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Is a Mini-Screen for Fetal Alcohol Spectrum Disorder Feasible?

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Abstract

Background: Fetal Alcohol Spectrum Disorders (FASDs) are a global public health concern with lifelong consequences for affected individuals. Recent prevalence studies suggest FASD prevalence rates range from 1-5% among school age children. Most people with FASD are not correctly diagnosed and inadequate screening to identify patients with increased risk may contribute to under-diagnosis. This study developed a 10-item screening tool for FASD and examined its feasibility. **Methods:** The sample consisted of 355 children who had been evaluated at an FASD clinic. Data from the 33-item Alcohol Related Neurodevelopmental Disorder Behavioral Checklist was used to develop a brief FASD screen by comparing the changes in Cronbach's alpha for different combinations of items. The validity of the brief scale was then further examined using receiving operating characteristic analyses. **Results:** The 10-item screen demonstrated acceptable sensitivity, specificity, and accuracy to identify children at high risk for FASD. The percentage correctly classified was 91.3 and the area under the receiving operating characteristic curve was 0.971. **Conclusions:** This feasibility study demonstrated that a screen for FASD consisting of 10 items with yes or no responses can be completed in 3 - 4 minutes. The tool is brief, with a low administration burden and has acceptable epidemiologic performance characteristics including accuracy. Future research should examine the performance of this tool when used in larger, community-based populations where screening for FASD would be appropriate.

Keywords

Fetal Alcohol Spectrum Disorders (FASDs), Children, Screening, Prevalence

1. Introduction

Among women of child-bearing age in the United States, 53.6% used alcohol in

the past month and 18.2% binge drank [1]. Since up to 50% of pregnancies are unplanned, many of these women will have children with prenatal alcohol exposure [2]. Despite increasing awareness of the risks of alcohol use during pregnancy, rates of alcohol use during pregnancy continue to be alarmingly high. A study from the Centers for Disease Control and Prevention reported that 13.5% of pregnant women reported ongoing alcohol use and that over 5% reported binge drinking, defined as four or more drinks in a sitting [3]. Upon confirmation of pregnancy, most women quit or reduce alcohol use, but 10.2% continue to drink [1] and recent studies indicate that over 8% of pregnant women are drinking during the last trimester of pregnancy [4]. The United States has 3.6 million births annually. Thus, every year in the United States alone 494,679 pregnancies have prenatal alcohol exposure and 183,215 will have been exposed to binge drinking. Each day 1350 infants are born with a history of prenatal alcohol exposure. Data on prenatal alcohol exposure have considerable variation due to the presence of common confounders, including variation in data collection methodologies, lack of systematic population-based screening, underreporting of use during pregnancy, and differences in thresholds for exposure. However, this data does demonstrate that prenatal alcohol exposure is a huge public health problem.

Prenatal alcohol exposure has been demonstrated to increase risk for multiple adverse outcomes impacting the normal developmental trajectories of infants and young children. The range of adverse outcomes is large but frequently includes acquisition of speech and language skills, cognitive development and both gross and fine motor skills [5]. Prenatal alcohol exposure also increases risk for fetal alcohol spectrum disorder (FASD). This is an important public health issue since FASD is the most common cause of noninheritable developmental disability in the United States.

FASD is typically considered to be a broad term for four categorical entities. The first entity is fetal alcohol syndrome, which is diagnosed by the presence of prenatal alcohol exposure, abnormal facial features, growth impairment (height, weight, or both), and brain dysfunction, based on deficits in three domains of brain function. The second is partial fetal alcohol syndrome, characterized by the absence of growth impairment and only one or two abnormal facial features. The third is alcohol-related neurodevelopmental disorder, defined as prenatal alcohol exposure and impairment in three or more domains of brain function. The fourth is alcohol-related birth defects, involving prenatal alcohol exposure and a birth defect that may be attributable to prenatal alcohol exposure. However, this category is very rarely used. More recently, the broad category of FASD has been utilized as a diagnostic entity in some countries [6]. This broad construct of FASD is used as a diagnosis for individuals with adverse outcomes attributable to prenatal alcohol exposure.

