

A COMPARATIVE STUDY OF THE HAEMAGGLUTINATION INHIBITION (HI) TEST AND SPOT TEST FOR DETECTION OF DAPSONE IN URINE

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ABSTRACT : *A comparative study of the HI test and paper spot test for screening dapsone in urine was carried out in 692 specimens of urine, 149 from the field and 502 from patients attending the C.L.T. and R.I. clinic or admitted in the wards and 41 control urines. A very good correlation was noticed between the 2 tests in relation to positivity or negativity as well as DDS concentration. Fifty one urine specimens showed a specific agglutination, 41 of them were considered HI positive and 10 of them HI negative based on DDS concentration. The results are discussed.*

INTRODUCTION

The application of paper spot test for screening dapsone in urine and its value in field situations have been well documented (1-5). A sensitive Haemagglutination Inhibition (HI) test employing SRBC coated with DDS and anti-DDS Ig was developed by Huikeshoven *et al* (6). A pilot study to assess the feasibility of this test under field conditions was carried out by De Wit *et al* (7). The present report summarises the findings on a comparison of the two tests—HI test and paper spot test—in urine specimens from leprosy patients receiving DDS as monotherapy or as a component of multidrug regimen.

MATERIALS AND METHODS

Urine samples

A total of 692 urine samples were employed in this study, comprising of 149 samples collected from leprosy patients from the field, 502 samples from the ward of this Institute and those attending in the C.L.T. and R.I. clinic and the remaining 41 samples from healthy individuals among C.L.T. and R.I. staff (not receiving DDS) as control specimens. Of the 149 samples collected from the field, 108 were from patients receiving 100 mg, 29 from those receiving 50 mg and 12 from those on 25 mg of DDS (dapsone) as monotherapy. The 502 urine samples collected from the

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ward and from the patients attending the C.L.T. and R.I. clinic were those maintained on 100 mg DDS daily either as monotherapy or as part of multidrug regimens. The control urine specimens were collected from healthy individuals after making sure that they had not taken any drug during the previous 7 days. All the urine samples were kept in refrigerator (at 4°C) until the investigations were carried out.

Assay Methods

The Haemagglutination Inhibition test was performed by the method of Huikeshoven *et al* (6). The paper spot test was carried out according to the procedure of Balakrishnan (1). The sensitivity of the test was increased by applying 0.1 M citric acid as described by Ellard *et al* (8). The DDS as well as the DDS/Creatinine in urine were determined by the method of Ellard *et al* (8).

RESULTS

The results of the comparative assessment of the spot test and HI test carried out in both field and ward urine specimens are shown in the following tables.

Table 1 summarises the DDS levels, DDS/CR ratio and percentage positivity by the HI test and spot test in 149 urine specimens collected from the field.

TABLE 1

RESULTS		DDS CONCENTRATION μG/MG AM ± SD	DDS/CREATININE RATIO μG/MG CR AM ± SD
HI Test + ve	111 (74.5%)	47.57 ± 37.45	70.33 ± 47.71
HI Test - ve	38 (25.5%)	7.61 ± 3.74	15.75 ± 11.85
Spot Test + ve	104 (69.8%)	49.17 ± 34.35	73.54 ± 46.07
Spot Test - ve	45 (30.2%)	9.39 ± 5.51	15.76 ± 10.74

In Table 2 are summarised the DDS levels, DDS/CR ratio and percentage positivity by the HI test and spot test in 502 urine specimens collected from patients from the ward of this Institute and those attending the CLTRI clinic.

TABLE 2

RESULTS		DDS CONCENTRATION $\mu\text{G/ML}$ AM \pm SD	DDS/CREATININE RATIO $\mu\text{G/MG CR}$ AM \pm SD
HI Test + ve	488 (89.24%)	55.44 \pm 33.50	70.98 \pm 30.76
HI Test — ve	54 (10.76%)	7.76 \pm 3.66	13.04 \pm 8.58
Spot Test + ve	429 (85.46%)	56.41 \pm 31.47	72.89 \pm 30.16
Spot Test — ve	73 (14.54%)	9.07 \pm 4.64	20.26 \pm 21.12

The results of the two tests carried out in all the 651 urine specimens collected from those who had taken DDS are recorded in Table 3.

TABLE 3

	POSITIVE	%	NEGATIVE	%
HI Test	559	85.37	92	14.13
Spot Test	533	81.87	118	18.13

DISCUSSION

The above findings show a close correlation between the positive/negative tests by the two methods. However, the mean DDS concentration of the negative urine specimens by the HI test was lower when compared to the spot test. This is understandable because the HI test is certainly much more sensitive (about 12 times) than the spot test. It may be mentioned here that the spot test is carried out on neat urine specimens, while the HI test is performed with specimens diluted 1 : 10.

The arithmetical means of the DDS concentration in spot test negative urine specimens from both field and ward were found to be very close to each other (9.39 and 9.07 respectively). Similarly, a close correlation was also noticed between the DDS concentrations of the HI negative urine specimens from the field as well as from the ward (7.61 and 7.76 respectively). A very good correlation was observed in the arithmetical means of DDS concentration and DDS/CR ratio of HI and spot test positive from both field and ward urine specimens.

Another interesting observation is that the percentage negativity by both tests for field urine specimens were higher than those from the ward, as may be evident from Tables 1 and 2. The ward specimens were mostly from THELEP patients who had completed their multidrug regimen and were presently on DDS monotherapy. Presumably they were more regular in their drug intake compared to those receiving dapsone from field clinics.

The observation on the 41 specimens obtained from controls indicate a 100% negativity by both the screening tests with a mean DDS concentration of $3.56 \pm 2.87 \mu\text{g/ml}$ and mean DDS/CR ratio $5.30 \pm 3.28 \mu\text{g/ml CR}$ respectively. These are in agreement with the findings of earlier workers (9 — 11)

Aspecific Haemagglutination

During the study, 51 out of 692 urine specimens showed aspecific or nonspecific haemagglutination. Of these, 43 were from the ward, 5 from the field and 3 from the control urine specimens (collected from healthy individuals). The aspecific haemagglutination was demonstrated by taking a control well where the anti-DDS Ig was replaced by an equal amount of phosphate buffered saline (PBS). Of the 51 specimens with aspecific agglutination, 41 were found to give true HI positive reaction and the remaining 10 were HI negative. For this purpose, urine specimens having a DDS concentration less than $7.7 \mu\text{g/ml}$ (arithmetic mean of all the HI negative urine specimens) were considered as HI negative and those having concentration above this as HI positive. Table 4 shows the details about HI positive and HI negative specimens showing aspecific agglutination.

The aspecific agglutination is due to the presence of certain nonspecific substances in the urine which probably act as anti DDS Ig. In the present study, the factors involved in this process have not been identified.

The specificity of HI test was also brought to light by showing that intake of sulphonamides such as Elkosin did not give a positive reaction by

TABLE 4

RESULTS		DDS CONCENTRA- TION $\mu\text{G}/\text{ML}$ AM \pm SD	DDS/CR RATIO $\mu\text{G}/\text{MG}$ CREATININE AM \pm SD
HI + ve with nonspecific agglutination.	41 (80.4%)	61.91 \pm 34.86	69.9 \pm 27.95
HI — ve with nonspecific agglutination.	10 (19.6%)	4.33 \pm 2.15	8.89 \pm 7.12

the HI test. Another interesting observation about aspecific agglutination is that most of the urine specimens showing aspecific agglutination have a high DDS concentration which was sufficient to inhibit the original antigen-antibody reaction in HI test.

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