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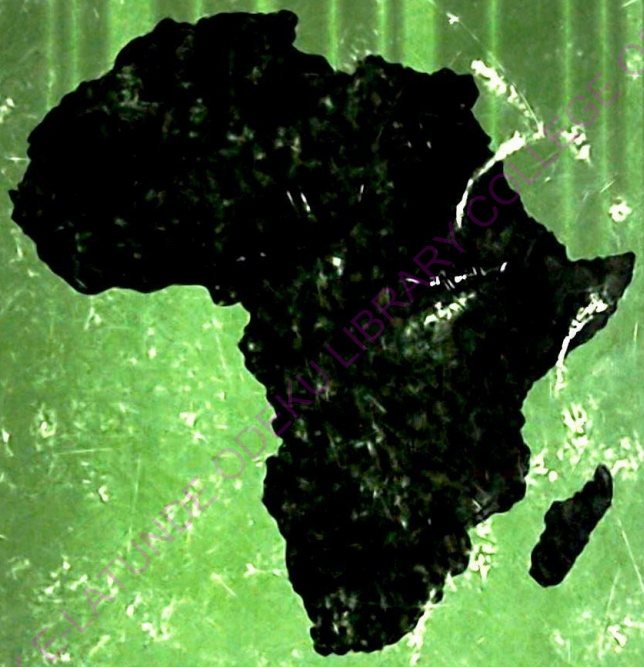

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Quantitative analysis of 1-naphthol in urine of neonates exposed to mothballs: The value in infants with unexplained anaemia.

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Abstracts

In Nigeria, severe NNJ is common in babies exposed to mothballs and other icterogenic agents.

High-performance liquid chromatographic (HPLC) method was employed for quantitative analysis of 1 and 2-naphtol in the urine of 50 neonates aged one to 19 days. Five of the 25 babies who had a history of exposure to mothballs, and none of the babies without a history of exposure had 1-naphtol in their urine. The value of 1-naphtol ranged between 0.75 and 11.69µg/ml with mean of 5 ± 5µg/ml. The overall correlation coefficient (*r*) between bilirubin values and 1-naphtol was 0.1 while it was 1 in the three G-6-PD deficient infants.

The procedure will be very useful in the evaluation of infants with unexplained NNJ, anaemia, acute haemolytic jaundice and haemoglobinuria if naphthalene poisoning is suspected.

Resumé

Nous avons remarqué, au Nigéria, une forte indidence de jaunisse chez les nouveaux-nés exponsés aux boules antimites et d'autres produits ictérogènes.

Nous avons recouru à la chromatographie d'un luquide à haute performance pour déterminer le taux de naphtol 1 & 2 dans l'urine de 50 nouveaux-nes âgés de 1 à 19 jours. Nous avons relevé des traces de naphtol — 1 chez 5 bébés sur 25 ayant eté exposés aux boules antimites tandis qu'aucun nouveau-né à l'abri des boules n'était touché. La valeur de naphthol — 1 variait entre 0, 75 et 11, 69 µg/ml avec une moyenne de 5 ± 5 µg/ml. Le coefficient total de corrélation (*r*) entre lex taux de bilirubine et naphtol — 1 était 0, 1 mais il a atteint 1, 0 chez trois enfants qui accusaient un manque de G - 6 - PD.

Cette méthode s'avérera très utile pour l'étude des cas mystérieux de jaunisse chex lex nouveaux-hés, d'anémie, de jaunisse hémolytique et d'haémoglobinurie s'il y a lieu de rdouter l' action nocive de la naphtalene.

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Introduction

Severe neonatal jaundice (NNJ) is now rare in term infants in the developed countries of Europe and North America, but is still a major cause of morbidity and mortality in Nigeria and other parts of Africa [1-8]. Glucose-6-Phosphate Dehydrogenase (G-6-PD) deficiency is the single most important factor associated with severe NNJ and kernicterus in Nigeria[3-5, 8].

Many of these babies with severe jaundice and kernicterus have been exposed to naphthalene and other possible icterogenic agents before admission into the hospital [8-10].

Inhalation of naphthalene vapour and dermal exposure to mothballs in garments and diapers by neonates have led to severe haemolysis of the red blood cells resulting in severe anaemia and jaundice and G-6-PD deficient infants are particularly at risk[11]. Exposure to naphthalene has resulted in kernicterus in many G-6- PD deficient infants [8, 12]. Naphthalene itself does not cause haemolysis but on absorption its metabolites (1 and 2-naphthols) cause haemolysis *in vivo*[13]. In babies exposed to mothballs, it is desirable to be able to determine the level of its absorption by estimation of it or its metabolites in the blood or urine because the degree of toxicity will depend on the quantity of the poisoning substance in the body[14].

