Monitoring Regularity of Drug Intake in Tuberculous Patients by Means of Simple Urine Tests

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SUMMARY

A study was undertaken to evaluate simple, reliable urine spot tests suitable for detection of the major anti-tuberculosis (TB) drugs rifampicin, isoniazid and pyrazinamide. The discrepancy between the actual ingestion of anti-TB tablets and prescribed medication was investigated in 234 hospitalized male and female patients as well as in 85 male and female outpatients with pulmonary tuberculosis. Several factors implicated in patient non-compliance are discussed, namely the degree of supervision, the independent administration of rifampicin before the other TB medication, and patient preference for certain TB medicines because of tablet size.

The efficacy of treatment in pulmonary tuberculosis (TB) depends on adequate chemotherapy and patient compliance for an extended period of time. Drug defaulting, especially by asymptomatic TB patients who need to take drugs for periods of 6 months - 1 year, is a serious problem. Irregular administration of anti-TB medication may result in the emergence of drug resistance in strains of Mycobacterium tuberculosis and/or patient relapse, previously administered drugs then have to be replaced by regimens which are more toxic and more expensive.

The three procedures which have mainly been used in attempts to control the drug intake of TB patients are direct supervision by nurses, the counting of dosage units, and urine testing. Of these only urine testing provides a reliable criterion of actual drug ingestion.

A number of urine tests have been developed for the detection of primary anti-TB drugs such as isoniazid (INH), rifampicin (RMP), ethambutol and streptomycin, as well as for second-line drugs such as pyrazinamide (PZA), ethionamide and cycloserine.

This study was undertaken to evaluate simple urine tests both suitable for the detection of the major anti-TB drugs and easy to perform even by paramedical teams. A further aim was to determine possible discrepancy between the medication prescribed and that actually taken by the patient.

Three simple, specific, reliable and inexpensive tests for demonstrating the presence of INH, RMP and PZA or their metabolites in the urine of tuberculous patients were used. The tests take little time to perform and require small amounts of urine. Standard laboratory glassware such as Pasteur pipettes and spot plates is used.

METHODS

Spot plate tests employing 3 - 4 drops of urine were used for the detection of INH and PZA. For the detection of RMP small aliquots (2 - 10 ml) of urine were extracted with water-insoluble butanol in test tubes. The detailed procedures were as follows.

Colour Test for N-Acetyl Isoniazid in Urine

The main metabolite of INH is N-acetyl isoniazid, which even in fast acetylators is present in the urine in larger quantities and for a longer period of time than any other INH metabolite.

The test is done in a white porcelain spot plate with hemispherical depressions which enables easy reading of results and examination of several specimens at the same time.

Reagents. Two solutions are made up and are stored separately: (i) a 10% solution of potassium cyanide (KCN), reagent grade, in distilled water; and (ii) a 10% solution of chloramine-T in distilled water. The reagents may be stored in the refrigerator at 4°C in amber bottles for 1 week.

Procedure. In each plate depression the following are added in strict sequence by Pasteur pipette and without shaking or stirring: (i) 4 drops urine; (ii) 4 drops 10% KCN solution; and (iii) 9 drops 10% chloramine-T solution. It is very important that the specified number of drops is used, that the sequence is strictly adhered to, and that there is no delay between additions of each successive reagent. Specimens should be tested in groups of 10 - 20 at a time so that the addition of the reagents is not delayed and there is no evaporation of urine or reagent — the method is so quick and easy that 300 urine specimens can be collected and processed in a day. Known INH-positive and -negative control urine is included in each test series to monitor the quality of the reagents.

A positive reaction is indicated by a cherry-red colour of varying intensity, which develops within 1 minute. A pink colour indicates that only traces of N-acetyl isoniazid are present, and negative urine remains yellow.

The estimated cost is 14 cents per 100 urine spot tests.
Colour Test for PZA and its Metabolites

The major metabolites of PZA are pyrazinoic acid and 5-hydroxy-pyrazinoic acid.

Reagents. Two solutions are made up and are stored separately: (i) a 2% aqueous solution of sodium nitroprusside; and (ii) a 2N solution of sodium hydrosulphide (NaOH 8.0 g/dl distilled water). The sodium nitroprusside reagent is stored in an amber bottle and keeps for 2 weeks in the refrigerator at 4°C.

Procedure. Before performing the urine tests, equal small aliquots (e.g. 2 ml) of each reagent are freshly mixed in a test tube, since the resultant final solution is only stable for 1 hour, after which it must be discarded. A fresh reagent solution must then be constituted if many specimens are to be tested.

