

CORRELATION BETWEEN SULPHADIMIDINE ACETYLATION TEST IN URINE AND PLASMA ISONIAZID LEVEL IN DETERMINING ISONIAZID ACETYLATION STATUS IN PULMONARY TUBERCULOSIS PATIENTS

C.P. SINHA and S. SINHA

(From Rajendra Medical College & Hospital, Ranchi)

Introduction

In the liver hepatic-N-acetyl transferase enzyme is present which takes part in the acetylation of Isoniazid. The larger the amount of this enzyme, the greater the speed of acetylation (Evans⁵, and White; 1964 and Hearse,¹⁰ and Weber; 1973).

The sulphadimidine is also acetylated in liver by the same enzymatic processes and its acetylation rate runs closely parallel to that of Isoniazid acetylation (Peters, et. al;¹² 1965 white,²³ and Evans 1968). The technique of estimation of both free and acetylated (Total minus free) sulphadimidine is comparatively simpler than those of Isoniazid estimation.

The present study has been carried out to determine the suitability of sulphadimidine acetylation test by the urine method as an indicator of Isoniazid inactivation status. For this purpose, a comparative evaluation has been made among the same tuberculosis patients by employing sulphadimidine acetylation test in urine as well as chemical extraction method for free Isoniazid level in Plasma.

Materials and Methods

(1) Selection of Subjects

The 60 patients of pulmonary tuberculosis who were having radiological lesions in lungs and A.F.B. in their sputum smears (W.H.O. expert committee on Tuberculosis)²⁴ - - were chosen. All of them were the patients of Rajendra Medical College Hospital, Ranchi. The patients with history of asthma, eczema, hay fever and major abdominal surgery, or like ones who reacted adversely to any drug were excluded from the present study. Chemotherapy was stopped for two days prior to investigation. The urine was tested early in the morning by S.N.P. test (Rao,¹³ et. al; 1967) to detect the presence of Isoniazid and acetyl-Isoniazid. Only such patients whose urine was free from Isoniazid and acetyl-Isoniazid were further investigated.

2. Doses of Sulphadimidine administered

The patients were administered sulphadimi-

dine 44 mg/kg. body weight early in the morning with a glass of water on empty stomach. They were not allowed to take anything by mouth for the next two hours. No other restriction was imposed on them (Rao,¹⁴ et. al; 1970 and Sen¹⁸ et. al. 1972).

3. Collection of Urine

The patients were asked to empty their bladders five hours after administration of sulphadimidine and were once again given a glass of water each to promote urine formation. The urine was collected in dry and clean glass vessels 6th hour after administration of Sulphadimidine and stored at room temperature. Rao¹⁴ et. al.; (1970 Sen¹⁸ et. al. 1972).

4. Estimation of Sulphadimidine

The total and free sulphadimidine in urine were estimated by Bratton and Marshall Method¹ Varley H. (1962). Total minus free sulphadimidine were estimated, is the acetylated form and their percentage was calculated. (The decimal figures obtained were rounded up to the nearest of the whole number to simplify the calculation).

5. Doses of Isoniazid

After 6 to 10 days all the above patients were administered oral Isoniazid 10 mg/kg body weight (Evans³ et. al; 1960) on an empty stomach. No other restriction was imposed on them.