FASD is a common condition with a broad severity with a typical course of increasing severity across the lifespan. Much of the impairment is due to lack of early recognition and inadequate interventions. The disorder varies widely, with cognitive ability ranging from average to intellectual disability. Common com-

orbidities include birth defects, sensory impairments, high rates of other neurodevelopmental disorders, such as attention deficit hyperactivity disorder, learning disabilities, speech and language deficits, and gross and fine motor impairments.

Recent research on the prevalence of FASD among first-grade students from four different areas in the United States found that the estimated prevalence rate of FASD ranged from 1.1% to 5.0% [7]. In the United States alone, this suggests that at a 1.1% prevalence rate, 100 new cases of FASD are born each day. If the prevalence rate is 5%, then 500 new cases of FASD are born daily, or about one new case every 20 minutes. Globally, the prevalence of FASD is at least 2.2% with wide variation by country [8]. Recent studies have consistently indicated that FASD is more prevalent than previously believed [9] [10]. A recent meta-analysis estimated the global prevalence to be as high as 8.5 per 1000 children [8]. These prevalence estimates suggest that FASD is more common than autism spectrum disorder and is a very important causal factor for attention deficit-hyperactivity disorder, intellectual disability, vision and hearing impairments, and multiple developmental neuropsychiatric disorders [9] [10].

While the prevalence rates from these studies demonstrate that FASD is a common developmental disorder, screening for prenatal alcohol exposure and FASD is still not routine in child health care settings [11]. Screening for prenatal alcohol exposure is often absent in obstetric, pediatric, and child welfare systems, especially when compared to screening for drug use. This may be due, in part, to concerns about the stigma against women who use alcohol during pregnancy [12]. However, stigma related to prenatal drug use is also a concern, yet screening for drug use in this population is common.

Obtaining information about prenatal alcohol exposure and exposure dosimetry is often complicated by the frequency with which children at the highest risk for FASD are separated from their biological parents (e.g., foster care, relative placement, adopted, or incarcerated in juvenile justice system or placed in a residential care facility). A recent study found that among 151 children and young adults screened for FASD, only four (2.6%) lived with their biological parents. This makes collection of data on prenatal alcohol use difficult in routine clinical settings, especially where health care for children at high risk for FASD may be delivered at multiple sites [13]. For example, 30.5% of children with FASD will be placed in foster care and over 18% of children in foster care have FASD (Engesether B, Hoffner M, Johnson E, Klug MG, Popova S, Burd L. Prevalence of fetal alcohol spectrum disorder in foster care: A scoping review. *Alcohol: Clinical and Experimental Research* (in press).). Foster care is an example of a system of care that utilizes routine systematic screening followed by referral for diagnosis and early intervention. These services already have existing funding streams to support them. While screening for FASD has not been widely adopted the foster care system does provide a useful example of a system where screening for FASD could take place. Children with a positive screen would have an existing system of care to support linking screening, diagnosis, and entry into diagnosis-informed interventions.

Comparisons of results from prevalence studies and rates of diagnosis of FASD in clinical settings demonstrate that both drinking during pregnancy and FASD are often undetected [14]. In a series of large prevalence studies, only two of the 222 cases of FASD diagnosed (1%) had been previously diagnosed [7]. An amazing 99% of children with FASD had not been correctly diagnosed. Another study in school-aged children found only one out of seven diagnosed cases (14.3%) had been previously diagnosed [15].

The low rates of diagnosis may be due to low levels of awareness among clinicians of FASD, uncertainty about how to diagnose FASD, and very limited routine screening in systems of care for children [16]. However, since 100 to 500 children with FASD are born every day in the United States, FASD may be much more prevalent than currently suspected by clinicians [17]. The lack of appropriate diagnosis suggests that many of these children were being treated for conditions other than FASD. Since FASD is frequently comorbid with many other developmental disorders, some may have been receiving care for these related disorders. However, they would not have been receiving diagnosis-informed care for FASD.

