Evaluation of acetylator phenotype of isoniazid among tuberculosis patients in delta state Nigeria: update on management of toxicity

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ABSTRACT

Background: In 1996, acetylator phenotype of thirteen consent ing tuberculosis patients who were being treated with isoniazid was briefly examined as part of an MSc biochemistry program. Initial agenda were to determine the half-life of isoniazid and evaluate potential toxicity associated with long-term treatment regimen. This report presents findings of the study, but with the aim of providing an update on diagnosis and monitoring of the treatment of tuberculosis by laboratory methods.

Methods: Firstly, the report submitted for the award of MSc Biochemistry was reviewed and reformatted. Secondly, a follow up survey was performed to determine the current practice at the general hospital and ‘tuberculosis and leprosy’ referral centres where the original study was carried out. Thirdly, a brief literature review was performed for any potential update on clinical practices.

Results: The study has shown that 53.8% of patients are fast acetylators (t₀<2 hr), 30.8% are intermediate acetylators (t₀: 2.0–2.5 hr), and 15.4% are slow acetylators (t₀>2.5 hr). The follow-up on current practice indicates that molecular technology has been introduced, but limited to susceptibility testing. Whether a patient could be a fast acetylator (requiring long term drug regimen) or slow acetylator (deserving short term drug regimen) is very much appreciated, but test is neither available, nor considered. Conventional diagnostic indices applicable for clinical laboratory monitoring in a drug-responsive patient in terms of efficacy and toxicity of the treatment are discussed.

Conclusions: Development of molecular technology has improved and revolutionized TB diagnosis, especially drug resistance testing. However, it is still limited to being an add-on test to conventional microscopy if a new patient is to be initially investigated for pulmonary TB.

Keywords: Clinical practice, Diagnosis and treatment monitoring, Isoniazid efficacy and toxicity, Laboratory methods, Tuberculosis

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. It predominantly affects the lungs (pulmonary TB), and can also affect some other parts of the body. Clinical symptoms and manifestations include chronic cough and abnormal breath, but active TB may also be asymptomatic. Infection by the pathogen is by inhalation. Initial localization and multiplication is at the lungs and nearby lymph nodes. This is primary infection, which could resolve on its own. If this self-healing fails, the primary infection will progress to involve the lymphatics, kidney, joints, and central nervous system among others – i.e. secondary infection. Some factors predispose individuals to infection. These include living with a sufferer, overcrowding and illiteracy. Others include smoking and alcoholism.
Laboratory diagnosis of the disease is by isolation of the causative pathogen microscopically. It also involves preliminary rapid tests like Ziehl-Nelson stain, and gas chromatography. Fluorescent microscopy using Rhodamine and Auramine stains are also applicable. Suitable specimens for such diagnosis include sputum, bronchial washing, transtracheal aspirate, gastric aspirate and cerebrospinal fluid (CSF).

Isoniazid (INH) is one of the principal anti-tuberculosis organisms. It halts the growth of resting organisms (bacteriostatic), but predominantly kills the bacteria (bactericidal). Other drugs include rifampicin, streptomycin, aminosalycylates, thiosemicarbazones, thioamides, nicotinamides and aliphatic amines. Isoniazid is used with one or more of these drugs for treatment of TB because of the problem of resistant strains. That is, either to avoid chances of missing the isoniazid resistant strains, or to prevent the susceptible strain becoming resistant.

**Isoniazid metabolism, mode of action and toxicity**

Metabolism of isoniazid is, it is easily absorbed after oral and/or intraparenetheral administration. The peak plasma concentration of 3-5ug/ml is reached within 2 hours of administration. It diffuses and distributes equally well. It is metabolized by acetylation and has a mean half-life of 1.8 hours. Rate of acetylation is considered to be slow only when T½ is>2.5 hours. Although, the half-life of the drug is defined on the basis of fast acetylators is 1 to 2 hours. Elimination is largely independent of renal function; however the half-life may be prolonged in liver disease. More than 75% of a dose is metabolised and excreted in urine within 24 hours.