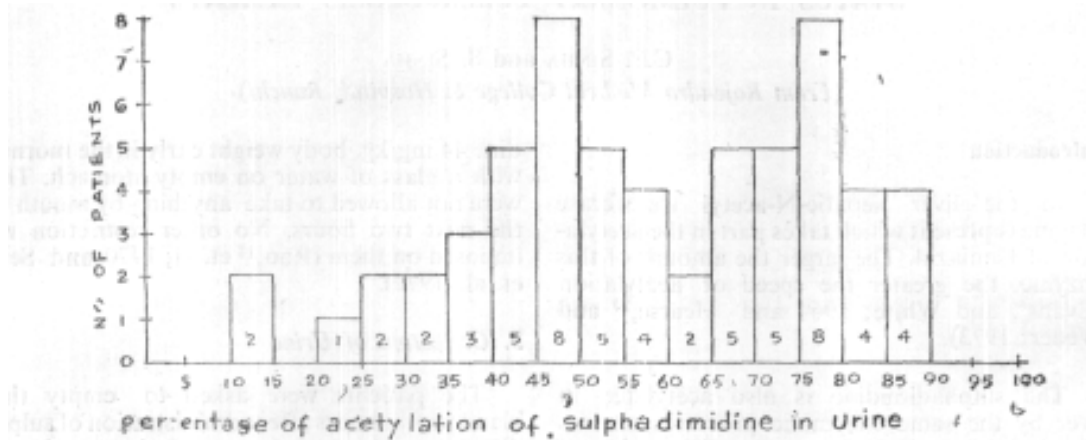
6. Collection of blood

10 ml. of venous blood was collected in heparinised vials after 6 hours of drug ingestion. The solid portion were allowed to settle and the supernatant fluid was transferred in clean centrifuge tube. The supernatant fluid was centrifuged for 5 minutes at a speed of 1000 rpm. The supernatant fluid was transferred thereafter in clean dry glass vessels and stored in deep freezing chamber of a refrigerator at -20°C .

7. Estimation of Free Isoniazid in Plasma

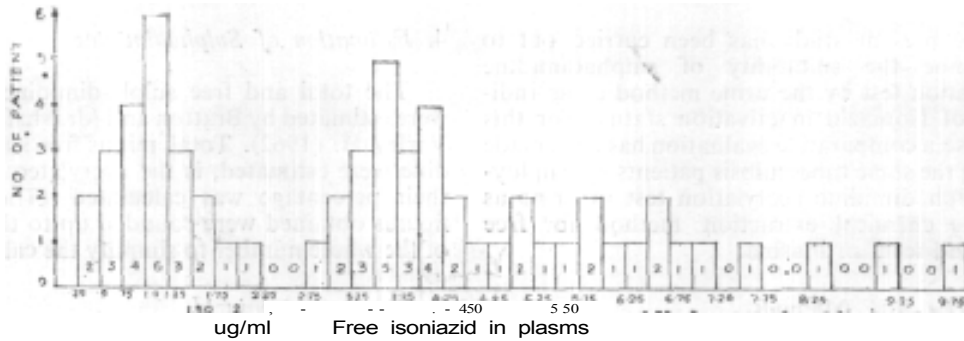
The free Isoniazid level in Plasma was estimated by Chemical extraction method of Rao¹⁵ et. al. (1971).

HISTOGRAM NO. 1



Histogram , showing percentage of acetylation in 60 subject of Pulmonary Tuberculosis in Urine

HISTOGRAM NO. 2



Histogram , showing free isoniazid µg/ml in Plasma of 60 subjects of Pulmonary Tuberculosis

Observation

In the 60 pulmonary tuberculosis patients percentage of acetylation of sulphadimidine in urine was estimated and plotted in histogram no. 2, which is bimodal in distribution. The discriminating point was observed in 65% of acetylation of sulphadimidine, which separated into two bimodal distribution

In the same 60 pulmonary tuberculosis patients free plasma Isoniazid level was determined and plotted in histogram No. 1 which is bimodal in distribution. The discriminating point was observed in 2.5 µg/ml, of free Isoniazid plasma level which separates into two unimodal distribution.

In the sulphadimidine acetylation test in urine 34 (57 %) patients out of 60, had 65 % or less of acetylation of Sulphadimidine in urine and they were classified as slow acetylators and

the rest 26 (43 %) out of 60 patients, had more than 65% of acetylation of sulphadimidine in urine and they were classified as rapid acetylators. In the isoniazid plasma level 22 (37%) out of 60 had free isoniazid plasma level of 2.5 (µg/ml. or less, and they were classified as rapid acetylators and 38 (63 %) out of 60 had free Isoniazid plasma level of more than 2.5 µg/ml. or less and they were classified as rapid acetylators and 38 (63%) out of 60 had free isoniazid Plasma level of more than 2.5 µg/ml. and they were classified as slow acetylators. In order to know the extent of association between the percentage of acetylation of sulphadimidine in urine and Isoniazid plasma level, the co-efficient correlation 'r' is work out and found to be (-1). The comparative value of estimate is shown in Table 1. Classification of the patients as slow and rapid inactivators obtained by both methods is shown in table 2.

Table 1

Shows in do pulmonary tuberculosis subjects distribution of slow and rapid acetylators by two different methods.

SI. No.	Item	Estimates of free Isoniazid in plasma.			Estimates of % of acetylation of sulphadimidine in urine;		
		Total no. of patients	Slow acetylator no. of patients.	Rapid acetylators no. of patients.	Total no. of patients.	Slow acetylators no. of patients.	Rapid acetylator no. of patients.
1	2	3	4	5	6	7	8
1.	No. of Patient	60	38	22	60	34	26
2.	Percentage	100	63	37	100	57	43
3.	Mean value	3.62 µg/ml	4.006 µg/ml.	1.13µtg/ml	59	45	77
4.	Standard deviation	2.2	2.01	0.86	19	11	7
5.	Standard Error of Mean	0.29	0.41	0.17	2.5	3	2

The co-efficient correlation $\mu = (-1)$

Table 2

Table 2 shows distribution of patients under two methods of estimations.