When FASD is undiagnosed or misdiagnosed, opportunities for early access to appropriate early interventions and support services are being delayed or missed [8] [18]. Identification of FASD is clinically important due to the very high rates of neuropsychiatric comorbidity, which increases over the lifespan. A large scientific literature on FASD has demonstrated an increased risk for a wide range of comorbid neuropsychological and mental health disorders over the lifespan of affected individuals [19].

Inadequate routine screening may be one of the primary factors contributing to underdiagnoses of FASD by healthcare professionals [17] [20]. Despite recommendations from organizations such as the American Academy of Pediatrics and the Centers for Disease Control and Prevention, screening for prenatal alcohol exposure and related developmental issues remains suboptimal [21] [22]. Physicians and other healthcare providers often face challenges in implementing comprehensive screening due to time constraints, competing priorities, and limited training on identifying and diagnosing FASD [23] [24]. Another factor may be the lack of an easy-to-use screening tool with acceptable epidemiologic performance criteria that could be used in routine office-based clinical care [25]. Screening appears to be an important step in both identifying children at increased risk for FASD and in constraining the demand for very limited access to FASD diagnostic clinics resulting from inappropriate referrals.

The rational for routine screening for FASD can be supported by the following:

- 1% - 5% of first grade students have FASD;
- Risk for placement into foster care for children with FASD is high;
- Rates of FASD in foster care populations are exceptionally high;
- Nearly all cases are undiagnosed;
- The mortality rate is increased;
- Mortality among siblings is also increased;
- FASD results in lifelong impairments;

- Early identification of FASD can reduce future developmental problems;
- FASD increases risk for learning disabilities and school related problems;
- Cost of care for FASD is very high;
- FASD increases risk for foster care placement and multiple foster home placements;
- FASD increases risk for involvement in the corrections system;
- FASD increases risk for substance use disorders;
- FASD increases risk for dependent living as adults.

Current diagnostic criteria for FASD have considerable variability, and require significant cost and staffing resources [26]. The multidisciplinary diagnostic process can be resource-intensive, time-consuming, and requires specialized expertise, making it challenging to implement in routine clinical practice [16]. However, a diagnosis of FASD has important benefits. The diagnosis of FASD can also impact care for the mother, the child with FASD and the child's siblings. The diagnosis of FASD offers multiple options to improve outcomes. A diagnosis identifies a mother in need of treatment. This can have multiple benefits, including preventing exposure in future pregnancies and reducing risk for mortality in the mothers [27]. A diagnosis also identifies siblings in need of assessment [28]. Additionally, a child with FASD is in need of increased surveillance for the development of multiple FASD associated problems, including increased risks for contact with juvenile justice systems. Lastly, a diagnosis identifies a need for a diagnosis-informed treatment plan [29]. This should include access to early developmental therapies, such as speech and language therapy, physical therapy, occupational therapy, behavioral interventions, treatment of sleep disorders, prevention of exposure to adverse childhood experiences, and prevention of multiple foster home placements.

One key area is to improve screening practices to identify children at increased risk for FASD. The current study examined the feasibility of development of a brief, easy-to-use screening tool for FASD, that could be used in screening programs in not only healthcare settings but community-based settings as well.

2. Materials and Methods

This study utilized a clinical sample of children ($N = 355$) who had been seen at a regional FASD clinic and, as part of the assessment, had been administered the 33 item Alcohol Related Neurodevelopmental Disorder Behavioral Checklist (ABC; **Figure 1**) [25]. A validation study of the ABC has been published [25].

Inclusion Criteria

Each child had been evaluated and had a diagnosis of either FASD or no-FASD. For those diagnosed with FASD, evidence of prenatal alcohol exposure was required. The study sample was limited to children from birth through 18 years of age.

Exclusion Criteria

Cases where the evidence of prenatal alcohol exposure status was unclear,

Alcohol Related Neurodevelopmental Disorder Behavioral Checklist (ABC)

Name _____ DOB ____/____/____ Age ____ Gender M F

In order to complete this checklist:
1) Behaviors must be impaired for the age of the person being assessed.
2) Interviewee needs to have known the person being assessed for at least one month.
3) After the reporter fills out the form, the clinician then adds other observed behaviors not already reported.

