Screening for Insecticide Overexposure under Field Conditions: A Reevaluation of the Tintometric Cholinesterase Kit

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A B S T R A C T

A semiquantitative tintometric field kit has been used in the developing world for almost 30 years to measure whole blood cholinesterase levels in persons exposed to organophosphate pesticides. The validity of this screening kit was evaluated among 79 workers heavily exposed to organophosphates by comparison with a reference assay for erythrocyte cholinesterase. Overall correlation between the two methods was good. However, either sensitivity or specificity of the tintometric kit was less than 75% for each of the three tintometric categories commonly used to define the limit of normal. Because baseline erythrocyte cholinesterase levels were not available for this population, the true sensitivity and specificity of the tintometric assay may be even lower. (*Am J Public Health.* 1994;84:479–481)

Introduction

Worldwide, it is estimated that there are approximately 3 million severe poisonings and 220 000 deaths every year due to pesticides.[1,2] Ninety percent of these poisonings and 99% of the deaths occur in developing countries, although these countries account for only 25% of world pesticide consumption.[2]

The measurement of cholinesterase in blood was one of the first biomarkers available as a screening test for a toxic exposure. It is used to identify asymptomatic workers who are overexposed to acutely toxic organophosphate insecticides.[3] In developing countries, an economical, "tintometric" field kit has been available for more than 40 years to measure whole blood cholinesterase activity semiquantitatively in nine intervals (from 0 to 100) as a percentage of the activity of blood from an unexposed control.[4] A previous evaluation of this kit concluded that differences from a standard laboratory assay were "not beyond those which might be anticipated as due to inherent differences in methods."[5] However, the sensitivity and specificity of the kit and its accuracy in identifying workers with mildly depressed erythrocyte cholinesterase were not presented.

In this study we reevaluate the tintometric kit, using as a reference assay a modification of the standard laboratory method (Ellman et al.'s[6]), as adapted for field evaluation of erythrocyte cholinesterase. The diagnostic validity of this assay has been established by laboratory tests and field trials with normal and pesticide-exposed populations.[7,8] In these studies, the assay exhibited a linear response as a function of erythrocyte cholinesterase concentration from 10% to more than 200% of mean normal activity. These measurements were proportional to the response observed on identical codeterminate samples using the method of Ellman et al.[6] ($R > .99$), which is considered to be the standard for cholinesterase assay.[9]

Methods

Seventy-nine male Hispanic workers at rural landing strips and at one large crop-dusting airport in Northern Pacific Nicaragua were examined. These workers' exposures to a wide variety of organophosphate insecticides have been reported elsewhere.[10,11] Each worker was instructed to wash his hands. Then the index finger or thumb was washed with 0.1 normal sodium hydroxide, wiped with 70% ethanol, and allowed to dry. A deep puncture was made with a lancet, and blood was drawn and assayed for whole blood cholinesterase (using the tintometric kit) and for erythrocyte cholinesterase (using the reference assay).

The protocol specified by the manufacturer of the tintometric kit (Tintometer Company, Williamsburg, Va) was followed:[12] the cholinesterase activity of each subject was compared with that of one of two adult male controls, who were unexposed to pesticides. The manufacturer makes the following recommendations for interpretation of the results:

- 100% to 75% of control cholinesterase activity: no action necessary.
- 75% to 50% of control cholinesterase activity: overexposure probable; repeat test. If results are confirmed, suspend subject from further work with organophos-

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TABLE 1—Mean Colorimetric Erythrocyte Cholinesterase,[a] by Tintometric Category: Crop-Dusting Airport Workers

Tintometric Result (Percentage of Control Activity)	No.	Low by Reference Assay		Mean Erythrocyte Cholinesterase (SD)	
		No.	%		
0.0	1	1	100	0.73	(0)
12.5	5	5	100	0.83	(0.28)
25.0	8	8	100	1.5	(0.6)
37.5	4	4	100	2.3	(1.0)
50.0	5	4	80	3.1	(0.4)
62.5	9	6	67	3.4	(0.7)
75.0	18	7	39	3.8	(0.6)
87.5	10	2	20	4.0	(0.4)
100.0	19	1	5	4.6	(0.5)
Within groups total	79			3.4	(0.6)

Note. Spearman R = .85.
[a]Reference assay in international units.

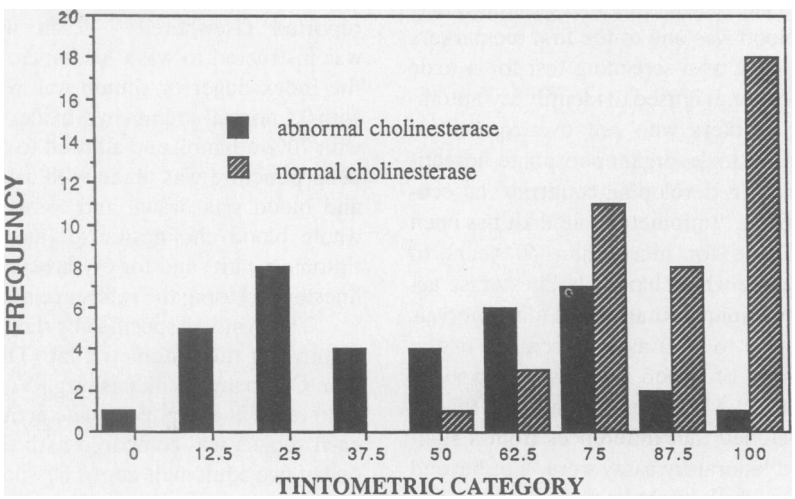

FIGURE 1—Frequency of normal and abnormal cholinesterase results (according to reference assay) in each tintometric category (graphed as percentage of control activity).

phate insecticides for 2 weeks; then retest to assess recovery.