Blood levels of INH vary despite administration of identical doses/kg body weight. This is due to genetic acetylation polymorphism phenomena, which has both therapeutic and toxic consequences. The patients with lower blood levels acetylate the drug more rapidly while those with higher level of INH acetylate it more slowly. The former are fast acetylators while the later are slow acetylators. The focus of this study is neither on N-acetyltransferase 2 (NAT2) polymorphisms, nor the risk of anti-tuberculosis drug-induced liver injury (ATLI). However, it is worth appreciating that slow acetylators had a higher risk of ATLI and it is acknowledged that knowledge of patient’s phenotype will be useful for the clinical prediction and prevention of ATLI.

Fast and slow acetylator statuses are inherited in simple Mendelian manner with the fast acetylation gene being autosomal dominant. This accounts for the difference in acetylation rate among individuals and ethnic groups, and thus the variation in blood levels of INH. It has been reported that percentages of rapid acetylators ranges from 40% of European and South Indian descent to over 85% of Japanese and Eskimos. Rate of acetylation is proportional to the amount of liver enzyme and it is clinically important as per therapeutic response and toxicity. Genetically determined slow acetylators are more susceptible to INH toxicity as well as drug interactions. INH metabolism could also be explained as a conjugation reaction. A polar substance (Acetyl-group) condenses with the drug to form a conjugated product (acetyl-INH).

Pyridoxine is a keystone in the management of INH toxicity. With pyridoxine in excess or high concentration in the blood, the toxic effects of INH, especially peripheral neuropathies will not be observed. It (pyridoxine), with or without diazepam is an antidote for INH toxicity/overdose.

The summary of what is known is that toxicity depends on the rate of drug metabolism, which on this occasion is by acetylation. Depending on the rate of metabolism, there are fast, intermediate and slow acetylators, which can be determined from half-life of the drugs. There is greatest toxicity in slow acetylators. The rate of acetylation in individuals and ethnic groups is due to genetic inheritance. What is unknown is whether the people within the catchment zone of Tuberculosis and Leprosy (TBL) Eku centre are fast acetylators to justify long term 9 month INH treatment regimen. The objective of this project is to (1) determine the acetylator phenotype of patients with TB using the biological half-life of the drug in the patients’ blood; and (2) assess the incidence of susceptibility to the isoniazid toxicity.

**METHODS**

After ethical approvals and the delivery of research information, only thirteen TB patients consented to participate in the study. The 13 consenting participants were in-patients, of which two were recruited from Eku Baptist Hospital and eleven from Tuberculosis and Leprosy (TBL) referral centre, of Delta State Hospitals Management Board, Eku. These patients were adhering to the prescribed drugs. Venous blood samples were collected in every half hour for 3hrs after medication in the morning. 4hr and 5hr samples were intended, but most of the participants withdrew from giving further blood – citing needle pain as well as ‘too much blood’ as reasons.

Isoniazid tablets were obtained from the pharmacy department of Eku Baptist Hospital. The brand used was a product of BCN PLC (formerly The Boots Co. Nig. Ltd.). Bromine, sodium thiosulphate and reagent grade starch were obtained from the Biochemistry Department of the University of Benin. Other materials were obtained from the laboratory department of Eku Baptist Hospital. Isoniazid solution was initially prepared as 0.1% stock and working solution of 1:100 dilution of the stock was made when required. Other solutions included 0.5% Bromine; 1.6% sodium thiosulphate; 10% potassium iodide; and 0.1% reagent grade starch – all prepared with distilled water.
Isoniazid assay

*Principle:* The quantitative assay followed the titrimetric method described in the British Pharmacopoeia of 1968. This method was adopted based on materials that could be afforded and sourced as well as considering the scope of the study. The principle was that:

- In water, bromine will displace iodine from potassium iodide and the displaced iodine will react with sodium thiosulfate in the titration reaction [control].
- In isoniazid solution or isoniazid-contained solution (such as blood), the INH will complex bromine and reduce amount of iodine displaced from potassium iodide. Thus, less sodium thiosulfate will be needed in the titration reaction (test).
- The difference in volume of sodium thiosulfate used at control vs. test represents the amount of bromine required by, and therefore a measure of the INH.

