Feasibility of Portable Fingerstick Cholinesterase Testing in Adolescents in South Texas

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Abstract

Adolescents are particularly vulnerable to the impacts of pesticide exposures, which can be assessed using surveys, environmental measurements, and biomonitoring. Biomonitoring of blood cholinesterase can be used to determine if an individual has been exposed to pesticides. A limitation of blood cholinesterase testing can be the use of a laboratory as well as time to receive results. In addition to laboratory tests, there are fingerstick cholinesterase (ChE) tests, which can eliminate the need for laboratory testing. Some populations, such as farmworkers, would benefit through fingerstick ChE tests. The objective of this pilot study is to determine the feasibility (eg, can the testing be used to assess ChE levels) of using fingerstick ChE testing in adolescent populations living along the Texas-Mexico border where adolescents who often engage in farm work live. A sub-objective was to explore differences in ChE levels by sex. The Model 400 Test-Mate ChE kit by EQM Research Inc (Cincinnati, OH) was used to assess for ChE inhibition in the participants, specifically acetylcholinesterase (AChE), which is I of the 2 ChE enzymes. During the postassessment, males had a mean AChE value of 3.75 U/mL (95% CI 3.51-3.98); whereas females had a mean AChE value of 2.86 U/mL (95% CI 2.64-3.08), which was statistically significant. Overall, the study supports the use of field ChE testing in adolescent populations with a small percentage (6.90%) refusing to complete ChE testing.

Keywords

cholinesterase testing, adolescents, pesticides

Background

The agriculture industry is one of the most hazardous in the United States.^{1,2} In 2016, the fatal occupational injury rate for those in the crop production industry was 20.9 per 100 000 full-time equivalent (FTE) workers, which is 5.8 times higher than all industries (3.6 per 100 000 FTE workers).² Agricultural hazards include vehicle rollovers or other crashes, heat exposure, falls, musculoskeletal injuries, unsafe work conditions (eg, confined space), and other physical and chemical exposures including pesticides.¹ Despite growth in organic farming, pesticides remain among the most commonly used chemicals in agriculture.³ Pesticides are defined by the US Environmental Protection Agency (EPA) as a substance or mixture intended to prevent, destroy, repel, or mitigate pests, as well as those used as plant regulators, defoliant, desiccants, or nitrogen stabilizers.⁴ Potential health effects due to pesticide exposures include a number of diseases and conditions, including various forms of cancer, as well as Parkinson's disease and other neurological disorders.⁵⁻⁷ Pesticide exposures also are associated with an increased risk of injury possibly due to their neurotoxic health effects.⁸⁻¹¹

According to the Occupational Safety and Health Administration (OSHA), there are more than 2 million youth younger than 20 years working in agriculture annually.¹ These youth may be particularly vulnerable to the impacts of pesticide exposures because they are not fully developed physically or mentally.¹² In the United States, adolescents younger than 18 years have various regulations

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on agriculture work they can perform.¹³ Those aged 12 to 13 years can only work in agriculture in nonhazardous tasks with parent or guardian written permission; whereas, those aged 14 to 15 years do not need written consent to perform nonhazardous tasks.¹³ However, once a youth turn 16 years old he or she can do any job in agriculture.¹³ The Agricultural Worker Protection Standard (WPS) aims to reduce pesticide poisoning and injury among those in the agricultural exposure.¹⁴ Specifically, the WPS restricts work of those younger than 18 years, by stating (1) any handler and early entry workers must be at least 18 years old and (2) employees entering treated areas during restricted-entry interval must be 18 years old.¹⁵ A pesticide handler is anyone who receives compensation that (1) mixes, loads, transfers, or applies pesticides; (2) handles open pesticide containers; (3) acts as a flagger; (4) cleans, handles, adjusts, or repairs mixing, loading, or application equipment; (5) assists with applying pesticides; (6) enters a greenhouse or enclosed area after pesticide application when inhalation exposure level is above WPS standards; (7) performs a task as a crop advisor during application when inhalation or ventilation exposure level is above WPS standards; or (8) disposes of pesticides or containers.¹⁶