In a white porcelain spot plate the following are added by pasteur pipette: (i) 3 drops urine; and (ii) 1 drop constituted reagent. The plate is gently shaken. In the presence of pyrazinamide the bright yellow colour of the solution changes to orange within 3 minutes. Known PZA-positive and -negative control urine is included in each test series to monitor the quality of the reagents.

The estimated cost is approximately 1 cent per 100 urine spot tests.

Colour Test for RMP and Desacetylrifampicin

RMP and its metabolite desacyl rifampicin are detected in urine by means of the method recommended by Dow Chemical Africa (Pty) Ltd (personal communication). A high concentration of RMP is indicated by the red insoluble n-butanol is used to detect RMP.

Procedure. A 10 ml urine sample is pipetted into a test tube and 2 ml n-butanol (reagent grade) is added. The liquids are mixed by inverting the tube gently twice. The tube is allowed to stand for at least 30 seconds to let the butanol separate again.

In positive urine a salmon-pink to cherry-red colour appears in the upper butanol layer. A light orange colour indicates the presence of only a trace of the drug and its metabolites. A false-positive result can occur if the urine sample contains an excess of urobilinogen.

The estimated cost is 68 cents per 100 urine spot tests.

Urine specimens can be stored in the refrigerator for as long as 24 hours before performance of all three tests if necessary.

MATERIAL

The urine specimens examined fell into two groups. In one group, timed urine specimens were collected from the same patient at different times over a 24-hour period to evaluate the reliability of the three tests. In the other group the regularity of drug intake was determined by one spontaneous urine specimen taken during surprise visits to two hospitals and two clinics.

Neither the staff of the hospitals or clinics nor the patients were informed of the purpose of the urine collection. Negative control urine specimens were obtained from healthy non-hospitalized subjects on each test day and were tested for RMP, INH and PZA. A healthy volunteer group ingested INH, PZA and RMP tablets in order to provide positive control specimens.

Timed Urine Specimens

Urine specimens were obtained from 53 hospitalized male patients with pulmonary TB, 2, 4, 6, 8 and 24 hours after strictly supervised drug intake. Regardless of the actual drug ingestion, urine spot tests for detecting INH, PZA and RMP were performed on all urine samples obtained. In addition, after the spot test the early-morning urine specimen from each patient was examined by means of indicator patch test strips (Combur-7 test; Boehringer Mannheim) to determine the pH and the protein, glucose, ketone, urobilinogen, bilirubin and haemoglobin concentrations.

The anti-TB regimens prescribed for these patients contained RMP (450 mg/d), INH (400 mg/d) and PZA (2 g/d), as well as streptomycin, ethambutol and ethionamide in varying combinations. RMP was administered before breakfast and approximately 2 hours before the remaining anti-TB drugs.

Spontaneous Urine Specimens

Hospitals. One spontaneous urine specimen was collected from each of 234 hospitalized male and female patients during late morning and early afternoon surprise visits on 3 different days. In hospital A, where there were several full-time doctors, these patients represented 37% of the 416 admitted TB patients. In hospital B, where there was one part-time doctor, these patients represented 71% of the 112 admitted TB patients.

The drug regimen prescribed contained RMP (450 mg/d), INH (400 mg/d), PZA (2 g/d), streptomycin and ethambutol in varying combinations. The drug intake in both hospitals was in theory supervised.

Spot tests for INH, PZA and RMP were carried out on each urine sample, and in addition the urine was examined with the Combur-7 test strips as described above.

Clinics. One spontaneous urine specimen was collected from each of 85 male and female pulmonary TB patients during the course of 3 mornings. These patients attended the TB clinics for: (i) supervised drug intake when a regimen containing RMP had been prescribed (24 patients); (ii) streptomycin injections and supervised drug intake (33 patients); and (iii) collection of their monthly anti-TB drug supply (28 patients).

RESULTS

The negative control urine specimens from healthy, non-hospitalized subjects not taking TB medication were all negative for the three anti-TB drugs tested. No false-positive or false-negative reaction for the detection of RMP, INH or PZA in urine could be verified.
Simultaneous administration of streptomycin, ethionamide, ethambutol or multivitamins did not affect the specificity of any of the three urine spot tests performed.

Timed Urine Specimens

The colour intensity serves as indicator for the presence of the respective drug metabolites, and fades with decreasing metabolite concentration. The intensity of the colour of the urine specimens taken 2, 4, 6, 8 and 24 hours after drug intake in each spot test is shown in Table I and as a histogram in Fig. 1.