*Protocol:* the titration followed a 3-step protocol, but the 2nd step was only for evaluation of the standard ‘working’ INH solution.

1. **Step 1:** As control, 5ml of solution (2) was added into 5ml of distilled water in a flat bottomed flask. 1ml of HCL was added and mixed for about 1 minute. It was then left for 15 minutes in room temperature before titration.

2. **Titration:** 2ml of potassium iodide solution was added followed with 3 drops of starch solution. The sodium thiosulphate solution was used to titrate the mixture until a colourless end point was reached. The volume of the sodium thiosulfate used was noted and recorded as the ‘control test volume’ (ctv).

3. **Step 2:** As standard, 5ml of bromine solution was added into 1ml of ‘INH working solution – 1:100 dilution’; dilute to 5ml with distilled water, 1ml of HCL was added and mixed for about 1 minute. It was then left for 15 minutes. Thereafter, titration was performed as in step 1. The volume of the sodium thiosulphate used up was noted and recorded as ‘standard test volume’ (stv).

3. **Step 3:** Venous blood samples were collected and were left at room temperature for about 20 before separation of the serum. The process was repeated as in step 2, but with serum instead of INH. The volume of the sodium thiosulphate used was noted and recorded as ‘blood test volume’ (btv).

Mathematical evaluation of results

The various results were obtained using the standard mathematical parameter of ‘1ml of bromine solution (0.1M) ≈ 0.0034g INH’ (British Pharmacopoeia, 1968); and the formulae:

- Concentration of INH in blood sample: (ctv-btv)/1000 x 3400µg/ml
- Where ctv = 653µl; stv = 652µl

Isoniazid Concentrations (µg/ml serum) were log transformed and afterwards presented in the graph plots. The participants’ phenotypes were then extrapolated from the graphs.

**RESULTS**

Descriptive statistics are usually presented as a table of averages not individual patient information. Due to very small sample size, the limited biodata of all the participants, comprising four females and nine males, with average age of 44±18 years are presented in table 1. The results of participant’s phenotype are presented in table 2.

### Table 1: Some of the biodata of participants.

<table>
<thead>
<tr>
<th>Home town</th>
<th>Duration of INH</th>
<th>Other drugs</th>
<th>Complain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uwheru</td>
<td>6 months</td>
<td>a, b, c, d</td>
<td>Fever</td>
</tr>
<tr>
<td>Ezionum</td>
<td>9 months</td>
<td>a, b</td>
<td>Trembling</td>
</tr>
<tr>
<td>Warri</td>
<td>1 week</td>
<td>a, b</td>
<td>Nil</td>
</tr>
<tr>
<td>Kokori</td>
<td>4 years</td>
<td>a</td>
<td>Hunch-back</td>
</tr>
<tr>
<td>Uwheru</td>
<td>1 week</td>
<td>a, b, c, d, e</td>
<td>Fever, edema</td>
</tr>
<tr>
<td>Eku</td>
<td>3 months</td>
<td>a</td>
<td>Nil</td>
</tr>
<tr>
<td>Ndoro</td>
<td>3 months</td>
<td>a</td>
<td>Nil</td>
</tr>
<tr>
<td>Orogun</td>
<td>2 months</td>
<td>a</td>
<td>Diabetes</td>
</tr>
<tr>
<td>Orogun</td>
<td>15 months</td>
<td>a</td>
<td>Joint pains</td>
</tr>
<tr>
<td>Obiaruku</td>
<td>2 years</td>
<td>a</td>
<td>Nil</td>
</tr>
<tr>
<td>Ogume</td>
<td>2 years</td>
<td>a, b</td>
<td>Nil</td>
</tr>
<tr>
<td>Ado</td>
<td>1 year</td>
<td>a, b</td>
<td>Nil</td>
</tr>
<tr>
<td>Obiaruku</td>
<td>18 months</td>
<td>a, b</td>
<td>Nil</td>
</tr>
</tbody>
</table>

