CLASSIFICATION OF CHILDREN AS SLOW OR RAPID ACETYLATORS BASED ON CONCENTRATIONS OF ISONIAZID IN SALIVA FOLLOWING ORAL ADMINISTRATION OF BODY-WEIGHT AND SURFACE-AREA-RELATED DOSAGES OF THE DRUG

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ABSTRACT

The acetylator phenotype of 180 children aged 3-11 years was determined on the basis of isoniazid concentrations in saliva collected at 5 hours after oral administration of body-weight and surface-area-related &sages of the drug in a syrup form. isoniazid 25 mglkg was administered on one occasion and 75 mg/m^2 surface-area on another, with an interval of 3 days between the occasions. A cross-over design was employed and the sequence was determined by random allocation. The distribution of the concentrations was bimodal with both procedures, indicating the presence of two groups namely, the slow and rapid acetylators. The criterion for a rapid acetylator was a concentration of 0.3 µg/ml or less by body-weight-related dosage and 0.4 µg/ml or less by that based on surface-area. Based on these criteria, 62 % of the children were classified as slow acetylators and 38% as rapid acetylators by body-weight, and 59 and 41 %, respectively by surface-area, and the findings were similar in children in the different age-groups. The agreement between the two procedures was 98%.

Key words: *Acetylator phenotype of children, Salivary concentrations of isoniazid, Surface-area and body-weight-related dosages of isoniazid.*

Information on the determination of acetylator phenotype of children is scarce, mainly due to difficulties in obtaining multiple blood specimens and timed urine collections from these subjects. A few investigators employing body-weight-related dosages of isoniazid, have reported a higher proportion of rapid acetylators among children than among adults of the same race(1-4). Isoniazid acetyltransferase, the concentration of which determines the speed of acetylation, is a constitutive enzyme and is not inducible; therefore, the proportions of slow and rapid acetylators in children should not be different from those in the adult population. It has been suggested that the age-related differences in the proportions of slow and rapid acetylators could possibly be due to the administration of inappropriate body-weight-related dosages and that the dosages should be based on body-surface-area instead, particularly in children aged less than 5 years (2,5) To the best of our knowledge, no investigations have been undertaken so far to compare the distributions obtained with the two procedures.

We have recently demonstrated that salivary concentrations of isoniazid are similar to those in serum(6), and that the concentrations in saliva at 5 hours following a uniform oral dose of isoniazid 100 mg could effectively discriminate between slow and rapid acetylators of the drug among adult subjects(7). An investigation was therefore undertaken to

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determine the acetylator phenotype of children aged 3-11 years (completed years) on the basis of concentrations in saliva at 5 hours following administration of the drug dosages based on body-weight as well as surface-area; this report presents the findings.

Material and Methods

Subjects: Healthy children aged 3-11 years and residing in Choolai, an urban crowded locality in Madras city, where a survey for the prevalence of tuberculosis was in progress were included in the study.

Calculation of the dosages of isoniazid: Calculation of body-weight and surface-area-related dosages of isoniazid to be employed in children was based on the successful experience in the classification of adult patients with pulmonary tuberculosis as slow or rapid acetylators on the basis of concentrations of isoniazid in saliva following a uniform oral dose of isoniazid 100 mg(7).

The mean body-weight, height and surface-area of 230 consecutive adult patients were 40.6 kg, 160.2 cm and 1.32 m^2 respectively, the surface-area having been calculated using the formula(8):

$$\log A = 0.725 \log H + 0.425 \log W - 2.144$$

where A=surface-area (m^2), H=height (cm) and W=body-weight (kg). If a test dose of isoniazid 100 mg were to be used, the mean dosages based on body-weight and surface-area would work out to be 2.46 mg/kg and 74.6 mg/m^2, respectively. Therefore, for investigations in children, the dosages employed were 2.5 mg/kg body-weight and 75 mg/m^2 surface-area.

Design of the investigation: The children were classified as slow or rapid acetylators on the basis of distributions of concentrations of isoniazid in saliva at 5 hours following oral administration of isoniazid 2.5 mg/kg body-weight on one occasion and 75 mg/m^2 surface-area on another occasion, with an interval of at least 3 days between the occasions. A cross-over design was employed and the sequence was determined by random allocation.