CHECK ALL THAT APPLY FOR THE APPROPRIATE AGE RANGE:

	Behavior	3-6 Years	7 Years +
1	Hyperactive		
2	Poor attention		
3	Impulsive		
4	Disorganized		
5	Seems unaware of consequences of actions		
6	No fear		
7	Would leave with a stranger		
8	Poor social skills		
9	Few friends		
10	Will talk or interact with anyone		
11	Easily manipulated and set up by others		
12	Socially inept (inappropriate speech or touching)		
13	Difficulty staying on topic during conversation		
14	Always talking		
15	Cocktail speech - little content		
16	Too loud		
17	Can't remember from one day to the next		
18	Below average IQ (<85)		
19	Poor school performance		
20	Suspended or expelled from school		
21	Poor sleeper		
22	Can't follow routine - needs reminders to get dressed, brush teeth, etc.		
23	Temper tantrums		
24	Extreme mood swings		
25	Requires constant supervision		
26	Been in trouble with the law		
27	Inpatient treatment for mental health or substance abuse, or in jail for a crime		
28	Inappropriate sexual behavior		
29	Poor motor skills		
30	Has or needs glasses		
31	Had foster care or was adopted		
32	Medication for behavior - ever		
33	Mother used alcohol or drugs during pregnancy (OPTIONAL)		

4) Calculate total score.

Total	Total
16	20

(Continue assessment if score is greater than or equal to above)

Figure 1. The 33-item Alcohol Related Neurodevelopmental Behavioral Checklist (ABC).

cases with possible or probable FASD diagnosis, and cases over 18 years of age were excluded. This study utilized a de-identified dataset and was developed under the University of North Dakota Institutional Review Board protocol (IRB 00006034).

Measures

The items used for development of the brief screening tool were from the 33-item ABC with yes or no response options (**Figure 1**) [30]. In this study, item 33 from the ABC was excluded from the analysis, since the data on prenatal alcohol exposure used in the diagnostic evaluation was obtained from a far more detailed exposure assessment.

Exposure Assessment

The development of improved exposure assessments has been an ongoing effort for over 30 years. An update on the ongoing effort and the current methodology for both detection of exposure and assessment of dosimetry is available [30]. As a part of this ongoing effort, the development of an effective screen for alcohol use during pregnancy has included the development of a one question screen, *i.e.*, The-One [30]-[32]. In a similar fashion, the development of the ABC has been underway for nearly 40 years and multiple iterations of the tool have been reported [30]. This includes five population-based studies, which included patients with a diagnosis of FASD [30].

Statistical Analyses

The original 32-item ABC scale was compared using combinations of different items. For the first reduction analysis, items were selected for removal using the change in Cronbach's alpha. An item was removed from the proposed scale if its removal would lead to an increase in alpha, or if the decrease from its removal was less than 0.003, equivalent to a reduction in alpha of less than 0.40%. This value was set primarily to facilitate the description of the reduction of items in the scale. After multiple iterations, a 10-item scale with yes or no response options was finalized and named the FASD MINI, which can be completed in 3 - 4 minutes.

The reliability of the 10-item scale was then compared to that of the 32-item screen using Cronbach's alpha. In addition, the validity of the brief scale was examined by comparing the percent correctly classified (%CC) and the area under the curve (AUC) with 95% confidence intervals (95% CI) obtained from the receiving operating characteristic (ROC) analyses.

Lastly, sensitivity analysis was completed by splitting the data into two groups, *i.e.*, children under 8 years of age and children 8 years or older, and again comparing the full 32-item ABC and the brief 10-item FASD MINI screen**.** To determine a cutoff score for the FASD MINI, a further sensitivity analysis was completed using children from 4 to 18 years of age. Different age groups based on this sample were analyzed with different cutoff scores**.** The analysis was completed with SAS v. 9.4. All references and computer code are available upon request from the corresponding author.

3. Results

The study included a final sample of 355 children, average age 8.52 (SD = 4.28; 47.89% under 8 years of age) and 223 (62.82%) were male. We identified 174 (49.01%) children who had a diagnosis of FASD and had a completed ABC available.