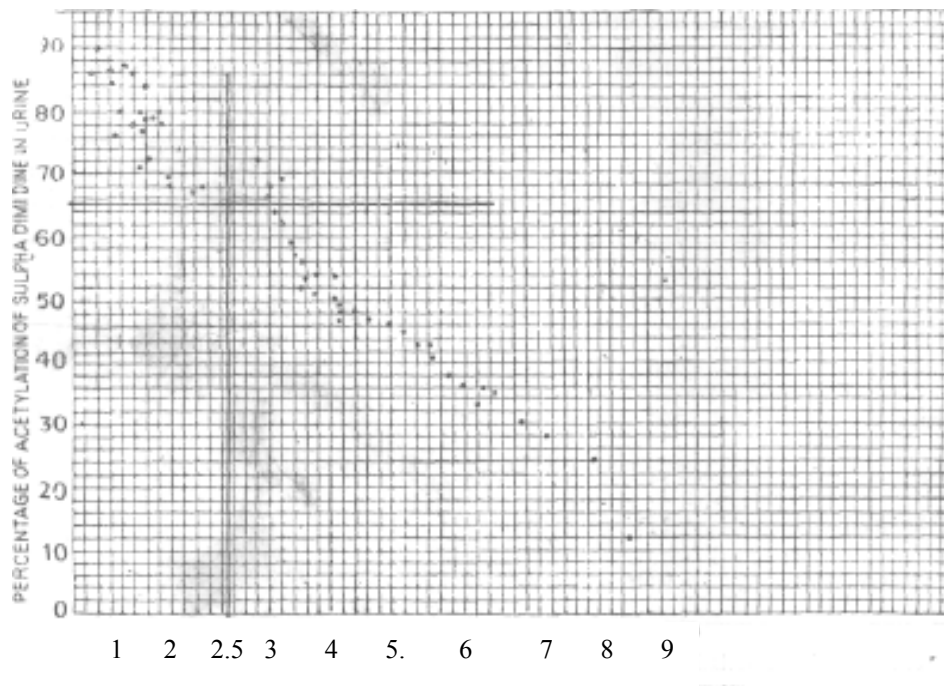
Sulphadiinidine acetylation test in urine	Isoniazid plasma concentration method.		Total no. of patient.
	No. of slow inactivators	No. of rapid inactivators.	
1	2	3	4
1. No. of slow inactivators	34	0	34
2. No. of Rapid inactivators.	4	22	26
Total	38	22	60

Misclassification of slow inactivators 7%.

Considering the merit of the sulphadimidine test in urine in comparison with the standard isoniazid plasma concentration method in classifying the patients as slow and rapid inactivators of isoniazid, it is seen that all the 22 patients classified as rapid inactivators by the isoniazid

plasma level test were also identified as such by the sulphadimidine test, an agreement of 100%. However agreement in respect of slow inactivator was obtained only in 34 (89%) out of 60 patients. Since 4 (11%) slow inactivators were misclassified as rapid inactivators. Considering

GRAPH NO. 1



the two groups together only 47%) out of 60 patients were misclassified.

As can be expected, low level of free Isoniazid in blood is associated with higher percentage of acetylation of sulphadimidine in urine and the same is evident from graph-1.

Discussion

The determination of Isoniazid acetylation (inactivation) status is of great therapeutic interest in once weekly therapy with isoniazid containing regimens. Subjects have been classified into two groups, slow and rapid acetylators. The rate of acetylation of sulphadimidine runs parallel to Isoniazid (Evans⁵ and White 1964 White²³ & Evans 1968, Evans⁶ 1969 and Weber and Brenner²² 1974). The present study is based on the presumption that sulphadimidine and Isoniazid acetylation in human body runs parallel (Evans⁵ & White 1964 and Weber²² and Brenner 1974).

In the present study 65% acetylation of sulphadimidine in urine is the discriminating point between slow and rapid acetylators according to histogram no. 2 and graph no. 1. The result of the present study confirms to White²³ and Evans (1968) Schroder^{16,17} (1972a and

1972b) Weber²² and Brenner (1974) and approximately conforming to Rao, et. al; (1970) British Medical Research Council⁷ (1973) but are dissimilar to Sen¹⁸ et. al. (1972). In the latter study, subjects have been divided in three groups Slow, Rapid and Intermediate group instead of the two, If slow and intermediate groups are clubbed together in the study of Sen¹⁸ et. al; (1972) their observations also become identical.