Conduct of the investigation: Administration of isoniazid and the collection of saliva were carried out in the Corporation Health Unit. The children were visited in their homes by field workers and given appointments to report to the health unit by 7 a.m. in a fasting state on the two days. Body-weight (kg) and height (cm) were recorded by field workers trained in anthropometry. After verifying that urine was negative for acetylisoniazid(9), isoniazid was administered in the form of syrup ("Isokin" stated content: 20 mg/ml) on the basis of separate dosage schedules based on body-weight *(Table I)* and a nomogram relating the dosage to height-weight measurements *(Table II)* for surface-area. The order in which the child received the body-weight or surface-area-related dose was by random allocation, which was supplied to the field workers in the form of a chart. The required volume of the syrup was delivered directly into the gullet (to avoid contamination of the oral cavity) with the help of a syringe, with graduation intervals of 0.2 ml, to which a siliconized rubber tubing was attached. The syringe and the tube were rinsed once with a few ml of water and the rinsings were also delivered into the gullet the same way. The children were asked to report to the

TABLE I– *Dosage Schedule Based on Body-weight (2.5 mg/kg)*

Body-weight (kg)	Volume (ml) of syrup to be administered*
≤ 5.0	0.4
5.1-10.0	1.0
10.1-15.0	1.6
15.1-20.0	2.2
20.1-25.0	2.8
25.1-30.0	3.4
30.1-35.0	4.0
35.1-40.0	4.8
40.1-45.0	5.4
> 45.0	6.0

*Isokin syrup (stated content: 20 mg/ml)

health unit about 4½ hours later. About 10 minutes before the scheduled time of collection at 5 hours, they were asked to wash their mouth thoroughly. To facilitate salivary secretion, the children were asked to chew a piece of unsweetened, unflavoured chewing gum and to spit out the initial salivary secretion. About 5 ml of saliva were then collected over 5-10 minutes in a universal container.

The saliva samples were sent soon after collection to the Tuberculosis Research Centre where they were kept frozen at –20°C overnight. The samples were then thawed and centrifuged, the residue containing mucoproteins discarded, and the clear supematants were stored at –20°C for not more than 48 hours. Isoniazid concentrations in the salivary supernatants were determined by a spectrophotometric method(10) after coding the samples. In brief, isoniazid was extracted from the salivary supernatants (3 ml) with a mixture of chloroform and n-butanol(7 : 3) and re-extracted into 0.1N sulphuric acid. The acid extract was reacted with a 2% solution of vanillin in 25 % ethanol and the extinction recorded at 380 nm using microcells of 2 cm path-length. The sensitivity of the method is 0.1 µg/ml and none of the metabolites of isoniazid namely, acetylisoniazid, isonicotinic acid, isonicotinylglycine, mono- and diacetylhydrazines nor any of the commonly used anti-tuberculosis drugs interfered with the estimation of isoniazid.

Results

A total of 182 children were studied. Findings from 2 of these were excluded from the analysis; the urine of one child was positive for acetylisoniazid and one child failed to turn up for the second occasion. Of the remaining 180 children in the analysis, 57 were in the 3-5 year age group, 66 in the 6-8 year age group and 57 were aged 9-11 years.

The mean body-weight, height, surface-area and doses of isoniazid administered are presented in *Table III.* The dose of isoniazid (mg) administered was always higher by the dosage based on surface-area than that based on body-weight, the differences being 31, 30 and 24% in the 3-5, 6-8 and 9-11 year age groups, respectively.

The distributions according to the concentrations of isoniazid in saliva at 5 hours with the two procedures are presented in the form of histograms in the *Fig.* Both the distributions are bimodal indicating the presence of two groups of children, namely the slow and rapid acetylators. On the basis of these histograms, the criterion for a rapid acetylator was taken to be a concentration of 0.4 µg/ml or less by surface-area and 0.3 µg/ml or less by body-weight. Based on these criteria, of the 180 children.

TABLE II– *Dosage Schedule Based on Surface-area (75 mg/m^2)–Nomogram Relating the Dose to Height- Weight Measurements*

