes delivered more dilute OR drug by 51% *vs* 10-mL ($P = 0.005$) and 83% *vs* 60-mL syringes ($P < 0.001$). Drug over-flows occurred in the 10- and 60-mL conditions, as noted above (Figure 3B-C). In subsequent time periods, no differences in concentration were observed between 3- and 10-mL syringes, but 60-mL syringes delivered more dilute OR drug in carrier fluid by more than 50% to a maximum of 106% greater dilution than 3- and 10-mL syringes in 30-60 and 60-90 min periods ($P < 0.01$ for each comparison). No differences in overall dilution variances were observed between syringe sizes.

Total drug delivered was calculated by multiplying volume times concentration (the inverse of dilution). Significant differences were observed only in comparisons of 60- *vs* 3- and 10-mL syringes. 60-mL syringes delivered less drug by 98% ($P = 0.031$) and

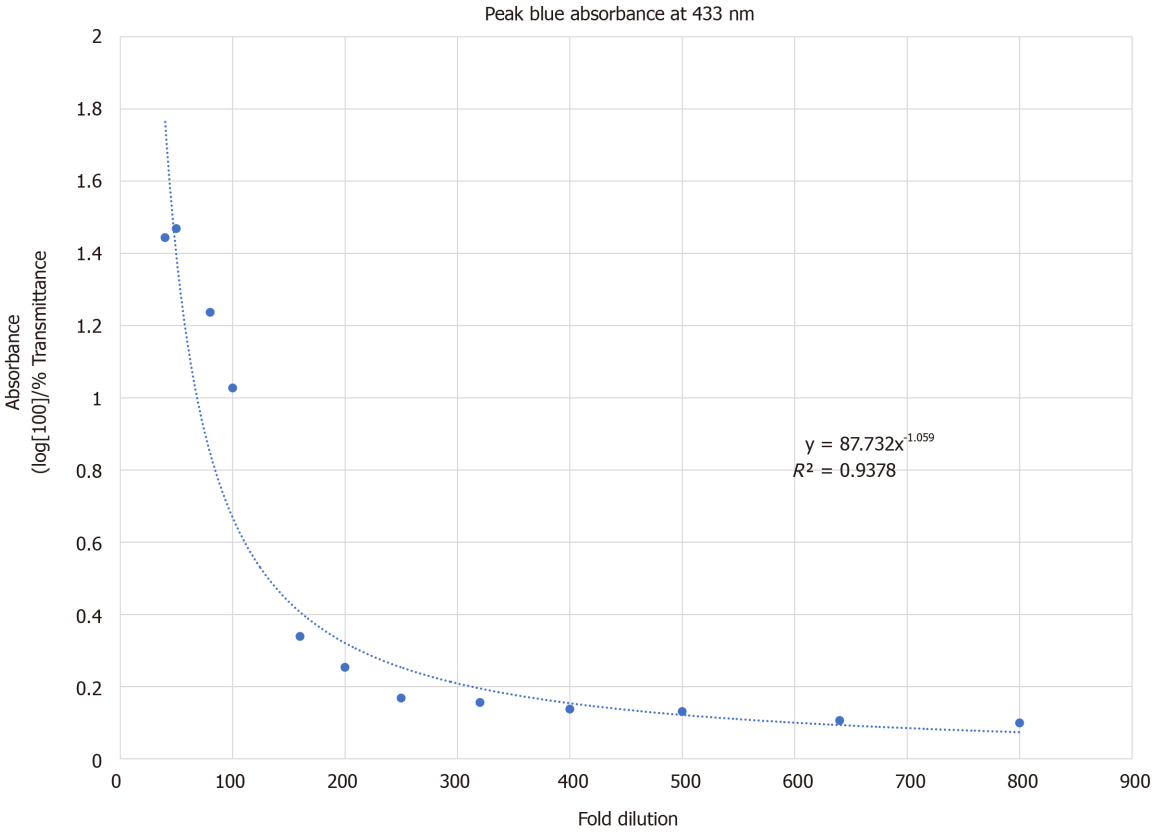


Figure 2 433 nm absorbance *vs* fold dilution of orange drug in blue carrier fluid.

115% (P = 0.039) *vs* 3-mL syringes in the 30-60 min and 60-90 min time periods, respectively, and 111% (P = 0.012) greater dilution *vs* 10-mL syringes in the 60-90 min time period.

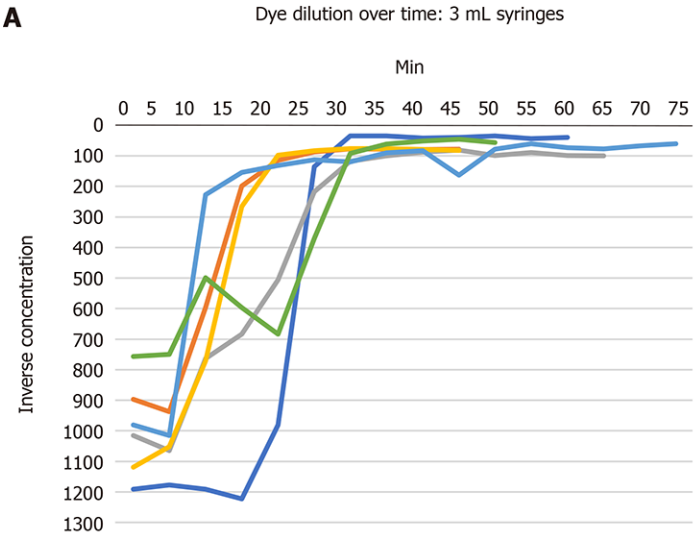
DISCUSSION

Carrier fluid, or primary infusion fluid, is a common pediatric intervention. Stable, infrequently titrated solutions for carrier flow include maintenance fluids or parenteral nutrition. These fluids maintain vascular access device patency[15], reduce drug delivery onset or offset times[13], and facilitate administration of multiple titratable drug infusions[16]. To our knowledge, our report is the first to demonstrate an interaction between carrier fluid and low flow infusion inaccuracies related to syringe size.

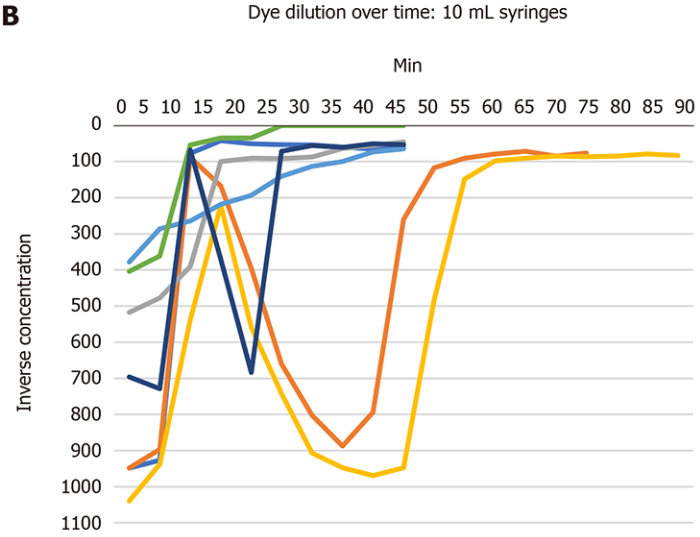
Ours and others' previous work demonstrated significant variability of low flow infusions related to syringe size. Methods used in syringe-only studies include linear fluid displacement in our previous study [1] and gravimetry in others[17]. These methods are largely incompatible with carrier flow studies, in which spectrophotometry is most frequently used[15,18]. Hence, direct comparisons of low flow infusions from syringe pumps alone *vs* with added carrier fluid are not readily accomplished. However, by using our previously established experimental low flow syringe model[1] to investigate carrier fluid interactions, we revealed unanticipated issues. We previously found deviations from steady state of two-fold in 10-mL and six-fold in 60-mL syringes at 0.2 mL/h[1]. With the same drug infusion rate but added carrier flow, we observed similar six to nine-fold deviations in 10-mL syringe flow and up to five-fold deviations with no clear steady state pattern in 60-mL syringe flow up to 90 min after start-up.

Carrier flow comprised 96% of total flow in our model. We observed < 5% variability in total flow per 5 min period, consistent with stable carrier flow. A small but statistically significant difference of lesser total flow was observed in the 60- compared to 3- or 10-mL syringe conditions, which may be accounted for by syringe flow. We observed multiple infusion anomalies occurring in interactions between larger syringes and carrier flow. Problems included introduction of air bubbles, backward drug flow at the carrier fluid connector, and lack of mixing.

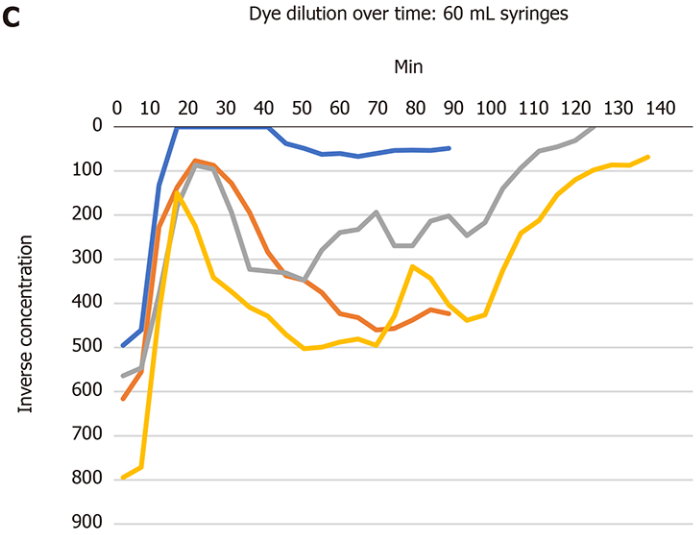
A


Dye dilution over time: 3 mL syringes
Min
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75
0 100 200 300 400 500 600 700 800 900 1000 1100 1200 1300
Inverse concentration

B


Dye dilution over time: 10 mL syringes
Min
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90
0 100 200 300 400 500 600 700 800 900 1000 1100
Inverse concentration

C


Dye dilution over time: 60 mL syringes
Min
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140
0 100 200 300 400 500 600 700 800 900
Inverse concentration

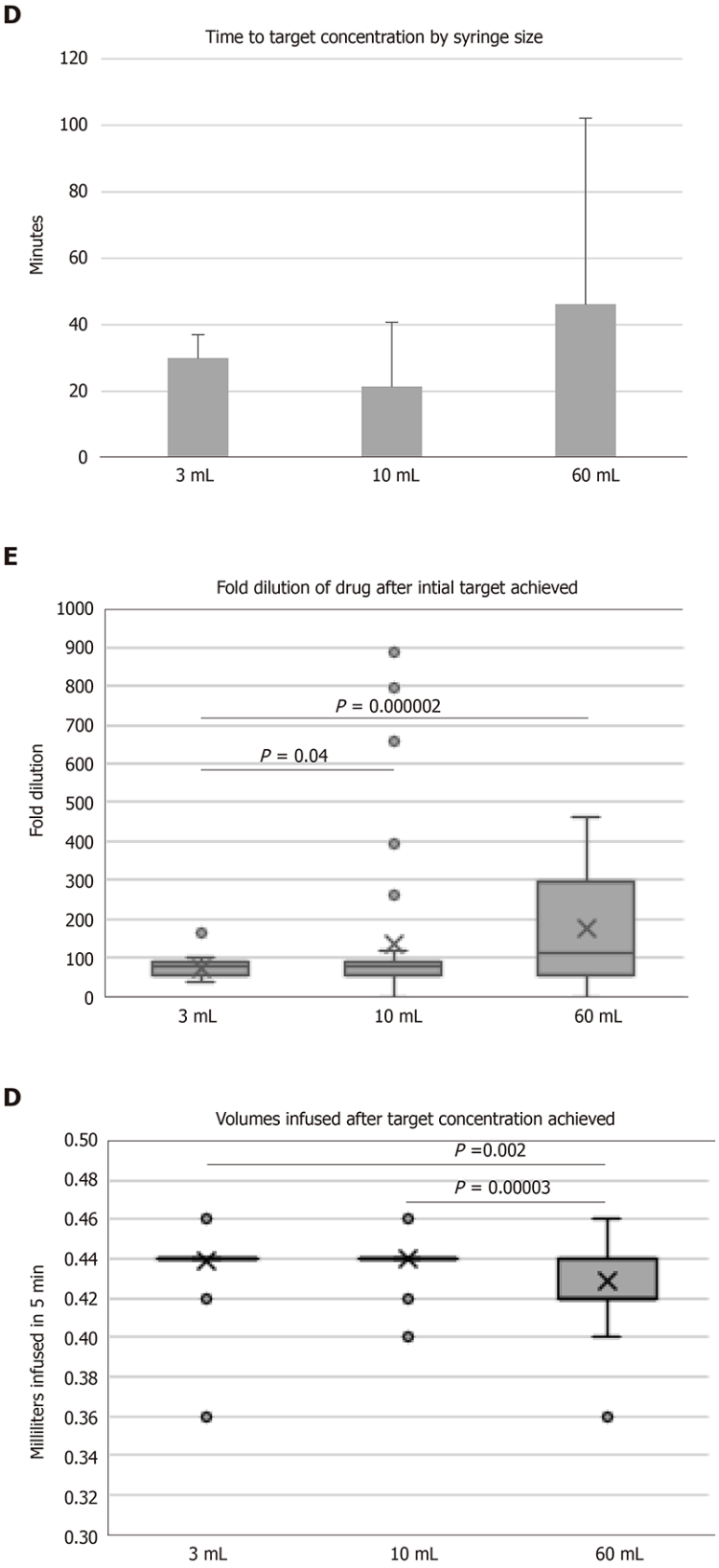


Figure 3 Low flow drug delivery at 0.2 mL/h with carrier fluid at 5 mL/h. A-C: Orange drug dilution over time; D: Time to target orange drug concentration of 100-fold dilution or less (no significant differences, $P = 0.42$). Average minutes shown with standard deviation error bars; E and F: Box and whiskers plots with outliers of (E) measured dye dilutions after target concentration achieved (ANOVA $P = 0.0067$) and (F) volumes infused per 5 min interval after target concentration achieved (ANOVA $P = 0.00006$).