Pesticide exposure is often assessed using surveys, environmental measurements, and biomonitoring. Surveys rely on self-reported pesticide exposures and self-reported injuries (eg, years of farm work, farm work in the past 12 months, farm injuries in the past 12 months). A recent systematic literature review found that biomonitoring through urine or blood measurements can provide indirect or direct evidence of pesticide exposures.¹⁷

For example, blood cholinesterase (ChE) testing can be used to assess for pesticide exposures, including organophosphates and carbamates.¹⁸ Cholinesterases are required enzymes that help the human body function by breaking down the neurotransmitter acetylcholine.¹⁸ There are 2 ChE enzymes, namely acetylcholinesterase (AChE) and butrylcholinesterase (BuChE). AChE breaks down acetylcholine, whereas BuChE is nonspecific and breaks down a variety of choline-based esters. Both enzymes can assess for acute organophosphate and carbamate exposures.¹⁹ ChE inhibition prevents the breakdown of acetylcholine.^{18,20} This leads to a buildup of acetylcholine, which results in repetitive nerve firing and symptoms, such as fatigue, dizziness, headache, sweating, nausea, vomiting, diarrhea, staggering gait, and death in extreme cases.^{18,21} This can occur with exposure to certain pesticide classes, including organophosphates and carbamates.^{18,21} Blood ChE testing has been used among farmworkers and other populations to explore cholinesterase depression.²²⁻²⁷ In addition to organophosphate and carbamate pesticides and their derived nerve agents, ChE can be inhibited by Alzheimer's disease drugs (donepezil, rivastigmine, and galantamine) that inhibit amyloid plaque formation.¹⁹ ChE-inhibiting drugs may also be used to alleviate symptoms in Down's syndrome, glaucoma, and myasthenia gravies.²⁸

Fingerstick ChE tests are available which have several benefits, including being minimally invasive (eg, fingerstick sample), relatively quick, and portable to a variety of settings. These tests can be used in nontraditional laboratory settings, including walk-in clinics and schools. Literature also supports field ChE tests, such as the Test-Mate ChE Field Kit, have strong agreement with laboratory tests.^{29,30} A recent study of patients with acute organophosphorus poisoning found the mean difference between the Test-Mate kit and a reference laboratory test was -0.62 U/g hemoglobin.²⁹ In the United States, Higgins et al³⁰ evaluated the Test-Mate kit in migrant farmworkers and their children in Oregon, which found a linear relationship between the Test-Mate kit and the reference Ellman method. Specifically, the results between the Test-Mate and Ellman method were parallel and the Test-Mate typically had 87% of the ChE level measured by the Ellman method.³⁰

A couple studies have been conducted in Egypt and Ecuador that involved fingerstick ChE testing to assess for ChE inhibition in children or adolescents.^{31,32} One study conducted in the United States used the Test-Mate OP Kit and a fingerstick sample to assess for ChE depression in migrant farmworkers in North Carolina.³³ This study found the mean AChE of farmworkers was significantly decreased (30.18 U/g hemoglobin) compared with nonfarmworkers (32.20 U/g hemoglobin; P = .01).³³ However, to our knowledge, there have been no studies using fingerstick tests, such as the Test-Mate ChE test, in adolescent farmworker populations in the United States. The objective of this pilot study was to determine the feasibility (eg, can the testing be used to assess ChE levels) of using fingerstick ChE testing in adolescent populations living along the Texas-Mexico border where adolescents who often engage in farm work live. In addition, a sub-objective of the study was to explore differences in AChE by sex. A recent systematic review found that existing studies on farmworkers have not adequately incorporated sex into study designs and analysis.³⁴ In addition, existing literature on sex differences have had conflicting findings. For example, López-Carillo and López-Cervantes²³ found that females had lower values of cholinesterase compared with males, but the findings were not statistically significant, whereas Jintana et al³⁵ reported higher mean AChE and BuChE for females.