In the present study 2.5 $\mu\text{g/ml}$ Isoniazid concentration in plasma is the discriminating point between slow and rapid acetylators according to histogram no. 1 and graph no. 1. Those having free Isoniazid concentration in plasma up to or less than 2.5 $\mu\text{g/ml}$ are rapid acetylators and those having over 2.5 $\mu\text{g/ml}$ in plasma are slow acetylators. These findings, too, are in close similarity with Evans⁵ et. al; (1960) Evans⁴ et. al; (1961) and Smith²⁰ and Kyi (1968). Indian patients suffering from pulmonary tuberculosis consist of 60 % Slow acetylators and 40% Rapid acetylators; Gangadharan, et. al; (1961a) and (1961b) Tuberculosis Chemotherapy Centre Madras²¹ (1973) Sharma¹⁹ et. al; 1974).

The present study on pulmonary tuberculosis subjects indicates that 57 % cases are slow acetylators and 43 % cases are rapid acetylators by sulphadimidine acetylation test in urine, and 63 %

cases are slow acetylators and 37% cases are rapid acetylators by estimation of free Isoniazid in plasma by chemical extraction method. The present finding is also marginally similar to Evans⁶ (1969) British Medical Council Research² (1973) and Viznerova²⁵ et al; (1973) on Pulmonary Tuberculosis by the sulphadimidine acetylation method and Evans,³ et al; (1960) Evans,⁴ (1961) Smith²⁰ and Kyi (1968) and Farhat⁷, et al; (1973) by the estimation of Isoniazid plasma level by different methods. A comparative study of sulphadimidine in urine and Isoniazid plasma level in sixty patients has revealed reverse correlation. The co-efficient correlation 'r' is (—1).

Sulphadimidine test detected all the (22) (100%) rapid in activators on the standard test and 34 (89 %) of slow inactivators. Considering all the 60 patients misclassification by the sulphadimidine acetylation test was only 4(7 %). The misclassification had been reported by Rao et. al; (1970) to be 1 % and Sen et. al; (1972) 9%.

Summary

Isoniazid inactivation status was compared in 60 pulmonary tuberculosis patients by sulphadimidine acetylation test in urine as well as free Isoniazid plasma level by chemical extraction method.

Out of 60 pulmonary tuberculosis patients 34 (57 %) were slow acetylators and 26 (43 %) were rapid acetylators by sulphadimidine acetylation test in urine. But 38 (63 %) were slow acetylators and 22 (37 %) are rapid acetylators by free Isoniazid plasma level determined by chemical extraction method.

Considering the merit of the sulphadimidine acetylation test in comparison with the standard Isoniazid plasamaconcentration method in classifying the subjects as slow and rapid inactivators of isoniazid, it was seen that all the 22 patients classified as rapid inactivators by the isoniazid plasma level test were also identified as such by the sulphadimidine test, and agreement of 100 %. However agreement in respect of slow inactivators was obtained only in 34 (89 %) out of 38 since 4 slow inactivators were misclassified as rapid inactivators. Considering the two groups together only 4 (7%) of 60 patients were misclassified.

Acknowledgement

Thanks are due to Dr. B. Singh, F.R.C.S. Superintendent, Rajendra Medical College Hospital, Ranchi who was kind enough to allow the authors to study the admitted indoor patients

in the Hospital. We are also grateful to Mr. M.P. Sinha, Assistant Director, Statistics and Evaluation, Government of Bihar, Patna for his help in various statistical calculations.