The buretrol was a frequent source of air bubbles, which in microfluidic systems contribute flow instability, increased compliance, and increased resistance[19]. While air

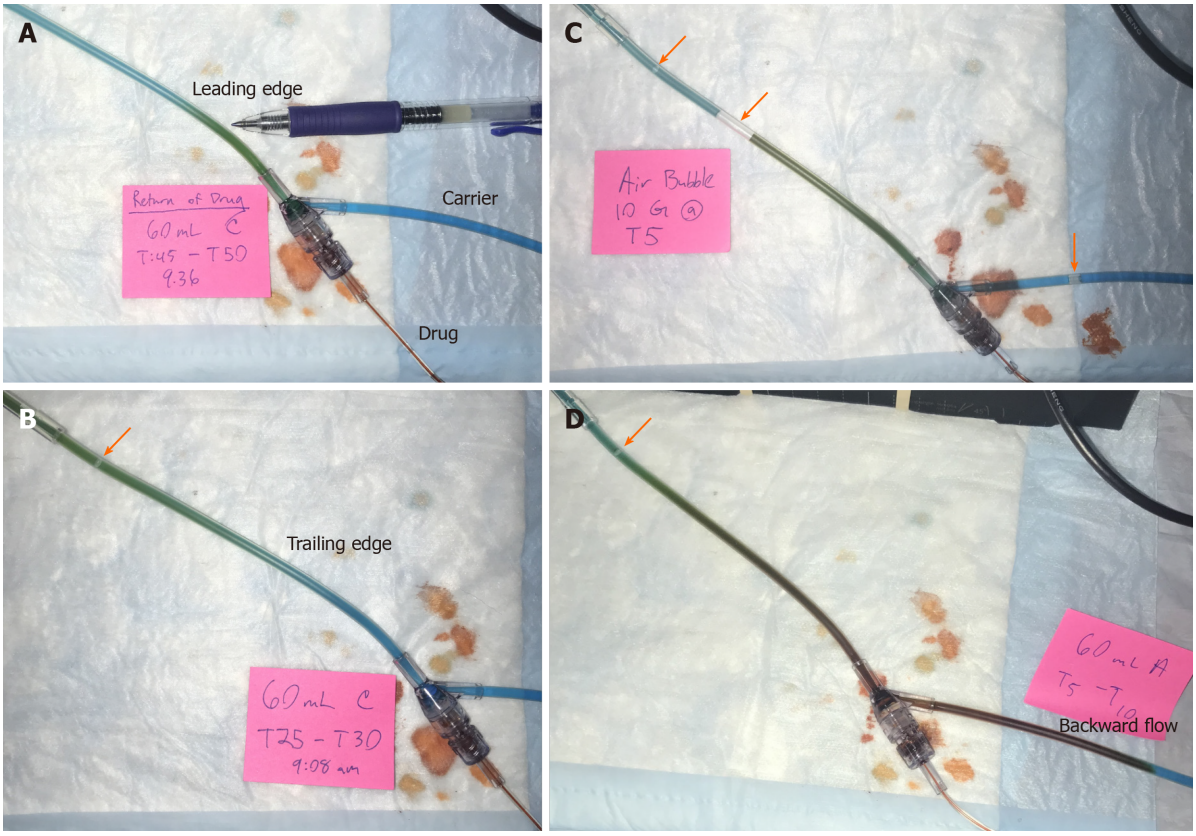


Figure 4 A leading edge of visible color change in the infusion tubing was observed as orange drug traversed the tubing. A: Green leading edge of admixed orange drug and BL carrier fluid seen after drug under-infusion during a 60 mL syringe trial; B: Trailing edge of green admixed drug and carrier solution during an under-infusion event with a 60 mL syringe, with BL carrier fluid proximally; C: Air bubbles introduced from the carrier line into the infusion set (arrows), which are also seen in (B) and (D); and D: Backward flow of orange drug into the proximal BL carrier fluid line in a 60-mL syringe trial. Note the orange color in the backward fluid column, suggesting lack of drug-carrier fluid mixing, as would be indicated by a transition to green color.

introduction in carrier tubing is independent of drug syringe size, bubble effects may exacerbate syringe size-related anomalies. Flow variability resulting from stiction and compliance of a larger plunger[20] may add to flow inconsistencies caused by air bubbles in the infusion tubing. Because pressure drop across gas bubbles is inversely proportional to channel radius[19], smaller radius microbore tubing as recommended for low flow infusions[3] may exacerbate bubble effects.

Backwards flow at the carrier fluid connector occurred inconsistently at the start of infusions with larger syringes despite ongoing carrier flow and persisted for many minutes, leading to below target drug delivery. Rapid initiation of syringe flow is a feature of modern syringe pumps[21] and is used to overcome problems of mechanical slack[22] or "breakfree force" of the plunger[20], both of which are proportional to syringe size. While this rapid startup would generally exert minimal clinical effect if infused directly into the patient, addition of a connector to carrier fluid allows for backward flow and, thus, unpredictable drug delivery. We observed persistence of OR color (instead of green from the mixture of BL carrier and OR drug) and delayed clearance of drug from the proximal carrier tubing, both of which suggest lack of mixing. This offers indirect evidence of fluid layering and laminar flow, which contrasts with previously described Plug Flow and Well Mixed models of fluid flow[14,22]. In laminar flow conditions, the fluid edge may flow at a slower rate than the center and maintain distinct fluid compositions[13]. Hence, drug entering from the edge of tubing may travel slower than faster carrier fluid in the center. To our knowledge, this observation is previously undescribed in the clinical literature.

Limitations of our methods include an emphasis on readily available and low-cost experimental equipment to encourage reproducibility testing in other institutions. We found a nonlinear relationship between food dye concentration and absorbance, which may be due to additives. For future studies, we would use pure dyes that conform to the Beer-Lambert Law. Due to changes in our hospital equipment, the smart syringe pump in our current study was different from our previous publication[1]. We observed different syringe infusion characteristics, notably a more rapid start-up in the larger syringe sizes. This improved uniformity of time to target concentration, but in

larger syringe sizes was associated with drug overdose, backward flow at the carrier fluid connector, and subsequent reductions in drug delivery. As backward flow was unanticipated and noted after trials of 3-mL syringes were completed, our study did not include spectral analysis proximal to the connector. We had no method to quantify air bubbles.

Despite the above limitations, our findings are qualitatively similar to previous publications on syringe size effects[1,3,4,17,23,24] while adding previously unreported problems of carrier fluid interactions with low flow infusions by syringe size. Importantly, our study provides no evidence to suggest that carrier fluid might reduce variability associated with low flows from larger syringes. This has important clinical implications for neonatal and small pediatric patients requiring critical short acting, high potency drug infusions such as epinephrine in settings where pharmacy standardization using prefilled or standardized syringes[10,12] may tend toward larger volume syringe sizes. Rather, we continue to recommend that syringe size be matched appropriately to the rate of infusion. In our health system, we now match syringe size to critical low flow pediatric infusions by using the smallest syringe capable of providing 12 h of infusion, or one nursing shift. Future studies will be needed to determine optimal carrier fluid to syringe flow ratios, the effects of tubing dead space on accuracy of low flow drug delivery with or without carrier fluid, and architecture of tubing connectors to reduce gas bubble introduction, improve mixing and reduce drug backflow.

CONCLUSION

Our study provides no evidence to suggest that carrier fluid might reduce variability associated with low flows from larger syringes. This has important clinical implications for neonatal and small pediatric patients requiring critical short acting, high potency drug infusions such as epinephrine in settings where pharmacy standardization using prefilled or standardized syringes[10,12] may tend toward larger volume syringe sizes.

ARTICLE HIGHLIGHTS

Research background

Critically ill neonates and pediatric patients frequently require drug delivery *via* low flow infusions below 0.5 mL/h. The use of carrier fluid has become common in clinical practice to facilitate delivery of these low flow drug infusions.

Research motivation

Flow continuity problems of low flow infusions are known to be related to syringe size. However, competing safety considerations encourage pharmacy standardization to the largest common syringe size. As such, in clinical practice, carrier fluids are commonly used to reduce variability of drug delivery from larger syringe sizes.

Research objectives

To evaluate whether carrier fluid improves continuity in low flow drug delivery.

Research methods

We simulated pediatric low flow infusions using dyed fluids in a drug infusion model. In-line spectrometry was used to measure drug concentrations. Administered fluid was determined volumetrically.

Research results

Low flow continuity errors were associated with larger syringe sizes and exacerbated by interactions with carrier fluid. Drug over- and underdosing, backward flow at the tubing connector, and frequent air bubbles from carrier fluid were observed.

Research conclusions

Our study provides no evidence to suggest that carrier fluid might reduce variability associated with low flows from larger syringes.

Research perspectives

Our study provides empiric data to suggest that continuity errors of low flow infusions are associated with larger syringes and not improved by carrier fluid. Syringe size should be matched to the rate of infusion. In our health system, we now match syringe size to critical low flow pediatric infusions by using the smallest syringe capable of providing 12 h of infusion.

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ORIGINAL ARTICLE

Retrospective Cohort Study

Gastroesophageal reflux disease in pediatric esophageal atresia: Assessment of clinical symptoms and pH-impedance data

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Author contributions: Aksionchyk M performed the pH-impedance testing and contributed to collection, analysis and interpretation of the patient's clinical data, final diagnosis, and conception, drafting and revision of the manuscript for important intellectual content; Marakhouski K performed the upper gastrointestinal endoscopy, collection and analysis of the literature data, and statistical analyses, and contributed to conception, drafting and revision of the manuscript for important intellectual content; Svirsky A contributed to the acquisition and analysis of data, design of the work, and drafting and revision of the paper for important intellectual content; All authors gave final approval of the version to be published.

Institutional review board statement: The study was reviewed and approved by The National Centre of Pediatric Surgery at Minsk, Belarus, No. 24.08.2017.

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Abstract

BACKGROUND

Esophageal atresia (EA) is the most common congenital anomaly of the gastrointestinal tract. Gastroesophageal reflux disease (GERD) is a frequent and lifelong problem in these patients. GERD can be asymptomatic and the incidence of esophageal gastric and intestinal metaplasia (Barrett's esophagus) is increased in adults with EA compared with the general population. Timely and accurate diagnosis of GERD is important to reduce long-term problems and this may be achieved by pH-impedance testing.

AIM

To assess symptoms and pH-impedance data in children after EA, in order to identify their specific features of GERD.

METHODS

This study was conducted from November 2017 to February 2020 and involved 37 children who had undergone EA *via* open surgical repair (51.35% boys, 48.65% girls; age range: 1-14 years, median: 4.99 years). GERD diagnosis was made based on multichannel intraluminal impedance/pH study and two groups were established: EA without GERD, n = 17; EA with GERD, n = 20. A control group was established with 66 children with proven GERD (68.18% boys, 31.82% girls; median age: 7.21 years), composed of a nonerosive reflux disease (referred to as NERD) group (n = 41) and a reflux esophagitis group (n = 25). Upper gastrointestinal endoscopy with a mucosal esophageal biopsy was performed on all patients.

Informed consent statement: All study participants and their legal guardian provided informed written consent prior to study participation, No. 37.

Conflict-of-interest statement: The authors declare no conflicts of interest.

Data sharing statement: No additional data are available.

STROBE statement: The authors have read the STROBE Statement–checklist of items, and the manuscript was prepared and revised according to the STROBE Statement–checklist of items.

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RESULTS

The most frequently observed symptom in EA patients with GERD and without GERD was cough (70% and 76.5% respectively). The number of patients with positive symptom association probability in the EA groups was significantly larger in the EA without GERD group (P = 0.03). In the control reflux esophagitis group, prevalence of gastrointestinal symptoms was significantly higher than in the NERD group (P = 0.017). For both EA groups, there was strong correlation with index of proximal events (IPE) and total proximal events (EA with GERD: 0.96, P < 0.001; EA without GERD: 0.97, P < 0.001) but level of IPE was significantly lower than in GERD patients without any surgical treatment (P < 0.001). Data on distal mean nocturnal baseline impedance were significantly different between the EA with GERD group (P < 0.001) and the two control groups but not between EA without GERD and the two control groups.

CONCLUSION

Mean nocturnal baseline impedance may have diagnostic value for GERD in EA children after open surgical repair. IPE might be an additional parameter of pH-impedance monitoring.

Key Words: Esophageal atresia; Gastroesophageal reflux disease; pH-impedance testing; Mean nocturnal baseline impedance; Proximal reflux; Reflux esophagitis; Nonerosive reflux disease; Pediatric

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Core Tip: Esophageal atresia (EA) is the most common congenital anomaly of the gastrointestinal tract. Gastroesophageal reflux disease (GERD) is a frequent and lifelong problem after EA repair. pH-impedance testing makes assessment of pH and other parameters of GERD possible, aiding disease diagnosis and management. Even asymptomatic patients should undergo monitoring of GERD to confirm the absence or the persistence of reflux, and the need to continue treatment. We analyzed data of children with EA open surgical repair to determine the features of GERD among them and propose some important issues for consideration in the follow-up program for these patients.

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INTRODUCTION

Esophageal atresia (EA), with or without trachea-esophageal fistula, is the most common congenital anomaly of the gastrointestinal tract and, given the increasingly successful surgical outcomes, it currently represents a lifelong issue[1-3]. Other than respiratory problems, nutritional and gastrointestinal issues are prevalent, not only in the first years of life but also in adolescence and adulthood. Gastroesophageal reflux disease (GERD), peptic esophagitis, esophageal gastric and intestinal metaplasia (known as Barrett's esophagus), anastomotic strictures, feeding disorders, dysphagia, and esophageal dysmotility are the most frequent gastrointestinal short-term and long-term complications encountered in children and adolescents with EA[4]. The incidence of esophagitis and Barrett's esophagus is increased in adults with EA compared with the general population[4].

The current gold-standard tests for the diagnosis of GERD are pH probe testing and pH-impedance testing, both of which measure the esophageal reflux burden[5]. Multichannel intraluminal impedance (MII) is an additional procedure for measuring the movement of fluids, solids and air in the esophagus. When combined with MII, pH recording is able to detect liquid reflux, independent of its pH, and gas episodes[6]. Twenty-four-hour measurement of esophageal MII combined with pH-metry (known collectively as MII/pH) makes possible the assessment of pH and other parameters of

gastroesophageal reflux together with disease symptoms and the diagnosis of GERD[6-7].

There remains a critical need for an effective way to diagnose and monitor reflux. pH-metry is able to quantify acid burden, ensure that acid suppression is adequate during long-term follow-up, and correlate acid reflux to symptoms. pH with impedance is additionally able to detect non-acid reflux as well as volume clearance, both of which correlate with patient symptoms. It is also able to correlate extra-gastrointestinal symptoms to reflux, which may help guide treatment. pH-impedance is also useful in quantifying the proportion of reflux reaching up to the proximal esophagus, referred to as "high reflux." EA patients frequently experience extraesophageal symptoms, and pH-MII has the unique ability to determine if these symptoms correlate with reflux episodes, regardless of whether they involve acid or non-acid[8].