Methods

The research was approved and monitored by the Texas A&M Institutional Review Board (IRB2011-0738D). This study was conducted as a pilot to a 3-year large study

that looked at occupational injury among adolescent migrant farmworkers in South Texas, an often hard to reach population. Data were collected in 2012 during the late spring (premigration season) and late fall (postmigration season). The spring samples were collected before farmworkers migrate for work (eg, baseline); whereas fall samples were collected when farmworkers should be done with migration season. The pilot testing of study procedures consisted of an interviewer-administered survey and a self-administered survey to obtain demographics, work history, injury history, and a health history. The survey items and format were based on our prior studies with this population and items from the National Agricultural Workers Survey (NAWS).36-38 The interviewers for the survey were bilingual and from the study community. In addition, trained clinical staff (a phlebotomist, certified nursing assistants and nurses who recently finished school, but were pending state certification) obtained fingerstick blood samples and completed the cholinesterase test. They also obtained physical measures (eg, weight, height, and foot size) and indicators of gross and fine motor control (eg, grooved pegboard test and postural sway analysis), but analysis of these data are not included in the present pilot assessment of ChE testing. All staff completed human subjects training and demonstrated mastery of data collection protocols.

Since this was a pilot testing of study procedures, we used a convenience sampling approach. Recruitment continued until we reached a sample size of approximately 50 participants. All students enrolled and attending school on campus and who did not have major physical or mental health problems or other limitations were eligible. The student body population was approximately 970 students at the time of recruitment. To ensure inclusion of farmworker students, students enrolled in the Migrant Education Program were recruited before other students. School staff helped to advertise the study and disseminate study information. Written parental consent documents were sent home with students who were asked to return the signed forms. Even if a parent provided consent, the student also had to provide their written assent in order to join the study. Written student assent was obtained before data collection commenced. Data were collected during a non-core class period in a private classroom. Participants were compensated for their time with school supplies (ie, computer stick drive, string backpack) that were printed with their school logo and valued at approximately \$15.00. Participants were compensated even if they did not complete all the study procedures.

The Model 400 Test-Mate ChE kit by EQM Research Inc (Cincinnati, OH) was used to assess for ChE inhibition in the participants through fingerstick blood samples, specifically AChE.³⁹ AChE tests were only done during the pilot project due to available resources. The Test-Mate 3

ChE Test System is based on the Ellman method.³⁹ AChE levels are reported in this article as AChE in units per milliliter (U/mL). At least 2 staff members worked together to collect and process the sample with at least 1 staff member fluent in Spanish and English. All testing was completed in a temperature-controlled setting, specifically a classroom with a consistent temperature. The Model 400 Test-Mate ChE kits recommended operating range is 59°F to 86°F.³⁹

Data were analyzed using Stata 15.1 SE (College Station, TX). Descriptive statistics and unpaired *t* tests were used to examine AChE values of participants.

Results

There were 58 participants in the pilot study. Of these, a total of 54 (93%) completed ChE testing. There were 15 participants in both the pre- and postassessments, 24 in the preassessment only, and 15 in the postassessment only that completed ChE testing. In addition, there were 4 (6.9%) participants that did not complete ChE testing at any assessment. There was also 1 (1.7%) participant who completed ChE testing during the preassessment and who refused the test during the postassessment.

Table 1 provides demographics of the 54 participants who completed ChE testing. A majority of participants in the pre- and postassessment test were males, 51.28% and 56.67%, respectively. In addition, the average age for participants in the pretest was 17 years with a range of 15 to 18 years, while the average age for the posttest was 16 years with a range of 14 to 18 years. All participants selfidentified as Mexican American, Latino, or Hispanic. Table 2 provides AChE values for all participants who completed ChE testing. The mean value during the preassessment was 3.23 U/mL compared with 3.36 U/mL during the postassessment. Next, sex differences were explored through unpaired t test (see Table 3). Unpaired t tests found that males had significantly higher AChE values during the preassessment compared with females, 3.67 U/mL (95% CI 3.37-3.97) and 2.76 U/mL (95% CI 2.48-3.5), respectively. In addition, an unpaired t test found males also had significantly higher AChE values during the postassessment, 3.75 U/mL (95% 3.51-3.98) and 2.86 U/mL (95% CI 2.65-3.08), respectively.