*different from duration of INH treatment that was indicated to be <3months; a ~ Rifampicin; b ~ Pyridoxine; c ~ Chloroquine; d ~ Panadol; e ~ Multivite;

**DISCUSSION**

Analysis of the results obtained reveal that of the 13 patients, $t_0$ of INH was <2hr in seven; between 2 and 2.5hr in four; and >2.5hr in the remaining two (Table 2). Thus approximately 53.8%, 30.8% and 15.4% are fast, intermediate and slow acetylators respectively. The values for $t_0$ reported here is in fair accordance with earlier observations by Reidenberg and Mastin. The significance of this observation is that up to 46% (or at least the slow acetylators comprising 15.4%) of the participants do not require the long-term regimen and may be prone to undue toxicity.
Table 2: Result of INH t1/2 and the participants’ phenotype extrapolated from graphs.

<table>
<thead>
<tr>
<th>T₁/₂ (hr)</th>
<th>Acetylation phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.25</td>
<td>Slow</td>
</tr>
<tr>
<td>1.90</td>
<td>Fast</td>
</tr>
<tr>
<td>1.20</td>
<td>Fast</td>
</tr>
<tr>
<td>1.25</td>
<td>Fast</td>
</tr>
<tr>
<td>2.80</td>
<td>Slow</td>
</tr>
<tr>
<td>2.10</td>
<td>Intermediate</td>
</tr>
<tr>
<td>1.80</td>
<td>Fast</td>
</tr>
<tr>
<td>2.35</td>
<td>Intermediate</td>
</tr>
<tr>
<td>2.30</td>
<td>Intermediate</td>
</tr>
<tr>
<td>1.70</td>
<td>Fast</td>
</tr>
<tr>
<td>1.60</td>
<td>Fast</td>
</tr>
<tr>
<td>2.25</td>
<td>Intermediate</td>
</tr>
<tr>
<td>1.55</td>
<td>Fast</td>
</tr>
</tbody>
</table>

One of the objectives of the work was to assess the incidence of toxic effects of INH in TB patients within the population. Evaluation of the clinical notes including discussion with clinicians indicated (beside diabetes in one) complaint of fever by the 2/13 slow acetylaters, joint pains in 1/13 and trembling in 1/13. Although this assessment intended to focus more specifically on the slow acetylaters by studying known symptoms associated with INH toxicity, the potential of observing toxicity in all the other participants was strongly limited by three reasons. First, most of the patients were on pyridoxine, as per standard practice, to prevents toxic reactions in an otherwise susceptible patient. Second, most of the patients (53.8%) are fast acetylators. Fast acetylators are known to be less susceptible to INH toxicity.

Third, although all the participants had various durations of the disease, they have been on treatment regimen for less than 3 months prior to the study. For instance, one has had the disease for 4 years but has been on the drug consistently for less than 3 months (Table 1). Nevertheless, he is a fast acetylator. It was therefore considered too early to assess the patients for chronic INH toxicity attributable to prolonged usage.

Despite the limitations, an examination of the bio data of the patient’s vis-à-vis their rate of INH metabolism leads to the following observations:

- The 2 patients that have t₁/₂ of INH >2.5hr out of the 13 are indigenes of the same village. Since the rate of acetylation is genetically determined, the significance of this observation needs to be ascertained – that is, whether slow acetylation phenotype is present in that particular community. The full implication in terms of INH toxicity for the community can be determined when the acetylation phenotypes of a reasonable number of members of that community are established. Nevertheless, this observation puts the results of this work in agreement with established facts [7, 19].

- One of the patients, a male developed Hunch-back within 2 months of commencement of disease/treatment. He discontinued treatment without consulting his physician and recommended <3months prior to the study. However, there is no evidence to show that hunch-back in pulmonary TB patients is due to INH toxicity. Instead, a possible explanation is tuberculous kyphosis due to the causative organism migrating to, and settling in the skeletal system.20,21

Among the 13 patients studied, none reported any serious symptoms except fever, which was successfully treated with chloroquine. This implies that the fever is not as a result of INH toxicity. Only one of the participants complained of joint pain. He was not on pyridoxine and the present work shows that he is an intermediate acetylator. It was known that joint pain is associated with INH toxicity in slow acetylators and in the absence of pyridoxine (Dr Ajayi: Personal Communication).