Height (cm)	Volume (ml) of syrup* to be administered for the following weight categories (kg)								
	≤ 5.0	5.1-10.0	10.1-15.0	15.1-20.0	20.1-25.0	25.1-30.0	30.1-35.0	35.1-40.0	> 40.0
< 80.0	1.0*	1.4	1.8	2.2	2.4	2.6	2.8	3.0	3.2
80.0-89.9	1.0	1.6	2.0	2.4	2.6	2.8	3.0	3.2	3.4
90.0-99.9	1.2	1.8	2.2	2.6	2.8	3.0	3.2	3.4	3.6
100.0-109.9	1.2	1.8	2.4	2.8	3.0	3.2	3.6	3.8	4.0
110.0-119.9	1.2	2.0	2.6	2.8	3.2	3.4	3.8	4.0	4.2
120.0-129.9	1.4	2.2	2.6	3.0	3.4	3.8	4.0	4.2	4.4
130.0-139.9	1.4	2.2	2.8	3.2	3.6	4.0	4.2	4.4	4.8
140.0-149.9	1.6	2.4	3.0	3.4	3.8	4.2	4.4	4.8	5.0
150.0-159.9	1.6	2.4	3.0	3.6	4.0	4.4	4.6	5.0	5.2
⩾160.0	1.6	2.6	3.2	3.6	4.0	4.4	4.8	5.0	5.4

*Isokin syrup (stated content 20 mg/ml).

TABLE III– *Mean Body-weight, Height, Surface-area and Doses of Isoniazid Administered*

Age group (years)	No. of children	Body-weight (kg)	Height (cm)	Surface.. area (m^2)	Dose of isoniazid administered (mg)	
					by body-weight*	by surface-area*
3- 5	57	13.7** (11.1-18.1)	97.9 (53.8-111.8)	0.61 (0.50-0.75)	**35 (32-44)**	**46 (40-56)**
6-8	66	17.5 (12.5-25.0)	113.7 (101.6-128.8)	0.75 (0.61-0.96)	**43 (32-56)**	**56 (44-68)**
9-11	57	22.3 (15.4-32.1)	126.7 (107.6-145.1)	0.90 (0.68-1.15)	**55 (44-80)**	**68 (56-88)**
All groups	180	17.8 (I 1.1-32.1)	112.8 (53.8-145.1)	0.75 (0.50-1.15)	**44 (32-80)**	**56 (40-88)**

* 2.5 mg/kg body-weight and 75 mg/m^2 surface-area.
** Mean, with range in parentheses.

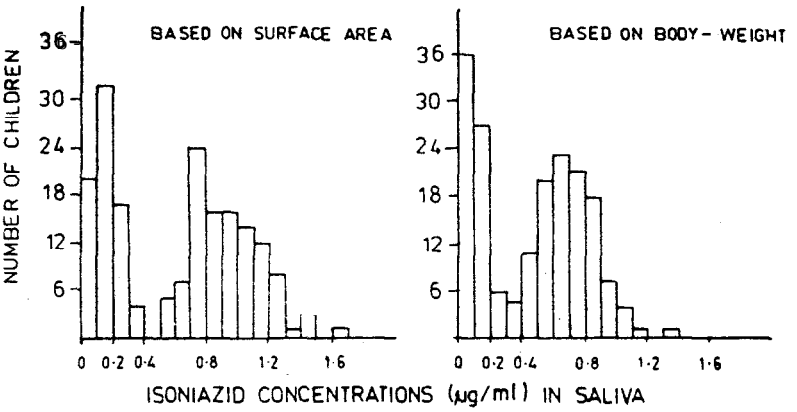

Fig. Distribution of 180 children according to the concentrations of isoniazid in saliva at 5 hours after oral administration of surface-area and body-weight-related dosages of the drug.

73 (41%), were classified as rapid acetylators by surface-area and 69 (38%) by body-weight, the proportions of slow acetylators being 59 and 62%, respectively. The classification by the two procedures was the same in 176 of the 180 children, leading to an agreement of 98%.

The mean isoniazid concentrations in saliva in slow and rapid acetylators and the indices of discrimination (i.e., the ratio of the difference between the mean values in slow and rapid acetylators to its standard error) and the proportions of the two phenotypes according to the age group of the children by the two procedures are given in *Table IV*. The mean concentrations of isoniazid were appreciably higher in the slow than in the rapid acetylators. The proportions of the two phenotypes were similar in the different age groups by both procedures. The index of discrimination by the procedure based on surface-area was higher than that based on body-weight in all age groups. Corresponding to the higher dose received with the surface-area-related dosage, the mean isoniazid concentration in the 180 children (amalgamating the findings in slow and rapid acetylators) was 0.62 µg/ml, which was significantly higher ($p<0.001$) than that based on body-weight (0.47 µg/ml). The differences between the mean values by the two procedures decreased with increase in age, the mean differences being 0.20, 0.15 and 0.12 µg/ml in the 3-5, 6-8 and 9-11 year age groups, respectively.