Variable analyses were conducted using Cronbach's alpha values for the full 32-item ABC scale. A receiving operating characteristic (ROC) analysis was also done to test the ability of the shortened scale to predict FASD, using the %CC and AUC. Table 1 shows that the alpha for the full scale was 0.893, with 89.0%CC and AUC of 0.979 (95% CI = 0.969 – 0.990).

The first step in reducing the 32-item scale involved excluding ABC items that, upon removal, would either increase alpha or cause a reduction in alpha by less than 0.003, corresponding to a decrease of less than 0.36%. This led to the exclusion of four items (18, 21, 23, and 30). Subsequently, 10 more items (1, 2, 15, 19, 20, 24, 26, 27, 28, and 29) were removed, leaving 18 items. The resulting Cronbach's alpha was 0.887 (a reduction of 0.006), with 90.7%CC and AUC of 0.981 (a slight increase). From the 32-item checklist, included as Figure 1, our analysis identified 10 items (4, 5, 8, 10, 12, 13, 16, 17, 31, and 32) that comprise the final FASD MINI Screen (Figure 2). The Cronbach's alpha for the 10-item FASD MINI was 0.822, with a %CC of 91.3 and an AUC of 0.971.

The validity of the FASD MINI was assessed by splitting the data into two cohorts: Children under 8 years of age (n = 170) and children 8 years and older (n = 185). Both the 32-item ABC and the shortened 10-item FASD MINI scales were compared between these cohorts. Figure 3 shows the %CC for each scale broken down by age cohorts. For the original 32-item scale, the group of children under age 8 was correctly classified 88% of the time, compared to 97% in the older group ($p < 0.001$). Using the 10-item scale, %CC was 88% for the younger group, and %CC was 91% for the older age group ($p = 0.214$). There was a significant difference within the 8 or older group between the 32-item scale (97%) and the 10-item scale (91%, $p = 0.004$). The optimal FASD MINI score to identify a positive screen was a score of six or more.

Table 1. The item elimination process for development of the 10-item fetal Aclohol spectrum screen (FASD) MINI.

Scale	Items Remaining	Items Removed	Cronbach's Alpha	%CC	AUC	LL	UL
Full	1-32		0.893	89.0	0.979	0.969	0.990
First Reduction	3-14, 16,17, 22, 25, 31, 32	1, 2, 15, 28, 19-21, 23, 24, 26-30	0.887	90.7	0.981	0.971	0.991
The final 10 items	4, 5, 8, 10, 12, 13, 16, 17, 31, 32	1-3, 6, 7, 9, 11, 14, 15, 18-30	0.822	91.3	0.971	0.958	0.984

Note. %CC = percent correctly classified; AUC = area under the curve, LL= lower limit and UL = upper limits of the confidence interval.

Fetal Alcohol Spectrum Screen (FASD MINI)

Name _____ Date _____

Age_____ Date of Birth ____/____/_____

Check all items that are concerns.

Disorganized	
Seems unaware of consequences of actions	
Poor social skills	
Will talk or interact with anyone	
Socially inept (inappropriate speech or touching	
Difficulty staying on topic during conversation	
Too loud	
Can't remember from one day to the next	
Had foster care or was adopted	
Medication for behavior – ever	
Total Score (positive if 6 or more)	

Figure 2. The final Version of the FASD MINI Comprised of 10 Items.

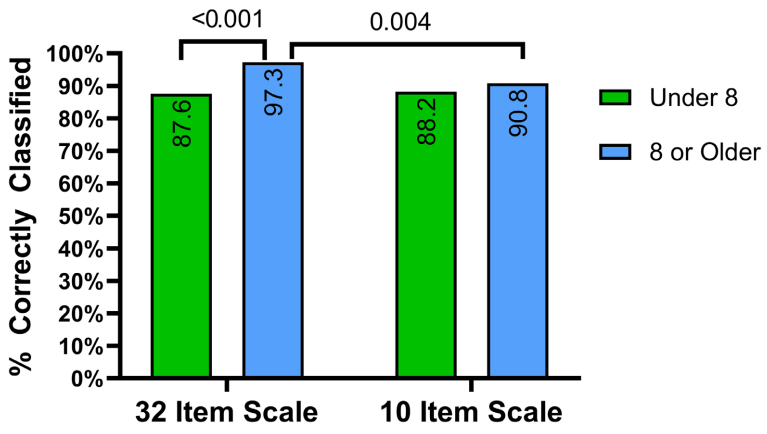

Figure 3. The percentage of the study sample (N = 355) correctly classified by the 32-item ABC and 10-item FASD MINI scale for each of the two age cohorts.