Updates and implications for respiratory medicine

Update from TBL Centre, Eku

When the World Health Organization attempted to determine the affordability and cost effectiveness of using Xpert MTB/RIF to diagnose TB, the wide variations in underlying assumptions and intended use of Xpert MTB/RIF made a systematic review impossible.22 Furthermore, it is known that the technology being for rifampicin resistance testing – i.e. not isoniazid.22 Therefore, one of the key objectives of revisiting the TBL Centre Eku for this update was to establish that GeneXpert technology is available at the rural TBL facilities; what the technology entails in terms of molecular methods; and how it has changed or could change the practice of diagnosis of disease as well as evaluation or monitoring of treatment.

First, we observed, with respect to diagnosis and monitoring of treatment, that the traditional microscopy method involving the use of the Ziehl-Nelson’s acid fast bacteria (AFB) test remains the major laboratory method in use at the Centre. However, since the establishment of a Cepheid GeneXpert technology unit at this Centre, this real time PCR-based molecular method is currently being used alongside the AFB test for diagnosis and to confirm rifampicin resistance among those undergoing treatment. Secondly, Staff at the centre confirmed that GeneXpert does not necessarily differentiate between rifampicin or isoniazid resistance.

Therefore, whether Cepheid GeneXpert is believed to be for rifampicin resistance testing only, or product information indicates that rifampicin and isoniazid resistance occurs concurrently, a sample giving a positive result for resistance is further subjected to a confirmatory
The GeneXpert® MTB/RIF test is currently the only molecular test of its kind and uses a cartridge containing all elements necessary for the reaction, including lyophilized reagents, liquid buffers and wash solutions. Target gene detection and characterization is performed in real time using a six-colour laser detection device. The technology is truly revolutionary, and is designed to avoid wet interface between instrument and cartridge to eliminate cross contamination; an integrated ultrasonic horn is used for rapid lysis of spores; it employs total internal control of reagent system, since no separate external positive or negative controls are required.

Despite obvious superior sensitivity, accuracy and speed of the GeneXpert technology compared with conventional microscopy, the World Health Organization (WHO) advises that microscopy will continue to play an important role in the diagnosis of TB for the foreseeable future, as well as the monitoring of a patient’s response to treatment, since the new technology has not been designed for this purpose. In other words, while the development of molecular technology may have improved and revolutionized TB diagnosis as well as drug resistance testing, it is still limited to being an add-on test to conventional microscopy if the patient is to be initially investigated for pulmonary TB.

With regards to the evaluation of therapy, it is pertinent to note that the molecular technology is identifying drug resistance. What happens in a drug-responsive patient in terms of efficacy and toxicity of the treatment is still subject to evaluation and monitoring by other clinical laboratory methods. Furthermore, whether a patient is a fast acetylator (requiring long term drug regimen) or a slow acetylator (requiring short term drug regimen) is now a subject of research interest, but acetylation phenotype has yet to be on the post-treatment or pre-treatment laboratory testing list; probably because there is still controversy over the necessity of acetylation monitoring including the role of genetics. The implication is that every patient in the population is still being treated in a similar manner without pathology-based evidence as if they are all fast acetyulators.