Many children with EA with chronic GER have no troublesome symptoms. Results from pH-impedance (pH-MII) studies as well as endoscopic evaluations in children with EA show that asymptomatic children can have severe abnormalities[7,9]. These data indicate that patients with EA should be evaluated regularly by a multidisciplinary team (pulmonology, gastroenterology and otolaryngology), even in the absence of GERD-related symptoms. Therefore, the European Society for Pediatric Gastroenterology, Hepatology, and Nutrition (known as ESPGHAN)-North American Society for Pediatric Gastroenterology, Hepatology, and Nutrition (known as NASPGHAN) Guideline (2016) recommends that all EA patients (including asymptomatic patients) should undergo monitoring of GERD (impedance/pH-metry and/or endoscopy) at time of discontinuation of anti-acid treatment and during long-term follow-up[4].

One of the limitations of pH-impedance testing in patients with esophagitis or esophageal motility disorders (both of which are commonly found in patients with EA) is that baseline impedances are 75% lower than in control patients[7]. This is important to realize, and manual revision of pH-MII tracings should be considered for all EA patients, especially in cases of unexplained symptoms or persistent growth impairment[9].

We designed this study to assess the clinical symptoms and pH-impedance data in children who underwent EA open surgical repair, with comparison to a control group of children with proven GERD[10], to find specific features of GERD in the group of EA patients and to provide data that will aid in the development of an effective and efficient national follow-up program for the EA patients.

MATERIALS AND METHODS

Study population and data collection

The study comprised 43 children with EA, ranging in age from 1 to 14 years (average: 5.09 years), treated within the first days of life *via* open surgical repair. All children were operated on in the Department of Pediatric Surgery of The National Centre of Pediatric Surgery. This study was approved by the ethics committee of the National Centre of Pediatric Surgery and registered in The National Centre of Pediatric Surgery Trial Registry. Written informed consent was obtained from the parent(s) or guardian of each patient on the day of the procedure. The National Centre of Pediatric Surgery is located in Minsk (Republic of Belarus) and serves a pediatric population (up to 18 years of age) of approximately 1865000, including treatment and follow-up of EA cases. The average number of children born with EA in Belarus is 15-17 per year. The total number of children with EA in Belarus over the last 5 years is 102.

Patient selection

All surgical repairs were carried out by thoracotomy in the early postnatal period (days 1-2), using primary direct anastomosis of the esophagus "end to end". There were no cases of gastric/colonic pull-ups in the group of studied EA patients. All patients were treated with proton pump inhibitors (PPIs) for at least 6 mo after the open surgical EA repair.

This study was a retrospective chart review involving 43 children with EA who attended The National Centre of Pediatric Surgery. All EA open surgical repair patients (ages 1-18 years), who were bothered with troublesome symptoms and contacted our clinic, underwent combined impedance-pH testing and upper gastrointestinal endoscopy (with histological study of biopsied mucosa samples), and were considered eligible for study enrollment. For all, acid suppression therapy had

been discontinued for at least 7 d before the impedance-pH testing. Between the enrollment dates of November 2017 and February 2020, the 43 children considered eligible included 23 boys (53.5%) and 20 girls (46.5%).

Exclusion criteria

Patients were excluded according to MII/pH monitoring carried out < 18 h (n = 2), eosinophilic esophagitis diagnosis (n = 2), esophageal replacement therapy (gastric pull-up, jejunal/colonic interposition; n = 0), and receipt of fundoplication (n = 2). Thus, a total of 37 patients with EA were enrolled in the study, including 19 boys (51.4%) and 18 girls (48.6%).

Diagnosis of GERD was established based on the result of the MII/pH study and according the recommendation of guidelines on pediatric gastroesophageal reflux clinical practice[10-12]. Depending on the result of the pH-impedance testing, the EA patients were divided into groups of those with GERD (n = 20, 54.1%) and those without GERD (n = 17, 45.9%). The clinical and demographic features of both EA groups are listed in Table 1.

Control group

At the same time, we retrospectively evaluated 66 patients with proven GERD (acid exposure time > 7%, total number of retrograde bolus movement > 70), sex- and age-matched to the EA group, who were enrolled in the study to serve as a control group. These patients were selected from among children with GERD-related symptoms, who had undergone pH-impedance testing for suspected GERD (with indications to confirm the diagnosis of GERD[10-12]) and who had undergone upper gastrointestinal endoscopy. Patients were excluded based on history of any abdominal surgery.

Based on the results of the 24-h MII/pH monitoring and endoscopies, control patients with proven GERD were divided into groups of those with reflux esophagitis (RE) (n = 25, 37.9%) and those with nonerosive reflux disease (NERD) (n = 41, 62.1%). The clinical and demographic features of both control groups are listed in Table 2.

Clinical assessment

We used the Gross classification system to define the type of EA, whereby long-gap EA was defined as any distance (> 2 vertebral bodies) between the esophageal (pouch) ends in a newborn too wide for a primary anastomosis[13-14].

A detailed clinical history and parental reported symptoms in all patients were analyzed. Patient data on GERD-related symptoms were collected *via* a study-specific questionnaire that queried the frequency, strength/intensity, relationship with mealtimes and body position related to GERD symptoms, the previous treatment(s) (*i.e.*, PPIs, alginates, antacids, histamine 2 receptor antagonists, prokinetics), the history of atopy, the birth history, and any accompanying illnesses. Also evaluated were predominant symptom(s) at presentation, timing of symptom(s) onset after EA repair, and type of EA. Parents were instructed to fill out the questionnaire and then, throughout the study period, to maintain a diary of written descriptions of any GERD-related symptoms, body position (prone and supine), and mealtimes (beginning and end). Patients and their parents were instructed to avoid extremely hot or ice-cold drinks and food, "acid" foods, and carbonated beverages.

pH-impedance monitoring

The study was performed in all patients while off PPI therapy, using a Digitrapper MII ambulatory system (Medtronic, Dublin, Ireland) and disposable MII/pH catheters adjusted for age and height. The study was performed according to standardized protocol and, therefore, correct catheter position was confirmed by X-ray or under visual endoscopic inspection[15]. A single patient-length appropriate catheter with at least 6 impedance and 1 pH channel was used to perform the MII/pH monitoring. Depending on the age of the patient, the pH channel was placed 2 cm to 5 cm above the lower esophageal sphincter. The MII-pH catheters used were of 2.13 mm (6.4 Fr) diameter. All refluxes were then registered *via* the Digitrapper pH/ZÔ.

The following pH-impedance parameters were analyzed in the study: acid exposure time (AET), as percentage; longest acid exposure, in min; total number of retrograde bolus movements (RBM); number of proximal events; symptom association probability (SAP); and, distal mean nocturnal baseline impedance (MNBI). The impedance data of all patients with EA and GERD were subject to automatic analysis by the Medtronic software but also reviewed manually.

Table 1 Demographic and clinical characteristics of esophageal atresia patients, *n* (%)

Characteristic	EA with GERD, *n* = 20	EA without GERD, *n* = 17	*P* value
Age in yr, as median	4.93 (95%CI: 2.78 to 7.08; SD: 4.59); $P < 0.001$	5.06 (95%CI: 3.49 to 6.62; SD: 3.05)	0.444
Male/female, *n*	12/8	7/10	0.26
Gross type of EA	Type C-20 (100)	Type C- 17 (100)	
Dysphagia	6 (30)	2 (11.8)	0.186
Vomiting	2 (10)	2 (11.8)	0.862
Heartburn	1 (5)	1 (5.9)	0.905
Cough	14 (70)	13 (76.5)	0.662
Recurrent pneumonia	2 (10)	-	0.186
Recurrent bronchitis	2 (10)	2 (11.8)	0.862
Asymptomatic	2 (10)	1 (5.9)	0.653
History of atopy	5 (25)	2 (11.8)	0.314
Esophagitis	9 (45)	7 (41.2)	0.819
Previously treated with PPIs	8 (40)	9 (52.9)	0.606

Data are presented as *n* (%), unless otherwise noted. CI: Confidence interval; EA: Esophageal atresia; GERD: Gastroesophageal reflux disease; PPIs: Proton pump inhibitors; SD: Standard deviation.

Table 2 Demographic and clinical characteristics of control patients, *n* (%)

Characteristic	RE, *n* = 25	NERD, *n* = 41	*P* value
Age in yr, as median	8.68 (95%CI: 6.5796 to 10.7804)	5.74 (95%CI: 4.4583 to 7.0295)	0.0113
Male/female, *n*	19/6	26/15	0.276
Gastrointestinal symptoms	10 (40)	6 (14.6)	0.017
Respiratory symptoms	7 (28)	16 (39)	0.366
Combined symptoms	8 (32)	16 (39)	0.569
Asymptomatic	-	3 (7.4)	0.167

Data are presented as *n* (%), unless otherwise noted. CI: Confidence interval; NERD: Nonerosive reflux disease; RE: Reflux esophagitis.

Distal mean nocturnal baseline impedance

MNBI is considered an accurate method for characterizing esophageal baseline impedance[16-17]. Its measurement consists of determining the baseline impedance at 3 cm or 5 cm above the lower esophageal sphincter during overnight rest, which represents the mean of values obtained during three 10-min time intervals in a period of no swallowing[16]. Even in EA patients without esophagitis, baseline impedances are known to be 44% lower than in control patients with esophagitis[18]. Low baseline impedances impair bolus detection, resulting in an underestimation of the reflux burden in EA patients. This is a major limitation of MII/pH in EA patients[7,9].

We determined distal MNBI in all patients at the same distance of the esophagus depending on age (1 year to 10 years: 3 cm above the lower esophageal sphincter; older than 10 years: 5 cm above the lower esophageal sphincter) and automatically calculated when neither reflux episodes nor swallowing were present, using a specific software function[16,17].

Proximal events

All of the reflux events were evaluated manually for their proximal extent. Retrograde bolus movements that reached at least channel 2 (the second most proximal channel) in the upper esophagus were considered high refluxes[7].

Index of proximal events

The index of proximal events (IPE) was calculated as the ratio of the number of proximal refluxes to the total number of refluxes per day.

Upper gastrointestinal endoscopy

The endoscopy procedure was performed using the Evis Exera III imaging platform (Olympus Corp., Tokyo, Japan) under pharyngeal anesthesia or deep sedation. Mucosal biopsies were taken from the esophagus (a minimum of at least four samples from various parts of the esophagus), the stomach, and the duodenum. RE was diagnosed based on the Los Angeles classification system[19].

Statistical analyses

Statistical processing of the results was carried out using MedCalc Statistical Software, version 19.2 (MedCalc Software Ltd., Ostend, Belgium; https://www.medcalc.org; 2020). Descriptive statistics included the arithmetic mean and median [both with 95% confidence interval (CI)], standard deviation, and standard error of the mean. Analysis of consistency of signs' distribution type to the normal distribution law was carried out using the Shapiro-Wilk test; the sign distribution was considered a departure from normality at $P < 0.05$. Depending on the consistency/inconsistency of the distribution of the analyzed signs to the normal distribution law, the parametric Student's *t*-test and the nonparametric Mann-Whitney *U*-test were used to evaluate differences between the groups.

For regression analysis, the type of regression equation was chosen according to the highest F-ratio and lowest *P* value, with maximum of $P < 0.01$. Measures of central tendency and data dispersion were subjected to one-way analysis of variance (ANOVA) to test the difference between the means of several subgroups of a variable; when the test was positive, post hoc testing (*i.e.* Student-Newman-Keuls) was performed. The Kruskal-Wallis test was used to analyze the effect of a classification factor on ordinal data; if the test resulted in a $P \leq 0.05$, post hoc testing (*i.e.*, Dunn's test) was performed.

The diagnostic performance of data or the accuracy of a test to discriminate diseased cases from normal cases was evaluated using receiver operating characteristic (ROC) curve analysis. The ROC method used was based on Delong *et al*[20] with binominal extracted CI for the area under the curve (AUC).

RESULTS

Esophageal atresia group

A little over one-half (20/37, 54.1%) of the EA patients were diagnosed with GERD. The EA patients with GERD and those without GERD showed similar clinical characteristics, history of atopy, and upper endoscopy data.

Endoscopic analysis of the upper part of the gastrointestinal tract demonstrated 16 EA patients had esophagitis grade A (according to Los Angeles classification) (43.2%), 1 patient had gastric metaplasia (2.7%), and 6 patients had chronic gastritis (16.2%). The upper endoscopy data revealed no significant differences between the EA patients with GERD and those without GERD ($P = 0.819$) (Table 1).

There were 8 children in the EA with GERD group (40%) and 9 children in the EA without GERD group (52.9%) that had been previously treated with PPIs (1-3 mo prior). After therapy, clinical improvement was observed in only 47.05% of patients in both EA groups who had received therapy. The PPI therapy was discontinued in all patients for at least 7 d before the impedance-pH testing.

Only 3 out of 37 patients with EA did not experience any symptoms during pH-impedance monitoring. Before pH-impedance testing their parents reported extraesophageal symptoms (cough and recurrent bronchitis) spontaneously. Thirty-four patients reported symptoms during pH-impedance testing. Positive symptom association was defined in children who had a symptom association probability (SAP) over 95%. SAP was positive in 3/20 (15%) in the EA with GERD group and in 8/17 (47.06%) in the EA without GERD group. The most frequently reported symptom for the EA patients was cough, for both groups.

Controls (patients with proven GERD)

The NERD and RE patients in the control group were subcategorized by their symptoms (Table 2), namely gastrointestinal (heartburn, vomiting and abdominal

pain), respiratory (cough and recurrent bronchitis), and combined (gastrointestinal and respiratory symptoms in the same patient). Only the gastrointestinal symptoms showed a significant difference between the two groups, with RE patients having significantly higher prevalence of these symptoms than the NERD patients ($P = 0.017$).

Only 3 out of the 66 controls did not experience any symptoms during pH-impedance monitoring. Their previously reported symptoms, from before pH-impedance testing, were respiratory (cough and recurrent bronchitis).

GERD features in groups

The EA without GERD group had significantly more patients with positive SAP (> 95%) compared to the EA with GERD group [3/20 (15%) *vs* 8/17 (47.06%), $P = 0.03$] (Table 3).