A high performance liquid chromatographic (HPLC) method for detection and quantitation of 1 and 2-naphthols in human urine was recently developed in the Department of Pharmaceutical

Chemistry, Obafemi Awolowo University, Ile-Ife. The method is rapid, sensitive and reproducible. This method was employed to screen 50 urine samples from 50 neonates. The preliminary reports is presented below.

Patients and methods

The patients were neonates admitted from home into our Neonatal Units (NNU) at Ife State Hospital (ISH), Ile-Ife and Wesley Guild Hospital (WGH), Ilesha in 1987. They consisted of babies with and without jaundice. Each group was subdivided into those with a history of exposure and those without a history of exposure to naphthalene.

For each baby a detailed history of antenatal care (ANC), delivery, medical problem before/or on admission and management including cord care was recorded. On admission, the age, sex, weight, gestational age and temperature were also noted. A record of exposure to known potentially icterogenic agents were made. Exposure to naphthalene means that either the clothes of the baby or mother or both had been preserved with mothballs.

Those with jaundice had a full work up for the jaundice. This included full blood counts, serum bilirubin, blood group of babies and mothers, culture of blood, cerebrospinal fluid (CSF) and urine if sepsis was suspected. Urine for 1-and-2-naphtols analysis were collected on admission, usually within 24 hours by urine bag.

Jaundiced babies with sepsis or blood group incompatibility were excluded. A sensitive and rapid high-performance liquid chromatographic (HPLC) procedure was used for the detection of urine levels of 1 and 2-naphtols. Generally, the urine samples were adjusted to pH 1.5 and extracted with dichloromethane. HPLC on a micropak CN-10 column at a detection wavelength of 254nm and hexane: dichloromethane: isopropyl alcohol (89:10:1) as solvent system allowed the measurement of 1 and 2-naphtols (Retention times are 8.3 and 10.6 minutes respectively).

The standard curve was prepared by spiking control urine with naphthol samples, 2, 4-dicholorophenol as the internal standards (IS) and subjecting it to the procedure above (Retention of IS is 7 minutes). The tests were also spiked with the IS and similarly treated to obtain the amount of 1 and 2-naphthols contained therein. Value of naphthols was expressed in μg/ml. The data obtained were analysed using student '*t*' test and X^2 test as appropriate. Correlation coefficient (*r*) was determined between bilirubin values and naphthol level in urine — using Spearman Rank correlation coefficient.

Results

Fifty neonates, ages 1 to 19 days (mean 6.1 ± 3.3), were studied. The distribution of the diagnosis in the 50 babies is shown in Table 1. The mean weight was 2.8 ± 0.5kg. The male:female ratio was 7:3. Fifteen (46.9%) of the 32 jaundiced babies had a history of exposure to naphthalene while 10(55.6%) of the babies without jaundice were exposed to naphthalene. The babies with and without jaundice are compared in Table 2. The only significant difference was in the mean age at which urine was collected and the proportion of babies with G-6-PD deficiency. Eleven (55%) of the 20 G-6-PD deficient babies and 14 (46.7%) of the 30 G-6-PD normal babies were exposed to naphthalene (>0.5).

It was only 1-naphthol that was detected in the urine of five babies. The value varied between 0.75 to 11.69μg/ml with a mean of 5 ± 5.2μg/ml. These five babies with 1-naphthol in urine were among the 25 babies with a positive history of exposure to naphthalene ($P < 0.05$). The major clinical data of the five babies are presented in Table 3. Four of the five babies had jaundice and three were G-6-PD deficient. Of the two babies with normal G-6-PD status, one did not have jaundice on admission and history of jaundice was denied. He was admitted at the age of nine days with bilirubin of 1.3mg/dl and haematocrit of 48% with 1-naphthol of 9.55μg/ml. The diagnosis in that baby was neonatal tetanus (NNT).

Table 1: The Distribution of the major diagnosis in the 50 babies

Diagnosis	Total	Percentage of Total
Neonatal jaundice alone	26	52
Neonatal tetanus alone	12	24
Neonatal jaundice + Neonatal tetanus	2	4
Neonatal sepsis alone	4	8
Low birth weight	2	4
Low birth weight + Neonatal jaundice	3	6
Birth asphyxia + Seizures + Neonatal jaundice	1	2
Total with jaundice	32	64
Total without jaundice	18	36
Total	50	100

Table 2: Comparison between the 32 babies with jaundice and 18 babies without jaundice

Features	Babies with jaundice $n = 32$	Babies without jaundice $n = 18$	*P* Values
Male: Female ratio	3:1	1:6:1	NS
Mean age days + ISD	5.2 + 3.3 (1 — 19)	7.8 + 2.8 (1 — 14)+	< 0.005
Mean weight Kg + ISD	2.9 + 0.6 (1.65 — 3.8)+	2.8 + 0.4 (1.6 — 3.5)+	NS
Exposure to naphthalene	15(46.9)++	10(55.6)++	NS
G-6-PD deficiency	19(59.4)++	1(5.6)++	< 0.001
Positive for Naphthalene in urine	4(12.5)++	1(5.6)++	NS