**Update from literature – brief narrative**

A national TB prevalence survey was completed in 2013, and it is believed that Nigeria now ranks 11th among the 22 high burden countries that account for 80% of the global TB burden, although the actual burden of TB in the country may be about five times more than what is being notified by the programme. As at 10 years after the original study – i.e. in 2006, Nigeria ranked 5th for the highest number of reports of 9.2million new cases registered worldwide. A recent descriptive study of secondary data from a TB control programme showed that the annual number of all TB cases increased from 914 cases in the year 2000 to 1684 in 2009; and that while the proportion of new sputum smear diagnoses of pulmonary TB declined, extra-pulmonary TB cases increased by about four fold. This is evidence that pulmonary TB as a clinical condition is being managed and monitored by the practitioners in the country. There is also a report that the Federal Ministry of Health has established ten reference laboratories and scaled up GeneXpert technology from 9 in 2011 to 52 in 2013 to enhance diagnosis of drug susceptible and drug resistant TB.

In the process of this review, it has been found that several studies on acetylation phenotype for isoniazid have been reported prior to the 1996 study. The reports indicated 41%-51% of slow acetylators were observed in Nigerian populations being treated with sulfamethazine, and highlighted a hypothesis that slow acetylator phenotype may be more prevalent in populations of people living nearer the Equator relative to the Arctic Circle. While our report from the 1996 study was not in line with the hypothesis of more slow acetylators (i.e. given that Nigeria is nearer to the equator), the observation of a higher proportion of the subjects being fast acetylators corroborated with a West African study.

**Implications for evaluation and management of toxicity by laboratory methods**

**Toxicity**: Isoniazid causes toxicity by enhancing an increase in pyridoxine excretion thereby causing deficiency of the latter. Rate of acetylation is dependent on availability of N-acetyltransferase and a genetic disorder where the enzyme is deficient potentiates toxicity as such in slow acetylators. However, lower efficacy and toxicity also occurs among fast acetylation. Major toxicities are known to include neuropathies, hyperlipidaemia and hepatitis. Other minor toxic reactions of INH include convulsions as well as agranulocytosis and pellagra, which develops within three months especially in INH overdose.
Management of toxicity

Isoniazid toxicities have been managed in different ways including pyridoxine therapy, which is useful in the control of seizures and overdose of INH. It is particularly recommended for the cure and management, with or without withdrawal, of peripheral neuropathies, poisoning, rash, fever and acidosis. Fluids and sodium bicarbonate administration is recommended for poisoning and acidosis. Nicotinic acid therapy and full balance diet is recommended for pellagra-like skin lesion. Above all, meticulous observation of asymptomatic individuals and withdrawal of INH therapy is recommended for peripheral neuropathies, poisoning, psychosis, agranulocytosis and others, except where any other form of aforementioned measure could suffice.

Implication for laboratory methods

From the foregoing, a minimum of four conventional diagnostic indices (lipid profile, liver function, renal function and white blood cell differential) are necessary for clinical laboratory monitoring in a drug-responsive patient in terms of efficacy and toxicity of the treatment. Firstly, toxicities are associated with hepatitis and hyperlipidaemia as well as agranulocytosis. This implies that changes in liver function and/or lipid profile tests as well as leucocytosis could indicate the level of toxicity. Secondly, fluids and electrolyte imbalance including acidosis are implicated in pellagra. This implies that serum electrolyte or renal function test could be useful in evaluating of drug toxicity.

It has been noted for a long time that "pellagra is a well-known complication of isoniazid (INH) therapy, and if the clinical diagnosis is missed or delayed then this may cause life-threatening consequences. Due to the diversity of pellagra-related signs and symptoms, the diagnosis can be made with an appropriate index of suspicion", which includes simple laboratory tests like routine full blood count to determine anaemia, aforementioned liver function test to determine hypoprothrominemia, and electrolytes to determine hypercalcaemia and hypokalaemia. Specific tests include niacin, N-methyl nicotinamide and pyridone whereby deficiencies confirm the diagnosis of pellagra.

CONCLUSION

Out of the 13 patients which represents a random small sample of population serviced by the TBL Referral Centre, Eku, 53.8% are fast acetylators; while 15.4% are considered to be slow acetylators. Development of molecular technology has improved and revolutionized TB diagnosis, especially drug resistance testing. However, it is still limited to being an add-on test to conventional microscopy if a new patient is to be initially investigated for pulmonary TB. Analysis of drug toxicity still benefits from available conventional diagnostic laboratory methods.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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