- ≤50% of control cholinesterase activity: serious overexposure; repeat test. If results are confirmed, suspend subject from all work with insecticides. If subject is ill, or if results are less than 24% of control activity, arrange medical examination.

The results of the reference assay for erythrocyte cholinesterase were used for evaluating the tintometric kit. The assay was conducted as previously described:[7] 46 healthy, unexposed male health workers were sampled to establish a lower normal 90% confidence limit of 3.7 IU/minute/mL of blood (mean = 4.6 IU; SD = 0.54).

Results

The number of workers in each tintometric (whole blood cholinesterase) category and the mean erythrocyte cholinesterase activity (reference assay) among workers in each tintometric category are presented in Table 1. In a previous comparison of the tintometric method, a similar pattern of increasing mean erythrocyte cholinesterase was seen as the tintometric categories increased.[11] Thirty-eight of the 79 workers (48%) had erythrocyte cholinesterase activity (reference assay) below the lower normal limit of 3.7 IU. Thirty-two of 79 (41%) had low tintometric cholinesterase activity, using tintometric category 75% as the criterion for abnormal. However, in the critical tintometric categories from 75% to 50% of control activity, there was considerable overlap of workers with normal and abnormal erythrocyte cholinesterase activity, as determined by the reference assay (see Table 1 and Figure 1). In addition, although all workers with tintometric activity of less than 50% also had abnormal activity according to the reference assay, 3 of the 29 workers (10%) with tintometric activity of 87.5% or 100% of control activity had low activity according to the reference assay.

The sensitivity, specificity, and predictive value of the tintometric kit were calculated considering as abnormal a tintometric category of 75% or below (Table 2), as recommended by the manufacturer. In this highly exposed population, predictive value positive was only 70%. When tintometric categories 62.5% and 50% were used as criteria for abnormal and the screening parameters were recalculated, sensitivity declined markedly and specificity improved dramatically. Unfortunately, for any of the three tintometric categories (75%, 62.5%, and 50% of control activity), either sensitivity or specificity was less than 75%, and predictive value positive or predictive value negative was less than 80%.

Discussion

Although the overall correlation between the reference assay and the tintometric tests was good, either sensitivity or specificity was less than 75% for each of the three critical tintometric categories commonly used to define abnormal (from 75% to 50% of the tintometric control). In addition, although there is good agreement between the two methods at tintometric values of less than 50% of control activity, in tintometric categories of 87.5% and 100%, 3 of 29 samples were low by the reference assay. According to the tintometric kit's manufacturers, these samples should all be normal.[12] These results are consistent with our previous observation that the tintometric kit substantially

TABLE 2—Sensitivity, Specificity, and Predictive Value of the Tintometric Kit, with the Use of Different Tintometric Categories to Define Abnormal: Crop-Dusting Airports, Nicaragua (n = 79)

Tintometric Abnormal	True-Positive	False-Positive	True-Negative	False-Negative	Sensitivity, %	Specificity, %	Predictive Value Positive, %	Predictive Value Negative, %
75	38	15	26	3	92	63	70	90
62.5	28	4	37	10	74	90	88	79
50	22	1	40	16	58	98	96	71

overestimated cholinesterase activity during recovery from acute poisoning.[7]

Although there is a disturbing lack of agreement between the tintometric cholinesterase assay and the Ellman assay, the results may actually overestimate the accuracy to that kit. The intraindividual variance of erythrocyte cholinesterase over time is much less than the interindividual variance (used in this study to establish a normal range). Because of the wide range of normal values in different unexposed individuals, it is recommended that a preexposure baseline cholinesterase activity be established for each pesticide-exposed worker for subsequent comparison. Any organophosphate-exposed worker whose erythrocyte cholinesterase falls to 70% of baseline should be removed from exposure.[3,13,14] Unfortunately, the tintometric assay does not have sufficient precision to establish such a baseline reliably. There might have been both more false-positive and more false-negative results if preexposure reference baseline levels had been available for these workers. In addition, it should be noted that this was a heavily exposed population with a very high prevalence of workers with depressed cholinesterase according to the reference standard. It is well known that predictive value depends on prevalence in the sample population.[15] In a population with lower prevalence, predictive value positive would decrease.

Caution should be used in interpreting results of this useful biomarker of organophosphate exposure, especially when screening populations with low prevalence of overexposure, unless appropriate validation and rigorous quality control guarantee accuracy. □

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