

Evaluation of the Arkansas method of urine testing for isoniazid in South Africa

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SUMMARY

SETTING: A South African hospital serving gold mine employees.

OBJECTIVE: To determine the sensitivity and specificity of the Arkansas method for detecting isoniazid (INH) metabolites among South African adults and to examine the effect of smoking status on positive results.

DESIGN: Urine specimens were collected from in-patients taking INH as part of tuberculosis treatment at 6, 12 and 24 h after a directly observed 300 mg oral dose. As a control group, a single urine specimen was collected from surgical in-patients not taking INH. Specimens were tested for INH using a commercially available dipstick.

RESULTS: A total of 153 patients on INH and 60 con-

trols were recruited. The sensitivity of the test was 93.3% (95%CI 88.1–96.8) at 6 h post INH, 93.4% (95%CI 88.2–96.8) at 12 h and 77% (95%CI 69.1–83.7) at 24 h. The specificity of the test was 98.3% (95%CI 91.1–>99.9). There was no association between smoking status and colour change of positive results.

CONCLUSIONS: This test is a useful method of monitoring adherence to TB treatment or preventive therapy among South Africans. However, it is less than 100% sensitive, especially with increasing time post dose, which should be taken into consideration when interpreting results for individual patients.

KEY WORDS: isoniazid; Africa; adherence; tuberculosis

TUBERCULOSIS (TB) control is failing in sub-Saharan Africa, largely driven by the escalating human immunodeficiency virus (HIV) epidemic in this region.^{1–3} Good adherence to TB treatment is essential not only to cure disease but also to prevent drug resistance, as highlighted by the recent outbreak of extensively drug-resistant (XDR) TB with almost 100% mortality in South Africa.⁴ Adherence to isoniazid preventive therapy (IPT) for latent TB infection is notoriously poor, and a significant proportion of individuals, including health care workers, HIV-infected patients and children, fail to complete treatment.^{5–9} Barriers to adherence to IPT amongst HIV-infected patients in South Africa include poverty, fear of stigmatisation, reluctance to take medication when asymptomatic and perception that isoniazid (INH) is unsafe.^{8,10}

Methods of measuring adherence to INH include patient self-report, tablet counting, urine testing for INH metabolites and electronic devices that record when tablet containers are opened. Urine testing is the most objective of these methods. The Arkansas method, which detects the metabolites isonicotinic acid and isonicotinoyl glycine, is widely used among urine tests. It utilises the chemical reaction between cyanogen chloride, barbituric acid and the urinary me-

tabolites, which generates a blue-purple polymethine dye responsible for the colour change indicative of a positive test result.¹¹ It is a simple and rapid test, providing results within 1 min.¹²

The Arkansas method is highly sensitive and specific in European populations, with a reported sensitivity of 96–99% up to 24 h post INH ingestion.^{11,13} Even at 48 h post INH ingestion, a sensitivity of 76% has been reported.¹⁴ The original method uses potentially hazardous reagents and requires laboratory facilities, but impregnated paper testing strips based on the same chemical change are commercially available, although expensive, and provide results within 15–30 min.^{11,12} In-house strips, with similar performance characteristics, can be made at a fraction of the cost.¹⁵

There are no published data on the performance characteristics of the Arkansas method in black African populations, where the prevalence of fast acetylator status appears to be higher than in European populations.^{10,15,16} The aim of the present study was to determine the sensitivity and specificity of the Arkansas method in a South African adult population.

The Arkansas method has been reported in one study as being able to identify with 100% accuracy the urine of smokers (the criteria for defining a smoker

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were not given), in individuals taking INH.¹³ The urine of smokers taking INH turned blue-green, while that of non-smokers taking INH turned deep blue. The colour difference was due to the presence of nicotine and its metabolites in smokers' urine which reacted with the reagents to produce a yellow colour, resulting in the blue-green colour in samples from smokers taking INH.¹³ A secondary aim of this study was therefore to examine colour change by smoking status in TB patients who had a positive urine test using this method.

METHODS

Study design and population

Between September 2005 and July 2006, a cross-sectional study was performed among consecutive eligible patients admitted to South African mining health services. Two groups of patients were recruited: one group (the TB patient group) consisted of hospitalised patients who had been taking 300 mg oral INH for at least 3 days as part of directly observed TB treatment; the other group (the control group) consisted of surgical in-patients who had not taken any INH in the previous month. Patients with diarrhoea or vomiting, or who were too unwell to participate, were excluded from the study.

Study procedures

A standardised questionnaire was administered to all participants to collect demographic data and information about smoking history and medications taken within the last month. Hospital records were reviewed for admission diagnosis and medications. Urine specimens were collected from patients in the TB patient group at 6, 12 and 24 h after the supervised INH dose. A single urine specimen was taken from patients in the control group.