**Fig. 1. Effect of time interval after intake of the drugs RMP, INH and PZA on demonstration of positive urine spot tests (information in Table I presented as a histogram).**

**TABLE I. EFFECT OF THE TIME FACTOR ON COLOUR INTENSITY IN URINE SPOT TESTS AFTER ORAL ADMINISTRATION OF RMP, INH AND PZA**

<table>
<thead>
<tr>
<th>Drug</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
<th>8 h</th>
<th>24 h</th>
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</thead>
<tbody>
<tr>
<td>RMP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Light</td>
<td>4</td>
<td>24</td>
<td>4</td>
<td>25</td>
<td>5</td>
</tr>
<tr>
<td>Dark</td>
<td>13</td>
<td>76</td>
<td>12</td>
<td>75</td>
<td>11</td>
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<tr>
<td>No colour</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>100</td>
<td>16</td>
<td>100</td>
<td>16</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>INH</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Light</td>
<td>22</td>
<td>48</td>
<td>2</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Dark</td>
<td>22</td>
<td>48</td>
<td>48</td>
<td>96</td>
<td>46</td>
</tr>
<tr>
<td>No colour</td>
<td>2</td>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>46</td>
<td>100</td>
<td>50</td>
<td>100</td>
<td>51</td>
</tr>
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</table>

<table>
<thead>
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<th>PZA</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Light</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>Light</td>
<td>23</td>
<td>58</td>
<td>14</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>Dark</td>
<td>17</td>
<td>42</td>
<td>30</td>
<td>68</td>
<td>33</td>
</tr>
<tr>
<td>No colour</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>100</td>
<td>44</td>
<td>100</td>
<td>45</td>
</tr>
</tbody>
</table>

RMP. Two hours after patients had ingested 450 mg RMP all the urine specimens were positive, and they remained positive for a further 4 hours. After a 24-hour period RMP or its metabolites could be detected in only 25% of the samples. These findings are in accordance with previous studies.

INH. After a dosage of 400 mg INH its N-acetyl-INH metabolite could be detected after 2 hours in 96% of the urine specimens tested, after 4 - 6 hours in 100% and after 8 hours in 80%. However, after 24 hours none of the urine specimens was positive. Eidus and Hamilton reported that after the administration of 300 mg INH the acetylated derivative could be detected in urine for the next 12 hours and that traces could be found in overnight samples. This discrepancy may be due to a high percentage of rapid inactivators in Black patients.

PZA. After the intake of 2 g PZA all the urine specimens tested were positive for a period of 2-8 hours, and in 68% of the samples the overnight urine specimens were still positive. This corresponds with the time interval mentioned by Eidus and Ling.

Simultaneous administration of RMP and PZA (with a resultant reddish colourization of the urine due to RMP) did not interfere with the interpretation of a positive PZA reading. It should be noted that it is not always easy to distinguish between the canary-yellow
TABLE II. DISCREPANCY BETWEEN PRESCRIBED MEDICATION AND PROVED DRUG INTAKE DETERMINED BY URINE SPOT CHECKS IN HOSPITALIZED PATIENTS AND OUTPATIENTS WITH TB

<table>
<thead>
<tr>
<th>Prescribed medication</th>
<th>Patients tested</th>
<th>Total No. of patients</th>
<th>RMP</th>
<th>INH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMP (450 mg/d) + INH (400 mg/d) + other drugs</td>
<td>Hospital A*</td>
<td>57</td>
<td>849</td>
<td>88</td>
</tr>
<tr>
<td>Hospital Bt</td>
<td>61</td>
<td>21</td>
<td>34</td>
<td>40</td>
</tr>
<tr>
<td>Outpatients</td>
<td>24</td>
<td>13</td>
<td>54</td>
<td>11</td>
</tr>
<tr>
<td>INH (400 mg/d) + drugs other than RMP</td>
<td>Hospital A</td>
<td>87</td>
<td>86</td>
<td>99</td>
</tr>
<tr>
<td>Hospital B</td>
<td>18</td>
<td>12</td>
<td>67</td>
<td>6</td>
</tr>
<tr>
<td>Outpatients</td>
<td>33</td>
<td>29</td>
<td>88</td>
<td>4</td>
</tr>
<tr>
<td>INH (400 mg/d)</td>
<td>Outpatients</td>
<td>28</td>
<td>28</td>
<td>100</td>
</tr>
<tr>
<td>RMP (450 mg/d) + drugs other than INH</td>
<td>Hospital A</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neither RMP nor INH</td>
<td>Hospital A</td>
<td>8</td>
<td>7</td>
<td>87</td>
</tr>
</tbody>
</table>

* Full-time medical staff.
† 1 part-time doctor.

colour of the negative control urine and the brick-orange of a weakly positive specimen.