\+ Represent ranges
++ Figures in parenthesis represent percentages NS = $P > 0.05$ (Not significant)

Table 3: The clinical features of the five babies* with 1-naphthol in their urine

Serial No.	Sex	Age (Days)	Weight (Kg)	SBR Maximum mg/dl	G-6-PD Status	1-naphthol** in urine ug/ml	Diagnosis/ Outcome
1	M	4	3.7	13.4	Deficient	1.14	NNT* and NNJ Died
2	F	3	2.6	24.2	Normal	0.75	NNJ/Satisfactory
3	M	5	3.2	21.4	Deficient	1.76	NNJ/Satisfactory
4	M	4	2.65	22.6	Deficient	11.69	NNJ/Kernicteric on admission
5	M	9	2.55	1.3	Normal	9.55	NNT/survived

\+ All were term babies
++ All were exposed to mothballs

NNT = Neonatal tetanus
NNJ = Neonatal jaundice
SER = Serum bilirubin.

The correlation co-efficient (*r*) using Spearman Rank Correlation Coefficient between the weight on admission and 1-naphthol levels, the age and 1-naphthol levels and between bilirubin level and 1-naphthol levels in the five babies were - 0.2: 0.5 and 0.1 respectively, while in the three G-6-PD deficient infants the correlation coefficient between the bilirubin values and 1-naphthol concentration in urine was 1. (Table 4). One of these three babies had clinical evidence of kernicterus on admission. The bilirubin and 1-naphthol values in that baby at four days were 22.6mg/dl and 11.69μg/ml respectively.

Table 4: The correlation coefficient (*r*) between 1-naphthol and weight; 1-naphthol and age and 1-naphthol and the maximum bilirubin levels.

	Parameters	Number and Type of Patient	Correlation Coefficient(r)+
1.	Weight Vs 1-naphthol	All five	-0.2
2.	Age Vs 1-naphthol	All five patients	0.5
	SER Vs 1-naphthol	All five patients	0.1
	SBR Vs 1-naphthol	Three patients with G-6-PD deficiency	1

+Spearman Rank Correlation Coefficient was used because of the small number involved.

Discussion

Naphthalene (mothballs) is most often used to preserve clothes of mothers and their babies. It is sometimes used as a constituent of body care lotions for babies or as ingredients of traditional cough mixture or *Agbo Jedi*[15].

The vapour pressure of naphthalene at room temperature is appreciable hence considerable inhalation may occur during exposure[16]. Daily baby oil rubdowns may also facilitate dermal absorption in the neonate because naphthalene is lipophilic and neonatal skin is also thinner when compared with that of adult.

Naphthalene itself is nontoxic but its metabolites (1 and 2- naphthols) cause severe haemolysis even at low blood level[17]. Naphthalene is metabolised in the liver to 1 and 2-nephthols[15] which are conjugated in the liver before excretion[16].

Many cases of severe toxicity from dermal exposure to naphthalene have been reported[10,11]. Acute haemolytic anaemia is a major clinical feature. Usually a day after moderate absorption, there is a Hainz-body formation followed by a fall in haematocrit and red blood cell count. This may be accompanied by leucocytosis and poiklocytosis which usually return to normal except in very severe cases. Acute ingestion of naphthalene results in fever, abdominal pain, vomitting and diarrhoea, lethargy, pallor, jaundice and dark urine. Red blood cell fragmentation, haemoglobinuria and methaemoglobinaemia may occur[2, 18]. Cases of neonates with jaundice and kernicterus following exposure to naphthalene have been reported from Nigeria and other parts of the world[8, 12]. The present report has provided additional evidence that babies in Nigeria are exposed to mothballs.

Though only 5 of the 25 babies with a history of exposure to mothballs had 1-naphthol in the urine, the results so far appears interesting. It is significant that none of the 25 babies with negative history of exposure had naphthalene in urine. That only 1-naphthol was identified from the urine is in agreement with the fact that 1-naphthol is almost the exclusive metabolic product of naphthalene[16, 19].

That 1-naphthol was not present in the urine samples of all the babies with a history of exposure does suggest that all exposed babies did not absorb or metabolise naphthalene to a detectable level. On the other hand, the method of urine collection may also be important since concentration of excreted

substance in urine depends on the amount of urine produced. A 24-hour urine collection to determine a 24-hour excretion of 1-naphthol may yield more cases of positive result, and may give a better correlation with bilirubin levels even among the G-6-PD normal babies.