Urine specimens were tested with the TAXO dipstick (Becton Dickinson, Franklin Lakes, NJ, USA), which uses the Arkansas method. All colour changes were recorded, but only blue, purple, green or blue-green coloration of the test strip after incubation for 15–30 min was interpreted as a positive test result as per manufacturer's instructions. A negative result was recorded if there was no change in colour, or if there was a colour change other than one considered positive.

Patients who reported smoking within 48 h of the directly observed INH dose were defined as 'current smokers', and for all positive urine test results in the TB patient group we looked for an association between green/blue-green colour change and current smoker status. Colour changes other than those regarded as positive, such as pink, have been reported as a result of concomitant administration of other drugs, such as aspirin.¹¹ We also looked at medications taken within the last month amongst patients with a

colour change other than those regarded as positive and amongst control patients who tested positive.

Statistical methods

To calculate sensitivity and specificity, we regarded a directly observed dose of INH as the 'gold standard' against which we evaluated the urine test result at 6, 12 and 24 h post dose as a measure of adherence. Based on studies in other populations, we estimated that 90% of urine specimens from patients on TB treatment would be positive for INH 24 h after the dose. To determine sensitivity with a precision of $\pm 5\%$, we estimated a sample size of 138 evaluable individuals in the TB patient group. If the anticipated specificity was 100%, using a one-sided 95% confidence interval (CI), we estimated a sample size of 60 individuals in the control group to determine specificity with a precision of 5%.

Categorical variables were compared using the χ^2 or Fisher's exact test, as appropriate. Continuous variables were compared using the Student's *t*-test for normally distributed variables and the Kruskal Wallis test for non-normally distributed variables.

Ethical approval

The study received ethical approval from the Research Ethics Committee of the University of KwaZulu-Natal and the London School of Hygiene & Tropical Medicine. Written informed consent, or witnessed verbal consent for participants unable to read or write, was obtained for all participants.

RESULTS

Participants

There were 153 participants in total in the TB patient group, of whom 136 gave all three urine specimens at the correct time; the remaining 17 patients gave fewer than three specimens. Sixty eligible patients were recruited to the control group. The demographics of the participants are summarised in Table 1. All participants were black African men. Compared to control patients, and in keeping with the demographics of TB patients, TB patients were older and had a lower body mass index (BMI) (the mean age of the TB patients was 43.7 years vs. 40.3 years in the control group, $P = 0.002$, *t*-test; the mean BMI of the TB patients was 22.3 vs. 23.9 in the control group, $P = 0.003$, *t*-test).

Sensitivity and specificity of the Arkansas method

Table 2 summarises the sensitivity and specificity of this test. Sensitivity was 93.3% (95%CI 88.1–96.8) at 6 h post INH, 93.4% (95%CI 88.2–96.8) at 12 h post INH and 77% (95%CI 69.1–83.7) at 24 h post INH. The test was also highly specific, with a specificity of 98.3% (95%CI 91.1–>99.9). The positive test result (purple colour) in the control group occurred in a patient admitted for a surgical reason, who was a

Table 1 Demographics of participants

Characteristics	Control group (n = 60) n/total (%)	TB patient group (n = 153) n/total (%)	P value
Age, years, mean ± SD	40.27 ± 8.24	43.7 ± 6.87	0.002*
Sex			
Male	60/60 (100)	153/153 (100)	
Country of origin			
South Africa	34/60 (56.7)	71/153 (46.4)	0.002†
Lesotho	13/60 (21.7)	67/153 (43.8)	
Swaziland	8/60 (13.3)	3/153 (2.0)	
Mozambique	3/60 (5.0)	6/153 (3.9)	
Botswana	2/60 (3.3)	5/153 (3.3)	
Zimbabwe	0	1/153 (0.5)	
Ethnic group			
Black/African	60/60 (100)	153/153 (100)	
Smoking history			
Smoked in the previous year	19/60 (31.7)	52/153 (34.0)	
Smoked within 48 h of INH dose		27/153 (17.7)	
Has not smoked in the previous year	41/60 (68.3)	101/153 (66.0)	0.872‡§
BMI, mean ± SD kg/m ²	23.93 ± 4.05	22.33 ± 3.3	0.003†

* t-test.

† χ^2 test.

‡ Fisher's exact test.

§ Compared with those who had smoked in the previous year.

TB = tuberculosis; SD = standard deviation; INH = isoniazid; BMI = body mass index.

current smoker, had smoked on the day of the urine test and had a history of laxative enema, reserpine and tramadol administration within the preceding 1 month.