**Spontaneous Urine Specimens**

The discrepancy between medication prescribed and proved intake of RMP and INH is demonstrated in Table II.

**RMP.** The urine spot test for RMP intake showed that 14% of 57 patients in hospital A reported to be on RMP, INH and other drugs did not in fact take RMP. On the other hand, in a group of 87 patients on a non-RMP-containing regimen, 1 patient (1%) was shown to have taken RMP. Out of 8 patients not supposed to be on RMP or INH medication 1 patient (13%) was in fact shown to have ingested RMP.

In hospital B, where there was one part-time doctor in charge, 34% of 61 patients supposed to be on RMP, INH and other drugs did not take RMP, while 33% of 18 patients on a non-RMP regimen did take RMP.

In outpatients who were supervised daily the discrepancy between prescribed RMP medication and actual drug intake was considerably higher — 54% of 24 patients did not take their RMP. In contrast, 12% of 33 patients received RMP without prescription.

**INH.** In hospital A the urine spot test for INH revealed that 96-98% of patients on an INH-containing anti-TB regimen took the prescribed INH. The urine samples of all 8 patients receiving neither RMP nor INH were negative for INH.

In hospital B the proven INH intake in patients to whom it had been prescribed varied between 78% and 84%.

In supervised outpatients the compliance rate for INH was markedly lower than in the hospitalized group, namely 61 - 75%.

In 28 outpatients attending the clinics for collection of their monthly drug supply only 54% of the urine specimens were positive for INH and no discrepant RMP results were found.

**PZA.** The urine spot test for PZA was performed only in patients in hospital A — the results are omitted from Table II because of the wide variety of PZA-containing anti-TB drug regimens.

Ninety-five per cent of urine specimens were positive for PZA in 122 patients who were supposed to be taking PZA, and 3% were positive in 33 patients who were not.

Routine diagnostic urine tests with Combur-7 strips indicated the presence of nitrate (2 patients), protein (8 patients), glucose (6 patients) and haemoglobin (12 patients), but these substances did not interfere with the specificity of the three spot tests. Eight patients with urobilinogen in their urine and those who were menstruating were excluded from this study.

**DISCUSSION**

Numerous publications point to the importance of patient compliance in the successful treatment of various diseases. Buchanan et al. recently discussed factors influencing compliance in ambulatory Black urban patients — non-compliance in 50 diabetic and 50 hypertensive adult patients was reported to be 60% in each group. In a study in India, Banerji found a high default rate of 50-60% in TB patients.

In accordance with that of Buchanan et al., our study indicated that the main factors which influence the compliance of Black TB patients are inadequate supervision and health education and complexity of therapeutic regimens. The most obvious fact that emerges from our study is that the degree of medical supervision is important. In a hospital with full-time doctors the overall compliance ranges from 86% to 100% (omitting the group of 3 patients on RMP and anti-TB drugs other than INH). However, in a hospital with one part-time doctor the percentage of patients who took the drugs prescribed is remarkably lower, the compliance ranging from 66% to 84%. The lowest percentage of precise drug
intake was found in patients attending clinics. Compliance in outpatients in whom drug intake was supposed to be strictly supervised ranged from 61% to 75%, and in patients attending clinics to collect their monthly INH supply the figure was only 54%.

The different compliance patterns (especially in hospitals) which were found for RMP, INH and PZA may be due to the administration of RMP in the early morning and independently of the other anti-TB drugs.

In hospital wards where the anti-TB drug regimens were more standardized and where the patient’s name and directions for prescribed daily drug intake were attached to each bed the compliance rates were higher.

Seventy-five patients who participated in this study were interviewed by means of a standardized questionnaire to identify common characteristics, especially regarding age, extent of information about the disease, and pill preference. The average age of the patients was 40 years. An average of 42% stated that they had been informed of the reason for their hospitalization or outpatient treatment, while one-third replied that they did not fully understand the reason. Only a minority, viz. 3%, admitted their dislike for certain drugs; in all cases the large size of certain tablets was given as the reason. These findings also indicate that the health education of all patients needs to be improved to a considerable extent.

Patient compliance may be improved by means of surveillance monitoring with the simple, specific and inexpensive urine spot tests described in this study.

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REFERENCES


Boeke Ontvang: Books Received