Figure 4 shows the ROC curves for the 32-item ABC and 10-item FASD MINI scales by age cohorts. The full 32-item ABC scale for children aged 8 and older has the highest AUC of 0.997 (95% CI = 0.993 − 1.00). The other three curves demonstrated similar performance, with AUCs of 0.968 for the 32-item ABC scale in younger group (95% CI = 0.947 − 0.989), 0.972 for the 10-item FASD MINI scale in the older group (95% CI = 0.953 − 0.990), and 0.980 for the 10-item FASD MINI scale in the younger group (95% CI = 0.965 − 0.995). The AUC of the 32-item ABC scale in the older group was significantly higher than that of the 32-item ABC scale in the younger group and the 10-item FASD MINI scale in the older group.

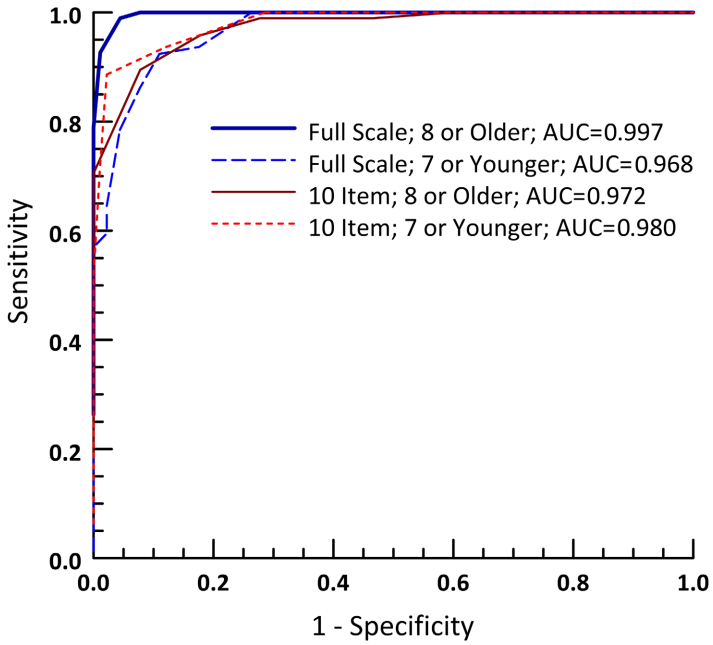

Figure 4. Receiver operating characteristics (ROC) for 32-item ABC and 10-item FASD MINI for the two age cohorts.

The 10 items on the FASD MINI screening tool were not all developmentally appropriate for very young children. In order to examine the most appropriate age range for the screening tool, we reviewed the 10 items and, based on our clinical experience, determined that an age range from four years of age to 18 years of age would be most appropriate. Table 2 presents the results of the sensitivity analysis for these age groups and the age-based cutoff scores for a positive screen on the FASD MINI when compared to the same age groups and the 32-item ABC. A cutoff score of six for all ages can be used and makes interpretation of the screening tool very simple.

4. Discussion

FASD represents a significant public health concern due to its high prevalence, impact across the lifespan, increased risk for multiple comorbid neurodevelopmental disorders, elevated cost of care and increased mortality rates. In current clinical practice, FASD is frequently undiagnosed, which can delay access to early

Table 2. The sensitivity analysis for the 10-item FASD mini by age cohorts.