A comparison of the pH-impedance parameters showed significant differences ($P < 0.001$) in AET, number of RBM, and duration of the longest reflux event between the EA with GERD group and EA without GERD group. However, Mann-Whitney test (independent samples) indicated no differences in either the number of proximal events ($P = 0.151$) nor in the IPE ($P = 0.939$) (Table 3).

Comparison of the MNBI data was carried out using the *t*-test since the distribution in the groups was normal (Figure 1).

ROC curve analysis used GERD as a classification variable (presence: 1; absence: 0) and MNBI as a variable, and the subsequent results were: AUC = 0.806, $P < 0.001$ with criterion – 1.69 kOhm, sensitivity 80.0% and specify 76.5% (Figure 2). It should be noted, pairwise comparison of ROC curves for AET (%) and MNBI in the EA group on GERD diagnosis did not show a reliable difference (AUC AET (%) = 0.89). The difference between two areas (calculated as AET~MNBI = 0.0838; 95%CI: -0.101 to 0.269; $P = 0.3743$) revealed a similar diagnostic value for AET (%) and MNBI, in relation to GERD.

Comparisons between groups (EA with GERD, NERD, and RE) were performed in order to identify specific GERD features. The pH-impedance parameters of the comparison groups are presented in Table 4. The NERD and RE groups were found to share some specific features; in particular, both groups showed a relationship between MNBI and AET, with the NERD group having Spearman's coefficient of rank correlation of -0.46 [$P = 0.002$; AET(%) = 206364 + -306169 Log(MNBI), $P < 0.001$] and the RE group having Spearman's coefficient of rank correlation of -0.68 [$P < 0.001$; AET(%) = 164401 + -243143 Log(MNBI), $P = 0.002$].

The Kruskal-Wallis test showed absence of difference in AET (%) ($P = 0.776$) and total number of RBM ($P = 0.697$) between the groups of EA with GERD, NERD, and RE. However, a significant difference was found in MNBI and the IPE (Figure 3). For the analysis of MNBI, we decided to increase the degree of freedom up to 3-times in the Kruskal-Wallis test, due to the introduction of the EA without GERD group. We based this approach on the fact that the data of MNBI from this group also has great scientific and practical interest when comparing to a group of patients with non-operated esophagus (Figure 4). The ANOVA gave an F-ratio of 6.69 ($P < 0.005$), and Scheffe test for all pairwise comparison (mean) confirmed the difference between NERD and EA with GERD groups ($P < 0.05$).

DISCUSSION

Limitations

First of all, the main limitation of our study is its high dispersion by age. We included children from 1-year-old to 14-years-old. Second, our institute has no follow-up program for EA open surgery repair patients. We currently examine patients with any GERD-related symptoms, and for this study only 11.7% of the eligible patients with EA open surgical repair were enrolled and included in the analysis. One more limitation of our study is the inability to rule out laryngopharyngeal reflux, because we use probes with one pH-sensor located in the distal part of the probe. This group of patients commonly complain of throat issues, such as chronic cough, throat clearing, or sore throat. Some of our patients had similar complaints. The most common tests in patients suspected of reflux-related laryngeal symptoms or laryngopharyngeal reflux are endoscopy and pH monitoring but these tests have poor sensitivity. The most popular examination of this pathology is proximal or hypo-pharyngeal pH monitoring, but these two probes have sensitivities of only 40%-50% at best, limiting their utility. Thus, there is a need for a better test with increased sensitivity for patients suspected of having laryngopharyngeal reflux[21].

Table 3 pH-impedance data of esophageal atresia patients

pH-impedance parameter	EA without GERD	EA with GERD	*P* value
AET (%)	2.59, 95%CI: 1.68 to 3.5	11.62, 95%CI: 7.54 to 15.7, $P = 0.0066$	< 0.001
Number of RBM	40.3, 95%CI: 34.3 to 46.3	67.3, 95%CI: 55.27 to 79.32	< 0.005
Longest acid exposure in min	9.37, 95%CI: 5.26 to 13.5, $P = 0.0104$	46.8, 95%CI: 28.39 to 65.26, $P = 0.0061$	< 0.001
Proximal events	6.59, 95%CI: 3.1 to 10.1, $P = 0.0249$	10.95, 95%CI: 6.24 to 15.56, $P = 0.0089$	0.151
Index of proximal events[1]	0.17, 95%CI: 0.09 to 0.27, $P = 0.0052$	0.16, 95%CI: 0.1 to 0.22	0.939
Distal MNBI in kOhm	1.99, 95%CI: 1.72 to 2.26	1.44, 95%CI: 1.21 to 1.67	< 0.005
SAP (> 95%/< 95%)	8/17	3/20	0.03

[1]In 4 cases in the group of EA without GERD, proximal events were not registered. Data are presented as mean with 95%CI by Shapiro-Wilk test. EA: Esophageal atresia; GERD: Gastroesophageal reflux disease; CI: Confidence interval; AET: Acid exposure time; MNBI: Mean nocturnal baseline impedance; RBM: Retrograde bolus movements.

Table 4 pH-impedance data of patients with nonerosive reflux disease, reflux esophagitis and esophageal atresia with gastroesophageal reflux disease

Parameter	NERD,	RE,	EA with GERD	Kruskal-Wallis test
	AM (95%CI)	AM (95%CI)	AM (95%CI)	*P* value
AET (%)	10.50 (8.05-12.95)	10.06 (7.33-12.79)	11.62 (7.53-15.7)	0.776
Longest acid exposure in min	21.8425 (17.14 to 26.55)	25.8560 (18.47 to 33.24)	46.8 (28.39 to 65.26)	< 0.05
Total number of RBM	74.61 (63.37 to 85.85)	82.28 (63.89 to 100.67)	67.30 (55.28 to 79.32)	0.697
Total number of proximal reflux	35.36 (20.76 to 29.97)	32.91 (21.35 to 44.48)	10.95 (6.23 to 15.66)	< 0.001
Index of proximal reflux	0.35 (0.29 to 0.4)	0.37 (0.29 to 0.46)	0.16 (0.1 to 0.22)	< 0.001
Distal MNBI in kOhm	2.25 (2.03 to 2.48)	1.95 (1.64 to 2.27)	1.44 (1.21 to 1.67)	< 0.001

AET: Acid exposure time; AM: Arithmetic mean; CI: Confidence interval; MNBI: Mean nocturnal baseline impedance; NERD: Nonerosive reflux disease; RBM: Retrograde bolus movements; RE: Reflux esophagitis.

In addition, this was a study, where in not all patients were included but only those who were treated for troublesome symptoms after applying the exclusion criteria and among patients who had contacted our clinic over the past 3 years. Controls were chosen from sex- and gender-matched children with proven GERD in order to find specific features GERD in EA patients. In Belarus, a national follow-up program for EA patients has not yet been developed. So, these patients come to our clinic for examination when they have symptoms. Some of them did not experience any symptoms during pH-impedance monitoring. Before pH-impedance testing their parents reported symptoms spontaneously. The research was carried out at a single institution and as a retrospective study. Further accumulation of study data is needed for a better comparison of data in EA with GERD patients and patients with GERD with nonoperative esophagus. Surely, these data should be evaluated and confirmed with a prospective multicenter study.

Clinical data

Detailed clinical history and parental reported symptoms were analyzed for all patients. Symptoms in study groups were recorded during the study as events and by means of a questionnaire prepared specifically for this study for patients with GERD-related symptoms. We asked parents of children (usually younger than 8 years) to fill out this questionnaire so that we could find out what worries parents of children who cannot explain the symptoms that bother them. Thus, one of the most common symptoms in children younger than 5-6 years are the symptoms noted by their parents, such as coughing, vomiting, feeding difficulties, recurrent bronchitis, and pneumonia. Evaluation of the patient's and/or parental questionnaires showed that the most frequently observed symptom in EA patients with GERD and without GERD

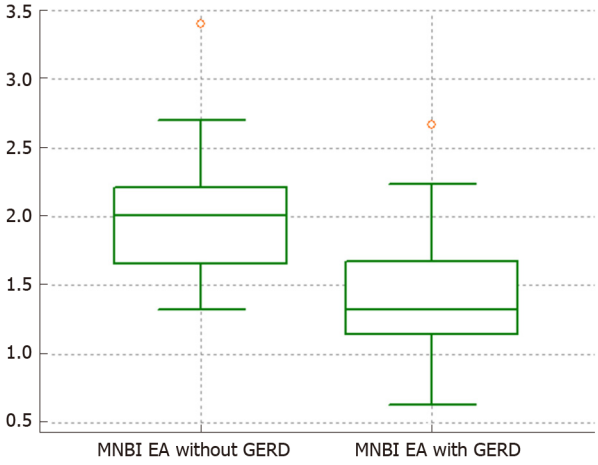


Figure 1 Differences in distal mean nocturnal baseline impedance values (kOhm) among the esophageal atresia study groups. Study groups are EA with GERD and EA without GERD. $P = 0.0024$ by *t*-test (assuming equal variances). EA: Esophageal atresia; GERD: Gastroesophageal reflux disease.

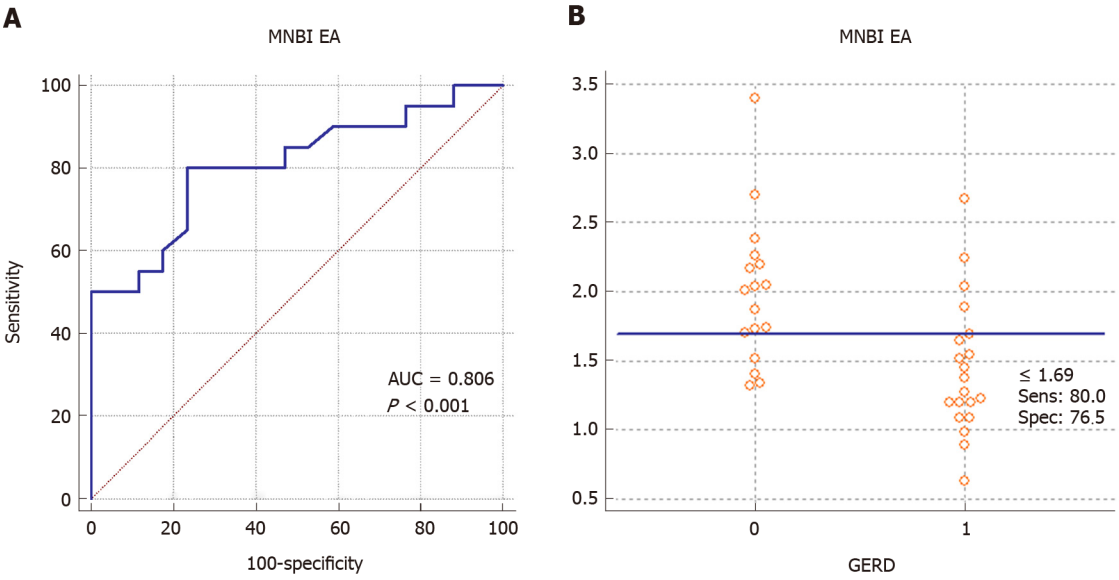
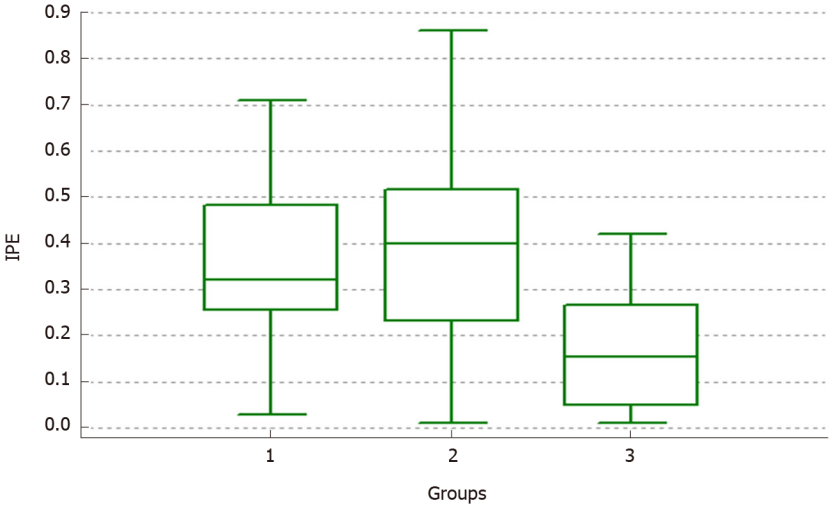


Figure 2 Mean nocturnal baseline impedance capabilities for gastroesophageal reflux disease diagnosis in patients with esophageal atresia. A: Area under the curve is 0.806, with $P < 0.001$; B: Diagnostic cut-off is 1.69 kOhm, with sensitivity of 80.0% and specificity of 76.5%. EA: Esophageal atresia; GERD: Gastroesophageal reflux disease; MNBI: Mean nocturnal baseline impedance.

in our groups was cough. We also found that EA patients in our study groups rarely had the typical GERD symptoms of heartburn, regurgitation, and belching. For all symptoms, the comparisons between the EA patients with GERD and those without GERD found no statistical relations.

An intriguing finding in our study was that the number of patients with positive SAP was significantly larger for the EA without GERD group. As such, these children are revealed to have more episodes of symptoms despite the normal data produced upon their pH-impedance testing. This fact can be very important for the accurate evaluation of GERD in symptomatic EA patients before prescribing antireflux medication and especially proceeding fundoplication. On the other hand, we found pathological pH-impedance data in 10% of the asymptomatic patients, meaning that we have to follow-up these patients correctly. Collectively, these results confirm the importance of pH-impedance testing in EA patients in order to evaluate GERD and to individualize the treatment to each patient.

The most frequently observed symptoms in our patients with RE were gastrointestinal (heartburn, vomiting, and abdominal pain). In the group of patients with NERD, respiratory (cough, recurrent bronchitis, and pneumonia) and combined



Factor	*n*	Average rank	Significantly different from factor nr[a]
(1) NERD	41	49.01	(3) EA with GERD
(2) RE	25	50.7	(3) EA with GERD
(3) EA with GERD	20	23.2	(1) NERD, (2) RE

Figure 3 Kruskal-Wallis test (*P* < 0.001) with post hoc analysis (Dunn's test) of index of proximal events in study groups. Study groups are: (1) NERD; (2) RE; (3) EA with GERD. [a]*P* < 0.05. nr: MedCalc numbers the factors. In the output you see a list of factors, with factor label, *n* and average rank. The factor label is preceded with a number between brackets. The "nr" in "Different (*P* < 0.05) from factor nr" refers to that number. NERD: Nonerosive reflux disease; RE: Reflux esophagitis; EA: Esophageal atresia; GERD: Gastroesophageal reflux disease.

gastrointestinal and respiratory symptoms were more frequently observed. A statistically significant difference was found when the clinical data on gastrointestinal symptoms were compared between the RE patients and NERD patients – showing a significantly higher rate in the former.

pH-impedance data

The prevalence of GERD in EA patients in our study was high (> 50%). Our results were similar to those from other studies of EA patients with pH-impedance monitoring[7,22-24]. The patients in our study were off PPI therapy for several weeks before examination (minimum being 1 wk).