Table 3 summarises the chronology of results for the 136 patients who gave all three urine specimens. Most patients with a negative result did not have negative results at all three time points, and these were not always biologically consistent (for example, a negative result followed by a positive result at a later time point post dose).

There was no change in the urine test result when the 6-h urine specimens (half of which were positive) of the final 10 TB patients were retested after further 6 h storage at room temperature.

Smoking status and colour change in TB patients with a positive test result

Amongst the 27 current smokers (defined as having smoked within the last 48 h) in the TB patient group,

Table 3 Comparison of urine test results at 6, 12 and 24 h post dose for patients who gave all three urine specimens

6 h	Result		Frequency (n = 136) n (%)
	12h	24 h	
Positive	Positive	Positive	99 (72.8)
Positive	Positive	Negative	19 (14.0)
Positive	Negative	Negative	6 (4.4)
Positive	Negative	Positive	3 (2.2)
Negative	Positive	Positive	2 (1.5)
Negative	Positive	Negative	6 (4.4)
Negative	Negative	Negative	1 (0.7)

no patients had a blue-green colour change, and one patient had a green colour change (at 12 h post dose).

Other colour changes

Any colour change other than blue, purple, green or blue-green was interpreted as a negative test result. In the TB patient group, two 24-h urine specimens that were tested developed a pink colour change. In addition to anti-tuberculosis medication, one of the two patients had taken pyridoxine and the other pyridoxine and multivitamin tablets. In the control group, two patients developed a red colour change. One patient, a current smoker, had taken ibuprofen, glycothymol mouthwash and opioid analgesia. The other patient, a non-smoker, had taken a homeopathic decongestant, ibuprofen, paracetamol, codeine and prednisolone eye drops.

DISCUSSION

This is the first study to describe the performance characteristics of the Arkansas method of urine testing for INH in a black African population. We found this method to be highly specific (98%) and highly sensitive (93%) up to 12 h post INH dose. Our sensitivities up to 12 h post INH dose are similar to findings from studies in Europe.¹⁴ In contrast, the sensitivity of 77% at 24 h post INH dose in our population is considerably lower than these studies.^{11,13,14} The main metabolic route determining the rate of INH elimination from the body is acetylation by the enzyme N-acetyl transferase, which is genetically determined. The pharmacokinetics of INH varies between popula-

Table 2 Sensitivity and specificity

	TB patient group (taking INH)			Control group (not taking INH) (n = 60)
	6 h (n = 150)	12 h (n = 151)	24 h (n = 139)	
Number positive	140	141	107	1
Sensitivity, % (95%CI)	93.3 (88.1–96.8)	93.4 (88.2–96.8)	77.0 (69.1–83.7)	
Number negative	10	10	32	59
Specificity, % (95%CI)				98.3 (91.1–>99.9)

Note: Two patients gave only 6-h urine specimens, three patients gave only 12 and 24-h urine specimens; 12 of the 24-h urine specimens were excluded due to logistical errors. In total, 136 patients gave all 3 urine specimens. TB = tuberculosis; INH = isoniazid; CI = confidence interval.

tions due to differences in the prevalence of fast acetylase status, ranging from 41% to 48% in European populations, 58% to 73% amongst black Africans in South Africa and 79% to 95% in Eskimo and Japanese populations.^{16–20} Although we did not assess acetylase status, the lower sensitivity observed at 24 h could be due to a higher prevalence of fast acetylase status in our study population, as reported in previous studies in black Africans in South Africa.^{16–20} Only one previous South African study has evaluated urine testing for INH metabolites, but this used a less sensitive urine test that detects N-acetyl INH. At 8 and 24 h post 400 mg INH dose, this test had sensitivities of 80% and 0%, respectively, which was also lower than in European populations. This discrepancy was also thought to be due to a higher proportion of fast acetylators in the African population, and is consistent with our findings.²¹ Our study supports the superiority of the Arkansas method for monitoring adherence and demonstrates that it is a useful method for monitoring adherence in our study population up to 12 h post INH dose. However, it is important to note that a negative test result does not necessarily indicate non-compliance. In a few patients, we found that the test results were biologically inconsistent (for example, being negative at 6 h post dose but positive at 12 h post dose), which could reflect variability of the amounts of INH metabolites in the urine or inaccuracies inherent in the test.

HIV prevalence in adult TB cases in South Africa is 60%; it is thus highly probable that the majority of the TB patient group were HIV co-infected.¹ HIV infection per se does not appear to affect INH pharmacokinetics, but there is conflicting evidence around the impact of HIV infection with concurrent diarrhoeal disease on INH pharmacokinetics.^{22–25} As all patients with a history of diarrhoea or vomiting in the previous 48 h were excluded from our study, our results would not have been affected by diarrhoeal disease.