Discussion

Use of saliva, where applicable, has obvious practical advantages, particularly in children, as the procedure is non-invasive, amenable for collection of multiple specimens, and also permits the conduct of the investigations under field conditions. Indeed, this investigation, employing a randomised cross-over design for drug administration and the collection of saliva on two different occasions was successfully carried out in a field area. Results presented in this report demonstrate that the test based on the determination of isoniazid concentrations in saliva at 5 hours after administration of

TABLE IV– *Mean Isoniazid Concentrations, Proportions Classified as Slow or Rapid Acetylators and the Indices of Discrimination*

Age group (years)	No. of children	By body-weight			By surface-area		
		Mean INH concentrations (µg/ml)		Index of discrimination	Mean INH concentrations (µg/ml)		Index of discrimination
		Slow	Rapid		Slow	Rapid	
3-5	57	0.68 (61)*	0.12 (39)*	11.6	0.99 (58)	0.19 (42)	13.8
6-8	66	0.69 (61)	0.09 (39)	18.8	0.93 (59)	0.14 (41)	19.3
9-11	51	0.72 (63)	0.10 (37)	14.9	0.91 (61)	0.15 (39)	16.2
All groups	180	0.70 (62)	0.10 (38)	25.6	0.94 (59)	0.16 (41)	28.0

*Figures in parentheses indicate the proportions (%) of slow or rapid acetylators.

body-weight or surface-area-related dosages of isoniazid can be successfully employed for classifying children as slow or rapid acetylators.

Several studies undertaken in 3,000 adult South Indian patients with pulmonary tuberculosis at our Centre(11) have shown that the proportions of rapid acetylators ranged from 32 to 46 % (mean 41%). In the present study in 180 children aged 3-11 years, the proportions of rapid acetylators were 41 % by the dosage based on surface-area and 38% by that based on body-weight, proportions very similar to those obtained in adult subjects. Even though the index of discrimination by surface-area is higher than that by body-weight in all age groups, employing the latter procedure would be more convenient as it avoids measurement of the height and the calculation of body-surface-area. However, the criterion adopted to discriminate between the two phenotypes must be based on distributions obtained in children and not that derived from adult population. This may be illustrated by comparing the distributions obtained in adult subjects(7) and in children (present study). The criterion for a rapid acetylator in 150 adults employing a uniform oral dose of 100 mg (mean dosage 2.25 mg/kg) was a concentration of 0.4 µg/ml or less. If this criterion were to be applied in the present study in children, 5 slow acetylators would be misclassified as rapid acetylators even though the dosage received (2.5 mg/kg) was higher than that in the adults. A similar observation has been made by Bartmann and Massmann(12) who compared the distributions of serum isoniazid concentrations following an oral dose of isoniazid 5 mg/kg in 39 children and 295 adults, the criteria to distinguish the two phenotypes being 0.3 and 0.5 µg/ml. respectively.

In a recent study, Bajaj *et al.* (4) determined the acetylator phenotype of 276 children based on plasma isoniazid concentrations at 5 hours following an intramuscular dose of isoniazid 10 mg/kg. They observed a trimodal distribution and classified 17% of their subjects as fast, 63 % as intermediate and 20% as slow acetylators of isoniazid. Since slow acetylators are homozygous for the recessive gene and rapid acetylators could be either homozygous or heterozygous for the dominant gene(13), it follows that 80% of the children investigated are rapid acetylators. They have not-mentioned the proportion of rapid acetylators among adult subjects of their region, but it will be surprising if a proportion of greater than 50% is observed. The main difference between our study and that of Bajaj *et al.* (4), apart from the dosages and the body-fluid employed, is that the age of their children was 1-12 years while that in our study group, was 3-11 years. It will be interesting to find out if they had observed any differences in the proportions of slow and rapid acetylators in the different age groups, particularly in children aged less than 3 years.

Children less than 3 years of age were not investigated in the present study due to difficulties in obtaining adequate volumes of saliva from these subjects. However, more sensitive methods such as those based on spectrofluorimetry or high-pressure liquid chromatography (which require smaller volumes of saliva) can be employed for determination of isoniazid concentrations even in these children. The dosage may, however, have to be based on surface-area rather than on body-weight for efficient discrimination, as results presented in this paper

show an increase in the mean difference in isoniazid concentrations between the two procedures with a decrease in age, and the difference between the indices of discrimination was highest in the youngest age group (3-5 years).

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