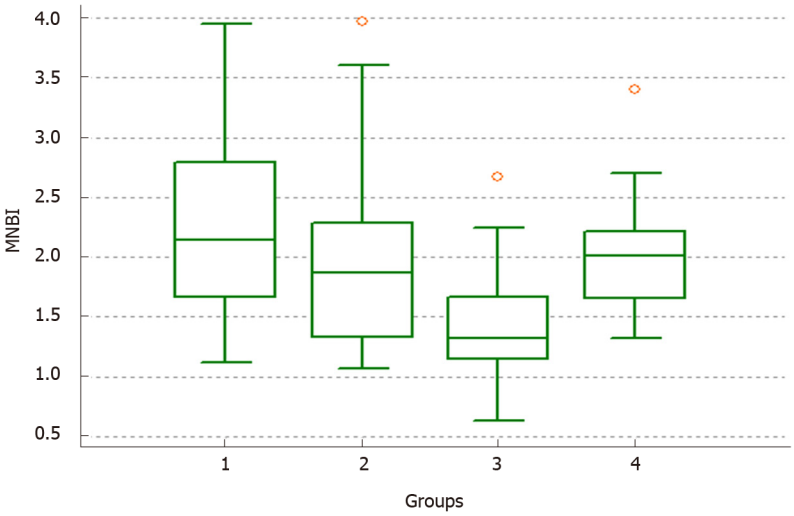
When we performed investigation into the features of pH-impedance data in our study groups with GERD (EA with GERD, NERD, and RE), we found no significant difference in the pH-impedance parameters (AET and total number of RBM). There were significant differences among all three groups for the longest acid exposure, total number of proximal events, and distal MNBI.

The esophagus is permanently compromised in EA patients, even when successful repair, sometimes under tension, has been achieved. Extrinsic and intrinsic innervations are abnormal and consequently motor function and sphincters are defective. The gastroesophageal reflux event is extremely frequent in patients treated for EA because of serious structural and functional deficiencies[22].

Mean nocturnal baseline impedance

MNBI, a novel pH-impedance metric, may be a surrogate marker of reflux burden. Investigations into the role MNBI in the diagnosis and phenotyping of reflux disease are relatively recent undertakings in the field. They have, however, revealed that MNBI can distinguish different GERD phenotypes from reflux-unrelated symptoms (*i.e.*, functional heartburn) and provides a good predictive value for antireflux therapy[16,17,25-28].

It is known already that EA patients have a significantly lower baseline impendence than normal children with suspected GERD[29,30]. In our study, the EA patients with GERD showed a significantly lower distal MNBI than either the EA patients without GERD or the patients with RE and NERD. Our results show that MNBI can be used as a diagnostic metric for GERD in EA patients after open surgical repair, having sensitivity of 80% and a specificity of 76.5%. We also found that distal MNBI at 1.69



Factor	*n*	Average rank	Significantly different from factor nr[a]
(1) NERD	41	63.78	(3) EA with GERD
(2) RE	25	49.68	
(3) EA with GERD	20	27.98	(1) NERD, (4) EA without GERD
(4) EA without GERD	17	55.26	(3) EA with GERD

Figure 4 Kruskal-Wallis test (*P* < 0.001) with post hoc analysis (Dunn's test) of mean nocturnal baseline impedance data in the study groups. Study groups are: (1) NERD; (2) RE; (3) EA with GERD; (4) EA without GERD. [a]*P* < 0.05. nr: MedCalc numbers the factors. In the output you see a list of factors, with factor label, *n* and average rank. The factor label is preceded with a number between brackets. The "nr" in "Different (*P* < 0.05) from factor nr" refers to that number. NERD: Nonerosive reflux disease; RE: Reflux esophagitis; EA: Esophageal atresia; GERD: Gastroesophageal reflux disease.

kOhm is the cut-off for diagnosis GERD in EA patients. These results highlight the potential utility/value of distal MNBI for designing a personalized follow-up program for EA patients without high AET or high number of RBM but who have level of distal MNBI < 1.69 kOhm. Such patients require constant monitoring and early treatment of the complications (special follow-up program).

There are many studies in adults and children which have shown a relationship between AET and baseline impedance[31-34]. While this result was found in our group of GERD patients with non-operated esophagus, our study extended the finding to a statistically significant association. However, the statistical analysis indicated the absence of a relationship (significant correlation and logistic regression) between any of the pH-impedance parameters and distal MNBI. This finding is similar to that from a recent study, in which Tong *et al*[29] proposed that their results could be due to the fact that a significant proportion of their EA cohort (87.9%) and controls (40%) were on PPI therapy during the study, which would have had an effect on the gastroesophageal reflux parameters[29]. In our study, EA patients and patients with proven GERD were off PPI therapy.

Proximal events

The role of reflux height in the clinical picture of GERD in general and extraesophageal symptoms in particular remains unclear. There are studies in EA patients which have shown no relevant correlation of high-reflux events and respiratory symptoms. Statistically, there has been no correlation between the amount of high reflux and symptom scores or reflux index[7]. Yet, as shown in infants by Wenzl[35], there was relevant correlation of high-reflux events with respiratory symptoms.

There was also, in our study, a significant difference between the total number of RBM and the number of proximal events in the same patient. So, one patient may have 100 episodes of RBM and 10 episodes of proximal events, and in another case, the patient may have 20 episodes of RBM and 10 episodes of proximal events; when we compare these cases, the difference will be significant. The first case has 10% proximal events of the total number of RBM, and for the second case it is 50%. We suggest using the IPE for estimation of more adequate assessment of proximal refluxes, as it reflects the share of proximal events in total number of RBM. We calculated this index as the ratio of the number of proximal refluxes to the total number of refluxes per day. Our

statistical analysis showed strong correlation with IPE and total proximal events for both EA groups, with and without GERD, and indicated that IPE was significantly lower in both compared to that in GERD patients with non-operated esophagus. Thus, it is obvious that factors other than proximal refluxes are involved in the pathogenesis of respiratory symptoms in EA patients. It is generally known that esophagus after atresia open surgical repair is restored anatomically, but is it restored functionally? An additional question is whether these motility disturbances will disappear with age?

CONCLUSION

GERD is the most common long-term complication of EA. These patients are predisposed to GERD as a result of the altered anatomy and motility of the esophagus. pH-impedance testing is an effective way to diagnose and monitor for reflux and to individualize the treatment strategy for each patient.

Distal mean nocturnal baseline impedance has good diagnostic value for GERD in children with EA after open surgical repair, with cut-off of < 1.69 kOhm. The difference between two areas (calculated as AUC AET~ AUC MNBI = 0.0838; 95%CI: -0.101 to 0.269; P = 0.3743) revealed a similar diagnostic value for AET (%) and MNBI, in relation to GERD. Distal MNBI can be used as an indicator to design a personalized follow-up program for EA patients.

The index of proximal events (IPE) is calculated as the ratio of the number of proximal refluxes to the total number of refluxes per day. There was strong correlation with the IPE and total proximal events in each of the EA groups, and our data showed that the IPE in both EA groups was significantly lower than in GERD patients with non-operated esophagus. IPE might be an additional parameter of pH-impedance monitoring.

ARTICLE HIGHLIGHTS

Research background

Esophageal atresia (EA) is the most common congenital anomaly of the gastrointestinal tract. Esophageal dysmotility and gastroesophageal reflux disease (GERD) are frequent and lifelong problems after repair of EA, even after successful surgical repair of the esophagus anatomy. It is important to diagnose and manage GERD to reduce subsequent related respiratory and gastrointestinal problems and their associated short-term and long-term complications. GERD can be asymptomatic and several studies have shown the absence of correlation between symptoms and esophagitis in this population. All EA patients (including asymptomatic patients) should undergo monitoring of GER (impedance/pH-metry and/or endoscopy) at time of discontinuation of anti-acid treatment and during long-term follow-up.

Research motivation

In Belarus, a national follow-up program for EA patients has not yet been developed. So, these patients come to our clinic for examination when they have symptoms. Some of them did not experience any symptoms during pH-impedance monitoring. Before pH-impedance testing their parents reported symptoms spontaneously.

This study was designed to assess clinical symptoms and pH-impedance data in children after EA open surgical repair, and to compare with a control group of children with proven GERD in order to find specific GERD features in these patients and to provide data that will support development of a national program for the follow-up of EA patients. This was accomplished *via* a retrospective chart review of EA open surgical repair patients with GERD-related symptoms in our clinic from November 2017 to February 2020 using pH-impedance data, upper endoscopy data, medical records and clinic letters.

Research objectives

The main objectives of this study were to assess clinical symptoms and pH-impedance data in children with EA open surgical repair and to compare with a control group of children with proven GERD in order to identify specific features of reflux disease in these groups of patients. According to the results, we hope to develop a national program for the follow-up of EA patients and to personalize their treatment.

Research methods

Patients with EA who received open surgical repair and combined impedance-pH testing while off proton pump inhibitor therapy and who underwent upper gastrointestinal endoscopy with histological study of mucosa biopsy samples were involved in the study. Data on patient symptoms were collected *via* a specially-prepared questionnaire for our study patients with GERD-related symptoms. We asked the parents of children (usually younger than 8 years) to fill out this questionnaire so that we could see what worries parents of children who cannot explain the symptoms that bother them. We used the index of proximal events (IPE), calculated as the ratio of the number of proximal refluxes to the total number of refluxes per day. We also determined distal mean nocturnal baseline impedance in all patients at the same distance depending on age (1 year to 10 years: 3 cm above the lower esophageal sphincter; older than 10 years: 5 cm above the lower esophageal sphincter).

Research results

We found a strong correlation with IPE and total proximal event in each EA group (EA with GERD: 0.96, $P < 0.001$; EA without GERD: 0.97, $P < 0.001$). The level of IPE in both EA groups was significantly lower than in GERD patients without any surgical treatment of esophagus (Kruskal-Wallis test, $P < 0.001$). Data on distal mean nocturnal baseline impedance in comparison of EA with GERD, EA without GERD, nonerosive reflux disease (commonly referred to as NERD) and reflux esophagitis (commonly referred to as RE) groups showed significant difference between EA with GERD (Kruskal-Wallis test, $P < 0.001$; one-way analysis of variance: F-ratio 6.69, $P < 0.005$) and the other two control groups but an absence of difference between EA without GERD, NERD and RE groups. We also found strong correlation with the IPE and total proximal events in each of the EA groups, and our data showed that the IPE in both EA groups was significantly lower than in GERD patients with non-operated esophagus.

Research conclusions

Distal mean nocturnal baseline impedance has good diagnostic value for GERD in children with EA after open surgical repair, with cut-off of < 1.69 kOhm, and can be used as an indicator to design a personalized follow-up program for EA patients. The IPE might be an additional parameter of pH-impedance monitoring.

Research perspectives

Not all patients were included in this study but only those who were treated for troublesome symptoms (after applying the exclusion criteria) and who had contacted our clinic over the past 3 years. In Belarus, a national follow-up program for EA patients has not yet been developed.

Our results confirm the importance of pH-impedance testing in EA patients in order to evaluate GERD and to individualize the treatment strategy for each patient. This finding has very important implications for the evaluation of GERD in symptomatic EA patients before prescribing antireflux medication and especially in the consideration of proceeding to fundoplication.

Although it is generally known that esophagus after atresia open surgical repair is restored anatomically, whether it is restored functionally remains unknown. Another important unknown for focus of future study is whether these motility disturbances will disappear with age? For such, correct and comprehensive follow-up of surgically-repaired EA patients (such as that designed upon the results of our study presented herein) is needed.

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Editorial board member of *World Journal of Clinical Pediatrics*, Dr. Rafael Denadai is a board-certified craniofacial and plastic surgeon, who offers both general and specialist cleft-craniofacial and plastic surgical care in Brazil. Dr. Denadai has been recognized among his national and international peers as a highly productive surgeon-scientist, who is involved in a broad range of research within the cleft-craniofacial field, ultimately aiming to improve the delivery of high-level surgical care. Dr. Denadai's extensive research experience includes outcome-based research, patient-reported outcome, patient-centered care, and patient-specific surgical planning and execution. He remains passionate about using patient-focused and data-driven elements to implement changes in private and public surgical practices throughout the care cycle within the fields of cleft-craniofacial, pediatric plastic surgery, and reconstructive plastic surgery. (L-Editor: Filipodia)

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MINIREVIEWS

Influenza B infections in children: A review

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Abstract

Influenza B (IFB) virus belongs to the Orthomyxoviridae family and has two antigenically and genetically distinct lineages; B/Victoria/2/87-like (Victoria lineage) and B/Yamagata/16/88-like (Yamagata lineage). The illness caused by IFB differs from that caused by influenza A. Outbreaks of IFB occur worldwide and young children exposed to IFB are likely to have a higher disease severity compared with adults. IFB mostly causes mild to moderate respiratory illness in healthy children. However, the involvement of other systems, a severe disease especially in children with chronic medical conditions and immunosuppression, and rarely mortality, has been reported. Treatment with oseltamivir or zanamivir decreases the severity of illness and hospitalization. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. IFB illness is less studied in children although its impact is substantial. In this review, the epidemiology, clinical manifestations, treatment, prognosis, and prevention of IFB illness in children are discussed.

Key Words: Children; Influenza B; Hospitalization; Oseltamivir; Respiratory infections; Severity

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Core Tip: Influenza B (IFB) outbreaks occur worldwide and young children exposed to IFB tend to have a higher disease severity compared with adults. The IFB virus belongs to the Orthomyxoviridae family and has two distinct lineages; Victoria lineage and Yamagata lineage. The illness caused by IFB is less severe than that caused by influenza A. IFB illness is less studied in children although its impact is substantial. IFB mostly causes mild to moderate respiratory illness in healthy children. However, the involvement of other systems, a severe disease especially in children with chronic medical conditions and immunosuppression, and rarely mortality, has been reported.