REFERENCES

1. Bratton and Marshall technique, Varley H; (1962) *Practical Clinical Biochemistry*. Third Edition P. 632-636.
2. British Medical Research Council (1973). Co-operative Controlled trial of a standard Regimen of Streptomycin P.A.S. and Isoniazid and three alternative Regimens of Chemotherapy in Britain. *Tubercle* 54, 99-129.
3. Evans, David. A. Price; Manley, Keith. A; and McKusick, Victor. A; (1960) Genetic Control of Isoniazid Metabolism in Man. *Br. Med. J.* 2, 485-491.
4. Evans, David. A. Price; Storey, Patrick, B., and McKusick, Victor. A; (1961) Further observation on the determination of the Isoniazid inactivator Phenotype. *Bui. Johns Hopkins Hospital*, 108, 60-66.
5. Evans, David A. Price; and White, Thomas, A; (1964) Human acetylation Polymorphism. *J. Lab. Clin. Med.*, 63, 394-403.
6. Evans, David A. Price (1969) An Improved and Simplified method of detecting the Acetylator Phenotype. *J. Med. Gene.*, 6, 405-407.
7. Farhat, S., Jordanoglou, J., Pall Karis, G., Lyberatos, K; Hadjhdanon, J. and Gardikas, C. (1973) Slow and Rapid inactivator of Isoniazid among greek patients. *Amer. Rev. Res. Dis.*, 108, 1242-1243.
8. Gangadharan, P.R.J., Selkon, J.B., Fox, Wallace; Ramchandar, K., Ramkrishnan, C.V. and Velu, S. (1961 a) Rate of Inactivation of Isoniazid in South Indian Patients with Pulmonary Tuberculosis. *Bull. W.H.O.* 25, 779-792.
9. Gangadharan, P.R.J., Devadutta, S., fox Wallace; Nair, C. Narayanan and Selkon, J.B. (1961b) Rate of inactivation of Isoniazid in South Indian Patients with Pulmonary Tuberculosis. *Bull. W.H.O.* 25, 793-803.
10. Hearse, D.J., and Weber, W.W. (1973) Multiple-N-acety transferases and drug metabolism. Tissue distribution. Characterization and significance of Mammalian N-acetyl transferase. *Biochem. J.*, 132, 519-526.
11. I.C.M.R. Bulletin September (1975) *Chemotherapy of Pulmonary Tuberculosis*, S, 9. p. 1-7.

12. Peter, J.H., Gordon, G.R. and Brown, P. (1965) The relationship between the capacities of human subjects to acetylate Isoniazid Sulphanilamide and Sulphamethazine. *Life Science* 4, 99-107.
13. Rao, K.V. Nageswara, Eidus, L., Jacob, C.V., Radha Krishna, S., and Tripathy, S.P. (1967). Sodium Nitroprusside test for the detection of Isoniazid and acetyl Isoniazid in urine. *Tubercle* 48, 45-50.
14. Rao, K.V.N., Mitchison, D.A., Nair, N.G.K., Prema, K. and Tripathy, S.P. (1970) Sulphadimidine acetylation test for classification of patients as slow or rapid inactivators of Isoniazid. *Br. Med. J.*, 3, 495-497.
15. Rao, K.V.N., Kailasam, S., Menon, N.K. and Radha Krishna, S. (1971) Inactivation of Isoniazid by Condensation in syrup preparation. *Bull. W.H.O.* 45, 625-632.
16. Schroder, Hasse and Evans, David, A Price (1972a) The Polymorphic Acetylation of sulphapyridine in man. *J. Med. Gene.*, 9, 168-171.
17. Schroder, Hasse (1972b) Simplified method for determining Acetylator Phenotype. *Br. Med. J.*, 3, 506-507.
18. Sen, P.K., Chatterjee, R. and Saha, J.R. (1972) Use of Sulphadimidine Acetylation test in determining Isoniazid inactivation rate in tuberculous patients. *Ind. J. Med. Res.*, 60, 28-39.
19. Sharma, G. Raghupati, Immanuel, Chandra, Kailasam, S., Kannapiram, M., Nair, N.G.K. and Radha Krishna, S., (1971) A modified method for the estimation of acetyl Isoniazid in urine. *Ind. J. Med. Res.*, 62, 6, 945-952.
20. Smith, S.E., and Kyi, Tun (1968) Inactivation of Isoniazid in Burmese subjects. *Nature* 207, 1273.
21. Tuberculosis Chemotherapy Centre, Madras (1973) A controlled Comparison of two fully supervised once Weekly regimens in the treatment of newly diagnosed Pulmonary Tuberculosis. *Tubercle* 54, 23-45.
22. Weber, Wendell W., and Brenner, William (1974) A filter paper method for determining Isoniazid Acetylator Phenotype. *Amer. J. Human Genetic*, 26, 467-473.
23. White, T.A., and Evans, D.A. Price (1968) The acetylation of sulfamethazine and sulfametoxy pyridazine by human subjects. *Clini. Pharma. Tiera.* 9, 80-88.
24. W.H.O. Expert Committee on Tuberculosis (1974) Ninth report Technical Report Series 552 p-16.
25. Viznerova, Anna; Slavikova, Zdenka and Ellard, G.A. (1973) The determination of the Acetylator Phenotype of Tuberculous patients in Czechoslovakia using Sulphadimidine. *Tubercle*, 54, 67-71.