The negative correlation between the weight and 1-naphthol values in urine suggests that the bigger babies will be less at risk of poisoining when exposed to the same level of naphthalene. Also the positive correlation between the age and the 1-naphthol levels probably means that the longer the period of exposure to naphthalene, the more naphthalene likely to be absorbed. The overall poor correlation coefficient between bilirubin values and 1-naphthol and the good correlation between the bilirubin value and 1-naphthol in the G-6-PD deficient babies is in support of the fact that G-6-PD deficient individuals are particularly at risk of haemolytic effect of 1-naphthol, and jaundice in the G-6- PD normal babies was due to other causes other than exposure to naphthalene[20].

Though G-6-PD normal cell may also haemolyse if exposed to high enough level of 1-naphthol, one baby in the present study with very high 1-naphthol in urine did not have jaundice. This is in keeping with the fact that G-6-PD normal babies are much less likely to develop jaundice when exposed to naphthalene and other icterogenic agents[19, 20].

Estimating 1-naphthol in the urine of infants with unexplained NNJ: haemolytic anaemia and jaundice; and haemoglobinuria may help in the evaluation of the setiology of such problems in many of the infants since children may be exposed to a toxic level of naphthalene either through inhalation, dermal exposure or ingestion in many of the concoctions given to children in form of herbal medicines[15] and the history may be denied.

References

1. Animashaun A. Aetiology of cerebral palsy in African children. Afr J Med Sci 1971; 2: 165-171.
2. Hendrickse R G. Problems and future of paediatrics in pre-industrial countries of Africa. Helv Paediatr Acta Supp 1972; 28: 23-31.
3. Ransome-Kuti O. The problems of paediatrics emergencies in Nigeria. Nig Med J 1972; 2: 62-70.
4. Effiong C E and Laditan A A O. Neonatal jaundice in Ibadan: A study of cases seen in the outpatient clinic. Nig J Paediatr 1976; 3: 1-8.
5. Azubuike J C. Neonatal jaundice in Eastern Nigeria. East Afr Med J 1979; 56: 320-324.
6. Nkrumah F K A and Neequaye J. Neonatal hyperbilirubinaemia in Ghana. West Afr J Med 1982; 1: 1-6.
7. Dawodu A H, Owa J A and Familusi J B. Prospective study of the role of bacterial infection and G-6-PD Deficiency in severe neonatal jaundice in Nigeria. Trop & Geogr Med 1984; 36: 127-132.
8. Owa J A, Dawodu A H and Familusi J B. Kernicterus in Nigerian infants. West Afr J Med 1987; 6: 11-20.
9. Olowe S A and Ransome-Kuti O. The risk of jaundice in G- 6-PD deficient babies exposed to menthol. Acta Paediatr Scand 1980; 69: 341-345.
10. Familusi J B, Dawodu A H and Owa J A. Some epidemiological aspects of neonatal hyperbilirubinaemia in Nigeria In: Fukuyama Y, Arima M, Maekawa K and Yamaguchi K (eds.). Child Neurology Series 579. Amsterdam. Excerpta Medica 1982; 272-280.
11. Vales T, Doxiadis S A and Fassas P. Acute Haemolysis due to naphthalene inhalation. J Pediatr 1963; 904-915.
12. Brown R W and Wong H B. Hyperbilirubinaemia and kernicterus in Glucose-6-Phosphate Dehydrogenase deficiency in Singapore. Pediatrics 1968; 41: 1055-1062.
13. Mackel J V, Rieders F and Brieger H L. Acute haemolytic anaemia due to ingestion of naphthalene mothballs. Pediatrics 1951; 7: 722-728.
14. Hepler B, Sugheimer C and Sunshinel. The role of the toxicology laboratory in suspected drug ingestions. Ped Clin North Am 1986; 33: 245-260.
15. Familusi J B and Dawodu A H. Neonatal jaundice in association with household drugs and chemicals. A survey of 450 Nigerian families. Nig Med J 1983; 13: 43-49.
16. Jerina O M, Jolly J W and Witkop B. 1-and-2-naphthalene oxide as an intermediate in microsomal hydroxylation of naphthalene. Biochem 1970; 9: 147-156.

17. Siegel E and Watson S. Mothball toxicity. Ped Clin North Am 1986; 33: 369-374.
18. Schafer W B. Acute haemolytic anaemia: Report of a case in a newborn infant. Pediatrics 1951; 7: 172-174.
19. Cock T C. Acute haemolytic anaemia due to naphthalene ingestion. Am J Dis Child 1955; 89: 612-614.
20. Owa J A. Relationship between exposure to icterogenic agents, Glucose-6-Phosphate Dehydrogenase deficiency and severe neonatal jaundice in Nigeria. Acta Paediatr Scand 1989; 78: 848- 852.

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