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Early treatment with antiviral agents decreases the severity of illness and hospitalization. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. In this review, the disease burden, clinical manifestations, treatment, prognosis, and prevention of IFB illness in children are discussed.

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INTRODUCTION

Influenza is a major public health problem worldwide[1-3]. It is one of the most common respiratory infectious diseases. The influenza viruses belong to the Orthomyxoviridae family and contain a single-stranded RNA genome. There are three types of influenza viruses A, B, and C based on different structural arrangements of internal nucleoprotein and matrix protein antigens. Influenza A (IFA) causes more severe illness and hence is well studied. Influenza B (IFB) is less studied in children although its impact is substantial. IFB has two antigenically and genetically distinct lineages, B/Victoria/2/87-like (Victoria lineage) and B/Yamagata/16/88-like (Yamagata lineage). Influenza C (IFC) is known to cause upper respiratory tract infection in younger children. It also causes community-acquired pneumonia. The severity of infection is less than that of IFA but more than that of IFB. C/Kanagawa/1/76-related lineages and C/Sao Paulo/378/82-related lineages are the IFC strains identified in the influenza seasons 2008-2009 and 2009-2010 in Milan[4]. IFB viruses have circulated worldwide since 1983 causing outbreaks now and then[5-8]. IFB usually causes mild to moderate illness in healthy individuals including children. However, severe disease in children, the elderly, and individuals with chronic medical conditions have been reported. Serious illness leading to mortality, although rare, has been described. Worldwide, young children exposed to IFB had a higher disease severity compared with adults[9-13]. Antiviral agents decrease the severity of illness and hospitalization[8]. Due to the enormous health and economic impact of IFB, these strains are included in vaccines. In this review, the disease burden, clinical manifestations, differences between IFA and IFB illness, treatment, prognosis, and prevention of IFB illness in children are discussed.

EPIDEMIOLOGY AND DISEASE BURDEN

IFB was first identified in 1940. The second lineage was discovered in 1983. Since then frequent outbreaks of IFB have occurred worldwide[1,3,5,6]. From 2001 onwards, both IFB lineages have been co-circulating each influenza season. Children are more susceptible to infection. In particular, children under 5 years of age are more susceptible to influenza illness as they are an immunologically naïve population. IFB caused significant morbidity in children in the United States during 2004-2011, and in the United Kingdom during 2010-2011 seasons[1-3,5,6]. The influenza-associated disease burden among infants and young children is likely to be underestimated because of the similarity of illness caused by other respiratory viruses during an influenza season. Annual IFA and IFB attack rates among children aged 5 to 9 years reach 35%, compared with those rates among the adult population which approach 13% for IFA and 6% for IFB. IFB outbreaks occurred worldwide from November 2017 to April 2018. The illness caused significant health and economic impacts worldwide[1-3,5-7].

Mancinelli *et al*[2] reported that among the IFB isolates, 91.54% were the B/Yamagata/16/88 lineage and 8.45% were the B/Victoria/2/87 lineage during the 2012-2013 influenza season in Italy. The B/Yamagata/16/88 lineage was most prevalent in children 3-6 years old. They reported the median length of hospital stay of 3 d for IFB viral illness. An Indian study reported high influenza-associated mortality in children aged less than 5 years in addition to the elderly population[10]. Studies on the incidence, clinical burden, and economic impact of IFB helped to include an additional B strain in the vaccine against influenza. A study involving IFB cases across

9 European countries reported wide variations in IFB cases during 1998-2013[5].

The Global Influenza B Study that included 1820301 influenza cases from 31 countries during 2000-2018 reported 419167 (23.0%) cases of IFB[14]. Cases from the United States (54.1%) and Australia (25.3%) contributed the maximum number of cases. In countries of the Southern hemisphere, IFA epidemics peak in July-September, and IFB peaks in August-September. In countries of Northern hemisphere, IFA peaks in January-February and IFB peaks in February-March (Table 1). However, there were exceptions to this pattern in some countries. In most countries, IFB/Victoria showed a unimodal curve with a peak below 10 years of age. B/Yamagata cases frequently showed two peaks, one peak below 10 years of age, and another peak between 25 and 50 years of age. In tropical countries, the timing of IFA and IFB epidemics differ. There appears to be no consistent pattern in the timing of the different epidemics. B/Victoria occurs more frequently in tropical countries, while B/Yamagata occurs frequently in temperate climate countries of the Southern and Northern hemispheres.

IFB-VIRAL CHARACTERISTICS

IFB viruses belong to the Orthomyxoviridae family[1,5,14]. They are single-stranded RNA viruses. They are classified into two lineages: B/Yamagata and B/Victoria. IFB viruses are further classified into specific clades and sub-clades. The B/Victoria clades include V1A and subclades include V1A.1, V1A.2, and V1A.3. B/Yamagata clades include Y1, Y2, and Y3. There are no subclades in B/Yamagata.

The virion of IFB virus consists of an envelope, a matrix protein, a nucleoprotein complex, a nucleocapsid, and a polymerase complex. The IFB virus has eight genome segments. The proteins of the polymerase complexes include PB1, PB2, and PA. The polymerase complexes are located at the ends of the nucleocapsids. These capsids are helical in nature. The nucleocapsids are encircled by the M1 matrix protein and a lipid bilayer envelope. The viral surface has two glycoproteins; hemagglutinin (HA) and neuraminidase (NA). The HA, NA and the M2 matrix protein are embedded in the lipid bilayer envelope. The IFB virus has 500 or more surface projections. These surface projections are made of HA and NA.

The IFB virus undergoes antigenic variation through genetic reassortment among co-circulating strains and antigenic drift from cumulative mutations[5,14]. IFB viruses generally change their genetic and antigenic properties more slowly than IFA viruses. IFB virus HAs have a mutational rate about five times slower than that observed for IFA virus HAs. The IFB virus is relatively vulnerable to certain environmental impacts. The virus can survive for several hours in the environment depending on humidity and temperature. In water, at < 20°C, the virus can survive for several months. Influenza viruses are sensitive to lipid solvents and detergents.

CLINICAL FEATURES

IFB illness in children ranges from subclinical illness to complicated disease involving multiple organs. The typical manifestations include respiratory tract and systemic signs and symptoms. IFB can present as croup, bronchiolitis, pneumonia and febrile disease mimicking bacterial sepsis. Central nervous system, cardiac, muscle, or renal complications can also occur[1-3,5-7,11-17]. IFB illness in children may predispose to bacterial superinfections. IFB viruses tend to persist across multiple seasons and exhibit complex global dynamics[18]. A peak between August and September has been observed in countries of the Southern hemisphere. In countries of the Northern Hemisphere, the peak occurs in February-March. There are exceptions to this pattern in certain countries. Generally IFB epidemics tend to peak three weeks after the IFA epidemics during winter in temperate countries of Southern and Northern hemispheres[14,18]. An Indian study involving children found more cases from January to May with a peak in March[12]. In younger children distinguishing influenza from other febrile illnesses based on clinical manifestations alone is often difficult.

IFB most often causes respiratory infections with cough, fever, myalgia, chills and malaise. The illness may last for two to eight days. The onset is typically rapid. A minority of patients, especially young children, and those with medical comorbidities may have severe disease. Respiratory and multi-organ failure may occur either due to virus or secondary bacterial pneumonia. IFB also affects children with underlying malignancies such as lymphoma, leukemia, solid tumor, or renal tubular disorders.

The highest frequency of IFB infections is said to occur in infants less than one year

Table 1 The differences between influenza A and influenza B illness in children[2,13-16]

Variables	Influenza A	Influenza B
Epidemiology	Constitutes about 75% of total influenza	Constitutes about 25% of total influenza
Epidemic/pandemic	Causes both epidemics and pandemics	Causes epidemics
Epidemic peak[1]		
Southern hemisphere	July-September	August-September
Northern hemisphere	January-February	February-March
Virology		
Family	Orthomyxoviridae	Orthomyxoviridae
Type	Single-strand RNA virus	Single-strand RNA virus
Subtypes/lineages	18 H subtypes and 11 N subtypes	Two lineages: B (Victoria) and B (Yamagata)
Common types	A(H1N1) and A(H3N2)	Victoria and Yamagata lineages
Pandemic strain	A(H1N1) pdm09 virus	/
Infection	Humans, pigs, horses, wild birds, *etc.*	Only in humans (possibly in seals)
Host factors (children)[13]		
Age (yr) (median)	2.0	4.2
Infants	Commonly affected	Less commonly affected
School-age	Can be affected	Commonly affected
Gender	Male predilection	Male predilection
Clinical features[13,16]		
Fever	High	< 38.5 °C
Febrile convulsion	About 5.4%	About 10.7%
Rhinorrhea	Common	Common
Underlying medical conditions	Not so common (26%)	More common (34%)
Length of hospital stay (median)	5 d	3 d
Gastrointestinal symptoms (abdominal pain, vomiting, diarrhea)	Less common	More common
Myositis	1.1%-6%	4.5%-15%
Otitis media	26%	19%
Laboratory parameters		
Leucopenia	Less common (8%)	More common (19%)
Leucocytosis	8%	7%
Elevated CRP	31%	15%-46%
Treatment		
Oseltamivir	Effective	Effective
Zanamivir inhalation	Effective in ≥ 5 yr of age	Effective in ≥ 5 yr of age
Prognosis	Good	Good
Complications	May occur	In young children/having comorbidities
Vaccine (Quadrivalent)	Effective	Effective

[1]Exceptions do occur in some countries.

of age. The median age of children with IFB viral illness is 4.2 years[13]. High fever especially an abrupt onset is common. Febrile convulsions are reported in 9% of children. Rhinorrhea and cough are the usual manifestations. Vomiting or diarrhea may occur in 25% of cases. Myalgia or myositis may be present in 15% and headache occurs in 25%. Pneumonia, otitis, and encephalitis may occur in a small percentage of children.

An Indian study involving IFB in children found upper respiratory tract infections in 78.5% cases followed by pneumonia in 19.6% and severe pneumonia in 1.7%[12]. The peak of the illness was observed in March. Male children predominated in the study. Male predominance in IFB illness has also been reported in other studies[14-19].

Gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting in IFB children were reported by Lennon *et al*[16]. Encephalitis is a rare manifestation of IFB. A 6-year-old girl with acute IFB virus encephalitis resulting in neurological sequelae was reported by McCullers *et al*[20]. IFB associated encephalitis, profound weakness, and response to oseltamivir in a 10-year-old boy was reported by Straumanis *et al*[21].

Chi *et al*[19] studied 118 cases of IFB in Taiwan with characteristics of Yamagata and Victoria strains. They reported a higher incidence of lower respiratory tract infections with Yamagata-like strains. All invasive diseases particularly occurred with this strain. Children infected with the Victoria-like group had the longest hospital stays.

HOST FACTORS

Influenza is a major cause of morbidity and mortality in humans globally. Certain age groups are more susceptible to influenza. Children and the elderly are more vulnerable to influenza. IFB commonly affects younger children. Eşki *et al*[22] reported that children less than 5 years of age had a higher hospitalization rate (82.9%). The strongest association between hospitalization and age was observed for children ≤ 2 years of age (63.6%) compared with other age categories (36.4%).

IFB related childhood morbidity and mortality increases with certain comorbidities. Underlying medical comorbidities such as asthma, neurologic deficits, or malignancies were documented in one-fourth of the children with IFA or IFB[13]. Congenital heart disease, neuromuscular disease, immunosuppression, presence of neutrophilia, lymphopenia, severe bacterial infections, and late initiation of antiviral therapy were found to be independent risk factors for prolonged hospitalization in patients with IFB-related lower respiratory infections. Prolonged hospitalization was more common in children with comorbidities (24.3%) compared to children without comorbidities (10.4%).

HOW IS IFB DIFFERENT FROM IFA?

Among influenza viruses, IFA virus causes more severe disease. Certain differences in median age, clinical manifestations, illness severity, risk factors, and length of hospitalization were observed between IFA and IFB illness in various studies[2,6,11,15]. Peltola *et al*[13] reported that the median age of children with IFA was 2.0 years and was 4.2 years in children with IFB. Infants accounted for 27% of children with IFA and 24% of those with IFB. Boys predominated in both IFA and IFB infected children. Underlying medical conditions were present in 26% of the children with IFA and in 34% of those with IFB. Fever is a common symptom in both IFA and IFB affected children. High fever and rhinorrhea were more common in IFA than in IFB. Respiratory symptoms were more prevalent in patients affected by IFA than by IFB.

Mancinelli *et al*[2] reported more IFA cases in children less than one-year-old and more IFB cases among school-age children. The length of stay in children with IFA was significantly longer than those infected with IFB. The median length of stay was 5 d (range: 0-59) for IFA and 3 d (range: 0-116) for IFB. IFB children mostly had a fever < 38°C. An Indian study also observed more IFB cases in the older age group[12]. Respiratory symptoms dominate in IFA children. Lennon *et al*[16] found that gastrointestinal symptoms such as abdominal pain, diarrhea, and vomiting were more common in IFB than IFA infection. Children with IFB were more likely than those with IFA to be diagnosed with upper respiratory tract infection, myositis, and gastroenteritis. Children with underlying malignancies such as lymphoma, leukemia, and solid tumor were more susceptible to IFB infection.

LABORATORY TESTS

Several different approaches are currently available for the diagnosis of influenza infections in children[23-25]. These include nucleic acid amplification tests (NAT), immunochromatography-based rapid diagnostic tests, immunofluorescence assays and viral isolation in cell culture[23]. Rapid molecular assays are the preferred diagnostic tests. The advantages of these tests include accuracy, faster results and ease of the test at the point of care[24].

Rapid molecular assays are more sensitive compared to antigen-based tests. They can detect viruses much earlier in clinical samples. The tests for the diagnosis of influenza infections include reverse transcriptase polymerase chain reaction (RT-PCR), next-generation sequencing, ligase chain reaction, sequencing-based tests including pyrosequencing, DNA microarray-based tests, nucleic acid sequencing-based amplification, loop-mediated isothermal amplification-based assay, simple amplification-based assay, *etc*.[23]. Most of these tests take 2 to 4 h to complete. They demonstrate higher sensitivity and specificity compared with antigen-based tests. RT-PCR, a gold standard assay for influenza diagnosis involves three essential steps: extraction of viral RNA from clinical specimens; reverse transcription of viral RNA to a single-stranded cDNA using the enzyme reverse transcriptase, and amplification of the PCR product is coupled to fluorescent detection of labeled PCR products[23,24]. RT-PCR is the most powerful NAT used to identify influenza viruses across many laboratories in the world.