Age Grouping	n	%CC	AUC	Cutoff
≤4	70	0.929	0.982	6
5 - 7	100	0.940	0.980	6
8 - 11	95	0.895	0.968	7
≥12	90	0.922	0.978	7
≤7	170	0.954	0.979	6
≥8	185	0.908	0.972	7
5 - 11	195	0.908	0.970	6
>4	285	0.909	0.972	6
All	355	0.913	0.971	6

Note. %CC = percent correctly classified; AUC = area under the curve.

intervention. Early diagnosis is also important for the prevention of FASD in younger siblings and has been demonstrated to be associated with substantial cost savings, compared to non-specific FASD prevention programs [33]. Screening for exposure-dependent conditions like FASD or neonatal opioid withdrawal has complex ethical implications which need to be considered. Does the screen have an unacceptable false positive rate and if so, how will this be managed? What are the implications of screening infants and children for maternal health disorders, including substance use/abuse? Can the screen increase the availability of supportive and confidential care for the children, their mothers, and perhaps their siblings? Will the screening increase the risk for contact with child protective services? For many infants and children, this should not be considered a negative consequence.

This study examined the feasibility of a brief screening tool to identify children at increased risk for FASD. The FASD MINI is both brief and has a low administrative burden, while demonstrating acceptable epidemiologic performance characteristics, including acceptable accuracy. The screen can be considered positive for FASD if the score is six or more. The 10-item version was demonstrated to have acceptable epidemiologic performance criteria. The accuracy of the screen, as assessed by the %CC, changed very little compared to the results from the 32-item original screen with the 10-item mini screen. The potential time savings and decreased patient burden from the brief scale would add additional support for the utilization of the FASD MINI. Furthermore, the tool also utilizes items describing easy-to-understand behavioral concerns, which are often part of the concerns expressed by parents and caretakers leading to referral for evaluation. The scoring system is very easy to use, and a single cut-point for a positive screen also simplifies the use of this tool in clinical settings.

Physicians and other health care providers face various challenges when it

comes to implementing comprehensive screening programs. The barriers to implementing screening for FASD includes time constraints, competing priorities, and limited training on identifying and diagnosing FASD [23] [24]. The development of the FASD MINI offers an opportunity to increases low-cost, low-burden screening in a variety of clinical care settings. The form could also be entered into the electronic health record for an automated approach to population-based screening.

Efforts to reduce the underdiagnoses and under-recognition of FASD, should include increasing awareness of the lifelong impact of FASD among healthcare professionals and emphasizing the importance of early identification, which has the potential to improve outcomes for mothers, children with the diagnosis, and their siblings. In addition to improved screening tools, strategies to improve screening methodologies in clinical care settings are also needed.

Although this study offers many benefits and proposes a potential solution to a common problem in clinical care, it also has multiple limitations. The data for this study were obtained from a single site and the subjects included in this study may not adequately represent children from other areas or cultures. A patient's diagnosis in our center may vary compared to diagnostic outcomes from other centers. These limitations should be considered in future studies to examine the feasibility of community-based screening using the FASD MINI. This should include multi-site feasibility testing of the tool in community settings, inclusion of larger and more diverse populations, and consideration of the cost of implementing a screening program. Additionally, longitudinal studies investigating the long-term outcomes of individuals identified with FASD through improved screening and diagnostic practices would provide valuable insights into the effectiveness of interventions and support services.

In conclusion, the underdiagnoses and under-recognition of FASD pose significant challenges to public health. By examining the feasibility of the FASD MINI, a brief 10-item FASD screening tool, this study has underscored the need for increased awareness, improved screening protocols, and enhanced diagnostic capabilities to address the hidden burden of FASD. By implementing these strategies, we can strive to identify and support individuals with FASD, possibly mitigating some of the adverse effects on their lives, the lives of their families and society as a whole.

Ethics Approval and Consent to Participate

This study was approved by The University of North Dakota Institutional Review Board. The protocol number is IRB00006034. The approval included a waiver of informed consent.

Availability of Data and Materials

The data for this study is available from the corresponding author.

Authors' Contributions

TM: Concept development, manuscript preparation; DE: Manuscript preparation and revision; JYK: Manuscript development and revision; MGK: Data analysis and initial draft of the results section; LB: Data acquisition, manuscript preparation, and manuscript revision.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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