Discussion

The study found that males had a higher mean AChE values compared with females at both pre- and postassessments. This finding adds to the existing literature, which has found conflicting findings in regard to ChE levels between sexes.^{23,34,35}

This study is one of the first in the United States that explored the feasibility of using a fingerstick ChE test kit in

	Prease	ssment	Postassessment		
	Number of Participants	Percent of Participants	Number of Participants	Percent of Participants	
Gender					
Male	20	51.28	17	56.67	
Female	18	46.15	13	43.33	
Not reported	I	2.56	0	0.00	
Age (years)					
14	0	0.00	2	6.67	
15	I	2.56	7	23.33	
16	8	20.51	4	13.33	
17	18	46.15	13	43.33	
18	10	25.64	3	10.00	
Not reported	2	5.13	I	3.33	
Ethnicity					
Mexican American, Latino, or Hispanic	All	100.00	All	100.00	

Table I. Demographics of Participants Who Completed Cholinesterase Testing.

Table 2. Acetylcholinesterase (AChE) Mean Values	for			
Participants Who Completed Cholinesterase Testing.				

	AChE Mean	AChE Median	SD
Preassessment (n = 39)	3.23	3.27	0.75
Postassessment (n = 30)	3.36	3.36	0.60

adolescent to assess AChE levels. The study had a very high completion rate with 93.10% of participants who took part in the survey also completed ChE testing. Overall, the study supports the use of field ChE testing in adolescent populations with a small percentage (6.90%) refusing to complete ChE testing. This finding needs to be validated with future research in a larger adolescent population. Field ChE tests improve on self-reported pesticide exposures measures through providing an option that is portable, minimally invasive, and produces almost immediate results. In addition, field ChE tests do not require lab transport or storage (eg, refrigeration) requirements. These characteristics are vital when conducting field studies assessing pesticide exposures or when needing to determine exposure relatively quick. For example, migrant farmworkers are often in locations for short periods of time based on availability of work; whereas, laboratory tests to determine AChE inhibition or pesticide exposures can take time to receive results which may not be ideal for this itinerant lifestyle. Field ChE tests provide almost immediate results that can be used for pesticide education or in extreme situations removal from exposure. In addition, fingerstick ChE tests can reduce the potential of sampling issues with immediate results. For example, Quandt et al²⁵ collected fingerstick blood samples to assess ChE levels, which were sent to a lab, but the researchers lost 6 participants (a total of 102 observations) due to either laboratory error or blood spots with inadequate saturation.

This pilot study had key limitations that should be considered, including a small sample size. The relatively small sample size may diminish the external validity of the findings. It may also increase the margin of error of the statistical tests and reduce the power to detect associations that truly exist. In addition, the AChE values from the fingerstick ChE test kit were not compared with a gold standard method, such as the Ellman method. Despite these limitations, the findings of this pilot study support field cholinesterase testing are acceptable in adolescent populations along the Texas-Mexico border and that the testing can be completed outside of a laboratory environment. Fingerstick samples may have been acceptable to our study population given the high prevalence of diabetes along the Texas-Mexico border. It is common for individuals living in the study area to monitor blood sugar levels using fingerstick blood samples. Therefore, study participants may view fingerstick blood samples to be a more "normal" part of life compared with youth living in areas with a lower prevalence of diabetes.

Future research should be conducted to validate the findings of this pilot study. In addition, future studies should involve developing protocols to gather AChE levels proximate in time to potential toxic exposures along with the collection of baseline levels during a period free of exposure to AChE inhibitors. Gold standard protocols for AChE testing to monitor for pesticide exposure requires a baseline sample for comparison of samples over time since levels need to be compared across time for the same individual with a nonexposure baseline. This would allow for a better assessment of exposure; for example, a 50% or greater depression of ChE is an indicator of an acute exposure.³⁹

	Number of Males	AChE Mean Males	95% Confidence Interval	Number of Females	AChE Mean Females	95% Confidence Interval	Р
Pre-Assessment	20	3.67	3.37-3.97	18	2.76	2.48-3.05	.0001
Post-Assessment	17	3.75	3.51-3.98	13	2.86	2.65-3.08	.0000

 Table 3. Acetylcholinesterase (AChE) Mean Values for Participants Who Completed Cholinesterase Testing at the Pre- and Postassessment by Sex.

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Declaration of Conflicting Interests

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