Nasopharyngeal and nasal swab samples should be obtained from influenza affected children and transported to the laboratory in a universal viral transport medium. Samples of throat and nasal swabs, nasopharyngeal aspirate, bronchoalveolar lavage, and sputum may also be taken depending upon the clinical situations. The samples should either be processed immediately or stored at -80°C before testing. Nucleic acid extraction and reverse transcription should be performed initially. Samples positive for IFB are further characterized by genotyping analysis to identify the subtype; the B/Yamagata/16/88 and B/Victoria/2/87 lineages. Different primers are used to identify the Victoria lineage and Yamagata lineage[23-25].

Other laboratory tests include white blood cell (WBC) counts and serum C-reactive protein (CRP) levels. The WBC counts and CRP levels may be helpful in detecting bacterial co-infections. Both WBC counts and CRP levels are low in uncomplicated IFB illness. A decreased WBC count of less than 4000/mm^3 or more than 15000/mm^3 can occur in approximately 10% of children with IFB[13]. Leukopenia and lymphopenia are common in IFB illness. Hence, these isolated findings need not be considered for further evaluation. IFB is more likely associated with leukopenia than IFA. One study reported an elevated CRP level in 46% of IFB children[2].

TREATMENT

The children affected by IFB need supportive care and antiviral agents[26-30]. The neuraminidase inhibitors are effective for IFB illness in children. Oral oseltamivir is effective in children aged 1-12 years. This drug efficiently metabolizes to the active carboxy metabolite. The oseltamivir carboxylate is primarily eliminated by renal excretion. A pharmacokinetic model in children indicated that a dose of oseltamivir 2 mg/(kg·dose) twice a day would be safe and effective[29].

Early administration of oseltamivir can reduce the risk of IFB virus-associated pneumonia[8]. In-field trials supported the benefit of anti-influenza drugs especially when they are administered within 48 h after the onset of symptoms[13,28,29]. Whitley *et al*[29] in their randomized controlled study enrolled 144 IFB infected children. In the subgroup analyses they showed a significant reduction in the median duration of fever, cough, coryza and other symptoms in the oseltamivir group (Placebo, 100 h; Oseltamivir, 73 h; P = 0.01). Other benefits of oseltamivir include a rapid decline in viral shedding and a significant reduction in complications, particularly otitis media. Oseltamivir is well tolerated in children. Vomiting may be observed in a few children. Oseltamivir treatment might also reduce the likelihood of the spread of influenza to close contacts. Matheson *et al*[30] also found that oseltamivir was effective in reducing secondary complications. Sato *et al*[26] in Japan found that oseltamivir was effective in IFA and IFB, and the benefit was higher among younger children. On the other hand, Suzuki *et al*[27] found that oseltamivir was less effective against IFB than IFA. If clinical suspicion is strong for influenza infection, it is also advisable to start antiviral treatment without waiting for laboratory confirmation in special categories of children;

those with comorbidities and unvaccinated children < 6 mo of age[22].

The main advantages of neuraminidase inhibitors compared with amantadine and rimantadine, are fewer adverse effects, activity against both IFA and IFB, and rare resistance. Zanamivir is another drug effective in shortening the duration and severity of influenza. A 5-d course of twice-daily inhaled zanamivir, 10 mg, was compared with placebo in symptomatic IFB children aged 5 to 12 years[28]. The zanamivir group had a reduction in the duration of IFB symptoms.

PROGNOSIS

Prognosis is generally good for children affected by IFB. Some of the affected children may require hospitalization for 2 to 8 d. The average length of hospital stay of 3 d has been documented[2]. The majority (78.5%) with upper respiratory infections recovered without any complications[12]. The authors reported pneumonia in 14.2% children aged > 5 years, and in 5.3% children aged between one to five years. The disease tends to be severe in young children and those with comorbidities. Mortality due to IFB in children remains low.

VACCINATION

World Health Organization and Centers for Disease Control and Prevention recommend that children between 6 mo and 5 years should be vaccinated against IFA and IFB. Inclusion of this age category in vaccination helps in decreasing hospital admissions for influenza. As there is no licensed influenza vaccine for children younger than 6 mo, alternative strategies including maternal vaccination during pregnancy and household vaccination are likely to reduce the burden of influenza[22]. Multiple influenza vaccine manufacturers have brought out quadrivalent seasonal influenza vaccines consisting of B strain to provide immunity against both lineages of IFB. The influenza vaccine including B strain is likely to offer benefit in children with chronic pulmonary, cardiac, or renal disease, diabetes mellitus, immunosuppression, or in those receiving long-term salicylate treatment.

CONCLUSION

IFB illness affects children worldwide during epidemics. The clinical manifestations vary depending on the lineages. The illness tends to be severe in younger children and those with comorbidities. The antiviral drug, oseltamivir is effective in children especially in reducing the duration of symptoms, viral shedding and secondary complications. The quadrivalent flu vaccine which includes IFB strain is recommended in children.

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ORIGINAL ARTICLE

Basic Study

Identification and characterization of resistance and pathogenicity of *Enterococcus spp.* in samples of donor breast milk

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Abstract

BACKGROUND

Breast milk is the primary source of nutrition for newborns. Hospitalized babies frequently need nutritional support from Human Milk Banks. As bacterial species of the genus *Enterococcus* are part of the microbiota of healthy donors, they may contaminate samples of pumped breast milk.

AIM

To identify and characterize the bacterial virulence and resistance in samples isolated from the nipple-areolar region, hands, and breast milk aliquots from donors at the Human Milk Bank of Municipal Hospital Esaú Matos in the city of Vitória da Conquista, Bahia State, Brazil.

METHODS

The personal hygiene and sanitation of donors were analyzed with the aim of identifying possible reasons for contamination of pumped milk. Cutaneous samples as well as aliquots of unpasteurized and pasteurized milk from 30 participants were obtained. Each *Enterococcus spp.* isolate underwent a disk diffusion susceptibility test and molecular biology techniques to determine resistance and virulence genes.

RESULTS

Enterococcus spp. were identified in 30% of donors (n = 9), and 11 specimens were

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isolated. Resistance to tetracycline was highly prevalent, being detectable in 63% of the isolates (n = 7) and followed by intermediate sensitivity to ciprofloxacin, observed in 27% of the specimens (n = 3). The *efa*A gene was found in 63% (n = 7) of the isolates, while the *ace* gene was detected in 27% (n = 3).

CONCLUSION

This study illustrates the importance of microbiological monitoring by Human Milk Banks and the need for alternatives to prevent the presence of *Enterococcus spp*. in hospital settings.

Key Words: *Enterococcus spp.*; Breast milk; Virulence; Human milk; Pasteurization; Antimicrobial resistance

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Core Tip: Human milk is the primary source of nutrition for newborns. Hospitalized newborns frequently require nutritional support from a Human Milk Bank. *Enterococcus spp.* can contaminate human milk, as they are part of the microbiota of healthy donors. This microbial genus is important in human diseases, mainly among newborns; therefore, this study aimed to identify and characterize the virulence and resistance of *Enteroccocus spp.* isolated from samples from the nipple-areolar region, hands, and raw and pasteurized milk aliquots from donors at a Brazilian Human Milk Bank.

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INTRODUCTION

There is no ideal substitute for human milk (HM) during early life despite current technological advances in baby food production, processing, preservation, and preparation[1]. Unquestionably, HM supplies newborns (NBs) with all the nutrients that adequately meet the physiological and metabolic needs in this age group[2].

Premature births and hospitalizations in neonatal intensive care units as well as maternal illness, death, and low milk production can hinder the introduction and maintenance of breastfeeding. In this context, the use of donated HM has become an efficient alternative to breastfeeding[2] and allows NBs to be fed with milk obtained from Human Milk Banks (HMBs)[3]. This strategy involves the collection, processing, and quality control of colostrum, transition milk, and mature milk for later distribution to NBs under a doctor or nutritionist prescription[4].

Enterococcus species are associated with important infections in NBs and are potential contaminants of milked human milk (MHM) samples. *Enterococcus spp.* are part of the gastrointestinal tract microbiota in healthy humans[5] and may be present in significant numbers on the hands and utensils of milk donors. The presence of these bacteria in human milk indicates that they are due to direct or indirect contact with fecal material, compromising the quality of the MHM[6]. Some *Enterococcus spp.*, especially *Enterococcus faecalis* (*E. faecalis*) and *Enterococcus faecium* (*E. faecium*), are highly pathogenic in NBs and must be effectively inactivated through pasteurization at HMBs.

To date, there are no published data on the isolation of enteric bacteria with hygienic-sanitary conditions of household collections performed by MHM donors. Considering the propensity of donated milk to be contaminated by these microorganisms, the clinical importance of such pathogens, and their ability to be dispersed through vectors such as health professionals and insects[7], it is important to investigate their virulence and dispersion capacity. Moreover, it is crucial to evaluate

the quality of the collection, preservation, and processing of the HM offered to NBs. These procedures are essential for a better understanding of the pathogenicity of *Enterococcus spp*. present in raw and pasteurized human milk samples and in samples taken from the hands and nipple-areolar region of donors linked to HMBs.

MATERIALS AND METHODS

Study design, period, region, and population

This is a cross-sectional study performed using samples from the nipple-areolar region, hands, and aliquots of raw and pasteurized MHM from healthy donors registered at the HMB of the Municipal Hospital Esaú Matos in the city of Vitória da Conquista, State of Bahia, Brazil.

The present study recruited donors registered at the above-mentioned HMB who had a donation history with a mean weekly volume of donated milk greater than or equal to 400 mL. Donated samples of raw milk with a volume less than 400 mL were pooled with milk samples from other donors for pasteurization, thus losing the traceability and specificity of the sample from a specific donor. The exclusion criteria were: donors with wounds on the hand or nipple-areolar region and donation of raw milk samples with a milk volume lower than that required for individual pasteurization.

The microbial isolates analyzed in the present study were obtained by the single collection of samples from the nipple-areolar region and hands as well as aliquots of MHM before and after pasteurization from 30 donors between March and August 2018. The collections were carried out at the donors' residences during scheduled visits. In addition to microbiological evaluation of the above-mentioned biological samples, questionnaires on hygienic-sanitary issues regarding the collection, packaging, and preservation of the donated milk were completed by the participants.

Ethical considerations

The study was approved by the Ethics Committee on Research with Human Beings of the Multidisciplinary Institute of Health of the Universidade Federal da Bahia, under the Certificate of Presentation for Ethics Apprehension number 80800717.8.0000.5556 and committee opinion number 2.475.023. The study followed all the conditions established by the Resolution 466/2012 of the Brazilian National Health Council.

Isolation, identification, evaluation of antimicrobial activity, and detection of constitutive, virulence, and resistance genes in Enterococcus spp.

After collection, the biological samples were immediately inoculated into enrichment brain heart infusion broth (HIMEDIA®) and incubated at 35-37ºC for 24 h. After that, they were placed in Petri dishes with 5% Blood Agar Medium (Base Agar, HIMEDIA®) and Chromagar Orientation (BD™ CHROMagar™ Orientation) in a bacteriological oven at 35-37ºC for 24 h. Both methods were important for increasing the isolation and identification of microorganisms of interest in the present study.

The colonies with presumptive characteristics of *Enterococcus spp*. selected in both media underwent Gram staining to evaluate the morphological characteristics as well as to perform the catalase test and subsequent tests to identify the genus *Enterococcus*, namely: hydrolysis of esculin in the presence of bile and growth in sodium chloride (NaCl) 6.5% media.

All *Enterococcus spp*. isolates underwent evaluation of antimicrobial activity using the agar diffusion method[8-10], according to the recommendations of the Clinical and Laboratory Standards Institute (2017)[11].

With the aim of detecting the constitutive[12], virulence[13-16], and resistance[17,18] genes in *Enterococcus spp*., all samples that were positive in the identification step underwent polymerase chain reaction (PCR) after reactivation in brain heart infusion medium.

Statistical analysis

A descriptive analysis of the study variables was carried out from a database constituted in Microsoft Excel through the Statistical Program EPI INFO (version 7.2.2.6). For the frequency comparison, the chi-square test was used, with values of $P < 0.05$ (95% confidence interval) considered statistically significant.

RESULTS

Milk samples were collected from 30 donors registered at the HMB of the Municipal Hospital Esaú Matos, in Vitória da Conquista, Bahia State, Brazil. A total of 81 samples were collected: 30 samples from swabs containing skin samples from the nipple-areolar region and donors' hands, 30 samples of raw MHM, and 21 samples of pasteurized MHM. Following the methodological criteria, it was not possible to obtain an appropriate aliquot of pasteurized milk from 9 (30%) participants as they did not donate enough milk volume to perform pasteurization in a single bottle.

Enterococcus spp. strains were isolated from 9 donors (30%), and a total of 11 strains were identified: 7 strains (63.6%) from skin samples and 4 strains (36.4%) from raw MHM samples. Although *Enterococcus spp*. were not detected in the pasteurized MHM samples, in 38% (n = 8) of these samples other microorganisms, mainly *Staphylococcus aureus*, were isolated.

The study participants were questioned about socioeconomic aspects and hygienic-sanitary conditions in order to link selected variables to the route of human milk contamination by *Enterococcus spp*. In this context, most participants were aged 30 years or less, were married or living with a partner, had a family income above two minimum wages, had one child, and were educated to high school level.

With regard to the hygienic-sanitary procedures performed by the donors for milk collection, most participants reported that they always performed the following procedures: hand washing before the collection of milk (96%); held back hair before milking (80%); breast washing before milking (86.7%); used a mask while milking (86.7%); avoided talking during milking (60%); discarded the first jet of milk (53.3%); milk obtained using a manual/electric pump (80%). However, only 12 (80%) participants declared that they checked the size of their nails and trimmed them when necessary before milking (Table 1).

Socioeconomic variables of HM donors such as age (above or below 30 years), marital status (single or living as a couple), family income (higher or lower than 2 minimum wages), number of children (one or more than one), and educational level (high school or graduate) as well as variables related to hygienic-sanitary procedures including hand washing before milking, talking during milking, and discarding the first milk jet at the beginning of collection were not significantly associated with the detection of *Enterococcus spp*. in samples ($P < 0.05$) (Table 1).

Among the 11 isolates of *Enterococcus spp*., antimicrobial resistance was found in 10 isolates (91%). Resistance to tetracycline was most common (63% of isolates, n = 7), and intermediate sensitivity to ciprofloxacin was found in 27% of the isolates (n = 3). Resistance to quinolones (ciprofloxacin, norfloxacin, and levofloxacin) was observed in 9% of the isolates (n = 1) (Table 2). All isolates were sensitive to vancomycin. All specimens were analyzed by PCR to detect quinolone resistance genes (*gyr*A and *par*C genes), but no positive results were obtained.