In a small subgroup, storage of specimens at room temperature for 6 h had no effect on the test result. In a very busy clinic setting with limited staffing, this may enable bulk testing of urine specimens and thus ease the workload.

We were unable to identify smokers amongst TB patients by the urine test colour changes, consistent with findings by Elizaga et al.¹⁴ Schraufnagel's study was conducted in the United States and did not report the ethnicity of the participants.¹³ It is possible that the ability of the Arkansas method to identify the urine of smokers taking INH may also differ according to ethnicity and to how soon urine testing is performed after smoking.¹³

Limitations

Our study population were entirely African male, which is in keeping with the demographics of the gold mining industry in South Africa. We cannot therefore be certain that the results of this study are generalisable to women. We are also unable to comment on

sensitivity and specificities more than 24 h since last INH dose.

CONCLUSIONS

This is the first study to describe the performance characteristics of the Arkansas method in a black African population. It performs well in this population and is highly sensitive and specific. Sensitivity does, however, fall with time; in particular, we found sensitivity 24 h post INH dose to be lower than in other studies, which may be explained by a higher frequency of fast acetylase status in our study population. Although an excellent and rapid test, its less than perfect sensitivity, especially with increasing time from INH dose, should therefore be taken into consideration when interpreting results for individual patients.

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RÉSUMÉ

CONTEXTE : Un hôpital d'Afrique du Sud desservant les agents d'une mine d'or.

OBJECTIF : Déterminer la sensibilité et la spécificité de la méthode Arkansas de détection des métabolites de l'isoniazide (INH) chez les adultes d'Afrique du Sud et examiner l'effet du statut tabagique sur la positivité des résultats.

SCHÉMA : On a prélevé des échantillons d'urine provenant de patients hospitalisés prenant de l'INH comme élément d'un traitement antituberculeux, respectivement 6, 12 et 24 h après une dose orale directement observée de 300 mg. Comme groupe contrôle, on a prélevé un échantillon unique d'urine chez des patients hospitalisés en chirurgie et ne prenant pas d'INH. Les échantillons ont été testés pour l'INH en utilisant un système de tigettes disponible dans le commerce.

RÉSULTATS : On a recruté 153 patients sous INH et 60 contrôles. La sensibilité du test a été de 93,3% à 6 h après l'INH (IC95% 88,1–96,8), de 93,4% à 12 h (IC95% 88,2–96,8) et de 77,0% à 24 h (IC95% 69,1–83,7). La spécificité du test a été de 98,3% (IC95% 91,1–>99,9). On n'a pas noté d'association entre le statut tabagique et une modification de couleur en cas de résultats positifs.

CONCLUSIONS : Ce test est une méthode utile pour suivre l'adhésion au traitement de la tuberculose ou au traitement préventif chez les sujets d'Afrique du Sud. Toutefois, sa sensibilité est inférieure à 100%, particulièrement lorsque la durée après la prise a augmenté, ce qui doit être pris en considération pour l'interprétation des résultats dans les cas individuels.

RESUMEN

MARCO DE REFERENCIA : Hospital de atención de empleados de las minas de oro en Sudáfrica.

OBJETIVO : Determinar la sensibilidad y la especificidad del método Arkansas de detección de metabolitos de isoniazida (INH) en adultos en Sudáfrica y examinar el efecto del tabaquismo sobre los resultados positivos.

MÉTODOS : Se recogieron muestras de orina de pacientes que recibían INH como parte del tratamiento antituberculoso 6, 12 y 24 h después de una dosis oral de 300 mg directamente observada. En el grupo testigo, se recogió una muestra única de orina de pacientes quirúrgicos hospitalizados que no recibían INH. La prueba de la INH se llevó a cabo con una tira reactiva obtenida en el comercio.

RESULTADOS : Se incluyeron 153 pacientes tratados con

INH y 60 testigos. La sensibilidad de la prueba 6 h después de la toma de INH fue 93,3% (IC95% 88,1–96,8); 12 h después fue 93,4% (IC95% 88,2–96,8); y 24 h después 77% (IC95% 69,1–83,7). La especificidad de la prueba fue 98,3% (IC95% 91,1–>99,9). No se puso en evidencia ninguna asociación entre tabaquismo y modificación del color en los resultados positivos.

CONCLUSIONES : Esta prueba representa un método útil de vigilancia de la observancia del tratamiento antituberculoso o del tratamiento preventivo en pacientes sudafricanos. Sin embargo, su sensibilidad es menor del 100% en particular cuando se aumenta el lapso después de la dosis, lo cual se debe tener en cuenta al interpretar resultados de pacientes individuales.