The *Enterococcus spp*. isolates were analyzed by PCR for detection of the constitutive *ddl* gene encoding the D-alanine ligases, which are species-specific for *E. faecalis* and *E. faecium*. This analysis showed that among the 11 enterococcal strains from 9 participants, 6 isolates were identified as *Enterococcus spp*. and 5 isolates were identified as *E. faecalis*. However, three *E. faecalis* isolates were found to be simultaneously colonized by *E. faecium* strains. Thus, three donors had *E. faecalis* and *E. faecium* strains. Such detection was possible because, after the initial identification of the *Enterococcus* genus, the stored colonies were collected from the CHROMagar Orientation medium (BD™ CHROMagar™ Orientation), which identified the genus and not the species of *Enterococcus*; thus, colonies of both species were collected.

All isolates of *Enterococcus spp*. underwent PCR for detection of the virulence genes *ace*, *efa*A, *gel*E, *as*, *cyl*A, *hyl*, *esp*, *as*, *cyl*A, *hyl*, and *esp* (Table 3). Among the 11 isolates, 7 isolates (63%) had the *efa*A gene that encodes the surface antigen A, which is commonly associated with biofilm formation and endocarditis, and 3 isolates (27%) had the *ace* gene, which encodes the protein that adheres to collagen and laminin, favoring bacterial adhesion commonly associated with endocarditis. The following enterocin genes were also genotypically investigated by PCR in *Enterococcus spp*.: Enterocin A, Enterocin B, Enterocin P, and Enterocin 31, also known as Bacteriocin. However, none of the isolates were positive for these genes.

DISCUSSION

Enterococcus spp. are present in the human microbiota and are associated with

Table 1 Descriptive analysis of the socioeconomic aspects and hygienic-sanitary conditions related to the collection of human milk and to human milk donors (*n* = 30) registered in the Human Milk Bank of the Municipal Hospital Esaú Matos in Vitória da Conquista, Bahia State, Brazil

Descriptive analysis of socioeconomic aspects of HM donors		
Variable	***n***	**(%)**
Age group		
Age less than 30 yr	19	63.3
Over 30 yr of age	11	36.7
Marital status/Conjugal state		
Married/living with spouse	20	66.7
Single or divorced/without a partner	10	33.3
Family income		
Less than 2 minimum wages[1]	9	30.0
Above 2 minimum wages	21	70.0
Number of children		
One child	16	53.3
Two or more children	14	36.7
Schooling		
High school	21	70.0
Graduate	9	30.0
Total	30	100.0
Descriptive analysis of hygienic-sanitary conditions of HM donors		
Variable	*n*	(%)
Hand washing prior to collection		
Sometimes/never	1	3.3
Always	29	96.7
Nail size check/trim before collection		
Sometimes/never	18	60.0
Always	12	40.0
Hair holding habit before collection		
Sometimes/never	6	20.0
Always	24	80.0
Breast lavage before collection		
Sometimes/never	4	13.3
Always	26	86.7
Use of mask during collection		
Sometimes/never	4	13.3
Always	26	86.7
Avoid talking during collection		
Sometimes/never	12	40.0
Always	18	60.0
Discard first jet of milk at the beginning of collection		
Sometimes/never	14	46.7

Always	16	53.3
Milk collection		
Manual/electric pump	24	80.0
Manual milking	6	20.0
Cleaning frequency of the pump[2]		
Sometimes/never	0	0
Always before using	24[1]	100.0[1]
Total	30	100.0

[1]Minimum wage in the period: BRL954,00. [2]Question applied only to donors who reported performing milking by hand and using an electric pump.

Table 2 Sensitivity profile of the isolates (*n* = 11) of *Enterococcus spp.* found in relation to the list of antimicrobials evaluated following the recommendations of the Clinical and Laboratory Standards Institute (2017)

Antimicrobials	Sensitive, *n* (%)	Intermediate, *n* (%)	Resistant, *n* (%)
Ampicillin 10 μg	11 (100.0)	0 (0)	0 (0)
Penicillin 10 un	9 (81.8)	0 (0)	2 (18.2)
Tetracycline 30 μg	4 (36.4)	0 (0)	7 (63.6)
Ciprofloxacin 5 μg	7 (63.6)	3 (27.3)	1 (9.1)
Norfloxacin 10 μg	9 (81.8)	0 (0%)	2 (18.2)
Levofloxacin 5 μg	10 (90.9)	0 (0)	1 (9.1)
Nitrofurantoin 300 μg	9 (81.8)	0 (0)	2 (18.2)
Linezolid 30 μg	11 (100.0)	0 (0)	0 (0)
Vancomycin 30 μg	11 (100.0)	0 (0)	0 (0)

un: Units.

Table 3 Distribution of the virulence genes of *Enterococcus faecium*, *Enterococcus faecalis*, and *Enterococcus spp.*

Virulence genes	*E. faecalis*		*E. faecium*		*Enterococcus spp.*		Total	
	N (isolated)	%	*N* (isolated)	%	*N* (isolated)	%	*N* (isolated)	%
Ace	1[a]	33	1[a]	33	2	67	3	27
*efa*A	3[b]	43	2[b]	28	3	43	7	63
*gel*E	0	-	0	-	0	-	0	-
As	0	-	0	-	0	-	0	-
*cyl*A	0	-	0	-	0	-	0	-
Hyl	0	-	0	-	0	-	0	-
Esp	0	-	0	-	0	-	0	-

[a]*ace* gene found in 1 isolate (containing strains of *Enterococcus faecalis* and *Enterococcus faecium*). [b]*efa*A gene found in three *Enterococcus faecalis* isolates (being a single isolate of *Enterococcus faecalis* and two isolates of *Enterococcus faecalis* and *Enterococcus faecium*). *E. faecalis: Enterococcus faecalis*; *E. faecium: Enterococcus faecium*.

important infections in NBs, and are potential contaminants of HM samples[8]. To date, there are no studies correlating NB infections and infant colonization with *Enterococcus spp*. in the city where this study was carried out. Moreover, there are no data associating socioeconomic aspects and hygienic-sanitary conditions regarding HM donors with the isolation of enteric bacteria. In the present study, *Enterococcus spp.*

were isolated from 30% of the participants. Similarly, *Enterococcus ssp*. were isolated from raw MHM samples within four[19] and seven[20] days after delivery. In such studies, gram-positive bacteria, including *E. faecalis*[21], *E. faecium*[21,22], *Staphylococcus spp*[23] and fecal coliforms[2] were identified in MHM[19], but they were not detected after milk pasteurization; however, other microorganisms continued to be detected after pasteurization.

Similar findings were observed in other HMBs in which the isolation rates were 2%[2], 6%[2], 7.5%[21], and even 70.4%[3] in non-pasteurized MHM samples and was 50.7% in pasteurized MHM[3] samples. These data reinforce the importance of strict hygienic-sanitary procedures in HMBs as well as the microbiological control of HM samples.

Among the 11 *Enterococcus spp*. isolated in this study, different types of resistance to antibiotics were found in 91% of the isolates (n = 10). In the phenotypical analysis of antimicrobial resistance, most isolates were susceptible to ampicillin, linezolid, and vancomycin, but resistance to tetracycline (63%), ciprofloxacin (27%), norfloxacin, levofloxacin, nitrofurantoin (9%), and penicillin was detected. *Enterococcus spp*. resistance has been previously reported in samples from miscellaneous clinical materials[22], food, and clinical samples[23], with resistance rates of 5% to tetracycline[22] and of 3%[22] and 70.5%[23] to ciprofloxacin.

Despite the identification of quinolone resistance, the resistance genes *gyr*A and *par* C were not detected. This phenomenon may be associated with interferences, including differences in the pH of the Mueller Hinton media, in mean temperature, and in the density of the inoculum applied. Due to *Enterococcus spp*. resistance to antimicrobials such as penicillin, ampicillin, aminoglycosides, tetracycline, and vancomycin and its association with nosocomial infections[24], it is crucial to identify these bacteria in different types of biological samples, including HM.

In this study, the presence of virulence factors in the isolated strains was also evaluated, and the genes *efa*A and *ace* were found in 63% and 27% of the isolates, respectively. These genes are associated with enterococcal biofilm formation and endocarditis, making the treatment of infections caused by these microorganisms difficult. With regard to species identification, of the three isolates with the *ace* gene, two were *Enteroccocus spp*. and one had *E. faecium* and *E. faecalis* strains. Among the seven isolates carrying the *efa*A gene, three were *Enterococcus spp*., two were *E. faecalis*, and two had *E. faecalis* and *E. faecium* strains.

The genes *ace* and *efa*A have been previously detected in *E. faecalis* and *E. faecium*[22] hospital strains and the *ace* gene has also been found in *Enterococcus spp*. of clinical origin[23]. The pathogenicity mechanisms of *Enterococcus spp*. are not yet fully understood. It is known that these microorganisms have virulence factors that are associated with the severity and duration of human infections as well as with the dissemination of these microorganisms in the hospital environment, where they mainly cause urinary infections, endocarditis, and bacteremia. The most frequently reported virulence factors are related to the adhesion to host tissues, invasion, abscess formation, modulation of the host inflammatory responses, and secretion of toxic products[25].

The high percentage of *Enterococcus spp*. isolates in HM samples associated with the high antimicrobial resistance rate (mainly to tetracycline and ciprofloxacin) and the positivity of virulence factors (detection of the genes *ace* and *efa*A) found in this study confirm the importance of carrying out strict control from the collection to the distribution of MHM. In this context, it is necessary to take utmost care during milk processing in order to avoid the dispersion of microorganisms, as the entry of these microorganisms into the hospital environment can increase the risk of infection in hospitalized patients, mainly in those with a weakened immune system.

CONCLUSION

The present study demonstrates the necessity for microbiological control in HMBs and the requirement to develop alternatives to avoid the entry of *Enterococcus spp*. species into the hospital environment, which can potentially lead to important NB infections that increase the length of hospitalizations and, consequently, health service costs. Considering that most premature NBs are transferred to intensive care units, thereby requiring food supplementation with MHM, and that the consumption of contaminated MHM can lead to mild-to-severe infections, the accurate identification of bacterial pathogens is an essential requirement for the detection of microorganism reservoirs, infection sources, HM distribution monitoring, and decision taking aiming to reduce health risks in NBs. Moreover, strict control from the collection to the

distribution of donated HM may reduce the dispersion rates of virulent and multidrug-resistant microorganisms that can cause several problems in a hospital environment, leading to increases in morbidity, mortality, and costs related to health care.

ARTICLE HIGHLIGHTS

Research background

Human milk is the primary source of nutrition for newborns. Hospitalized babies often require nutritional support from Human Milk Banks. As *Enterococcus spp*. are part of the microbiota of healthy donors, they are potential contaminants of pumped breast milk.

Research motivation

Donor Human Milk is an important alternative that allows the appropriate feeding of hospitalized newborns. A number of steps are needed to ensure the safe collection, processing, and quality control of colostrum, transition milk, and mature milk for subsequent distribution to newborns under a doctor or nutritionist prescription. However, such milk can be contaminated by *Enterococcus spp*. species that are highly pathogenic in newborns, and can increase morbidity, hospitalization time, and mortality among neonates.

Research objectives

The present study intended to identify and describe the bacterial virulence and resistance in samples obtained from the nipple-areolar region, hands, and breast milk aliquots of donors at the HMB of Municipal Hospital Esaú Matos in the city of Vitória da Conquista, Bahia State, Brazil.

Research methods

This cross-sectional study used samples from the nipple-areolar region, hands, and aliquots of raw and pasteurized milked human milk from healthy human milk donors. The study recruited donors who had a donation history of a mean weekly volume of donated milk equal to or higher than 400 mL. The microbial isolates from milk samples were analyzed before and after pasteurization. A total of 30 donors were assessed between March and August 2018.

The samples were immediately inoculated into enrichment brain heart infusion broth (HIMEDIA®) and incubated at 35-37°C for 24 h after collection. Thereafter, they were placed in Petri dishes with 5% Blood Agar Medium (Base Agar, HIMEDIA®) and Chromagar Orientation (BD™ CHROMagar™ Orientation) in an incubator at 35-37°C for 24 h.

Colonies in which *Enterococcus spp*. were observed were selected from both media and underwent Gram staining to evaluate the morphological characteristics and to perform the catalase test and subsequent tests to identify the genus *Enterococcus*.

All *Enterococcus spp*. isolates underwent evaluation of antimicrobial activity by the agar diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute (2017).

To detect the constitutive, virulence, and resistance genes in *Enterococcus spp*., all samples that were positive in the identification step underwent polymerase chain reaction after reactivation in brain heart infusion media.

A descriptive analysis of the study variables was carried out from a database constituted in Microsoft Excel through the Statistical Program EPI INFO (version 7.2.2.6). For the frequency comparison, the chi-square test was used, with values of $P < 0.05$ (95% confidence interval) considered statistically significant.

Research results

Eighty-one samples were collected, with 30 samples from swabs containing nipple-areolar region skin samples and donors' hands, 30 samples of raw milked human milk, and 21 samples of pasteurized milked human milk. *Enterococcus spp*. strains were isolated from 30% of the donors and 11 strains were identified: 7 strains (63.6%) from skin samples and 4 strains (36.4%) from raw MHM samples.

The majority of participants declared that they washed their hands before the collection of milk (96%), held back hair before milking (80%, washed the breasts before milking (86.7%), used a mask while milking (86.7%), avoided talking during milking

(60%), discarded the first jet of milk (53.3%), and obtained milk using a manual/electric pump (80%).

No statistically significant associations between demographic and hygienic characteristics and the detection of *Enterococcus spp*. were observed. Antimicrobial resistance was identified in 10 isolates (91%), mainly to tetracycline.

Research conclusions

Here we demonstrate the importance of appropriate microbiological control in Human Milk Banks in order to prevent *Enterococcus spp*. in hospitals as these bacteria can lead to important newborn infections that increase morbidity, mortality, and hospitalizations among these patients, and increase health service costs.

Research perspectives

This study adds information to the microbiological control of donated milk, which, if well-performed from human milk collection to its distribution, may reduce the dispersion rates of virulent and multidrug-resistant microorganisms in a hospital environment, avoiding unwanted clinical outcomes among newborns. Putting such measures into practice may improve the health of children around the world.

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