

Clinical Study

Performance of Clinical Criteria for Screening of Possible Antiretroviral Related Mitochondrial Toxicity in HIV-Infected Children in Accra

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Mitochondrial damage is implicated in highly active antiretroviral therapy (HAART) toxicity. HIV infection also causes mitochondrial toxicity (MT). Differentiating between the two is critical for HIV management. Our objective was to test the utility of the Mitochondrial Disease Criteria (MDC) and the Enquête Périnatale Française (EPF) to screen for possible HAART related MT in HIV-infected children in Ghana. The EPF and MDC are compilations of clinical symptoms, or criteria, of MT: a (+) score indicates possible MT. We applied these criteria retrospectively to 403 charts of HIV-infected children. Of those studied, 331/403 received HAART. Comparing HAART exposed and HAART naïve children, the difference in EPF score, but not MDC, approached significance ($P = 0.1$). Young age at HIV diagnosis or at HAART initiation was associated with (+) EPF ($P \leq 0.01$). Adherence to HAART trended toward an association with (+) EPF ($P = 0.09$). Exposure to nevirapine, abacavir, or didanosine increased risk of (+) EPF (OR = 3.55 (CI = 1.99–6.33), 4.76 (2.39–9.43), 4.93 (1.29–18.87)). Neither EPF nor MDC identified a significant difference between HAART exposed or naïve children regarding possible MT. However, as indicators of HAART exposure are associated with (+) EPF, it may be a candidate for prospective study of possible HAART related MT in resource-poor settings.

1. Introduction

Since the advent of highly active antiretroviral therapy (HAART), morbidity and mortality have been greatly reduced in children living with HIV [1]. Unfortunately, long-term use of HAART, specifically of nucleoside reverse transcriptase inhibitors (NRTIs), causes mitochondrial dysfunction manifesting clinically as lipodystrophy, lactic acidosis, peripheral neuropathy, myopathy, and pancreatitis [2–13]. Moreover, HIV infection also causes mitochondrial damage [14–16]. Differentiating between the two could influence HAART regimens, especially in resource-poor settings where the early generation NRTIs are still considered first line

therapy [17]. In addition, there are other sequelae of mitochondrial dysfunction such as anemia, poor growth, and cognitive delay [18] that may have a greater impact on developing children than adults, and unfortunately reports of mitochondrial toxicity in children are increasing [6, 19]. These side effects are a particular problem in those children who have acquired HIV perinatally, as they will likely remain on HAART throughout their lifetime. Therefore, a need exists for a sensitive and low-cost screening tool to detect HAART associated mitochondrial toxicity in children living in resource-limited settings in order to help guide the allocation of further diagnostic resources where they are available or distribution of second-line HAART regimens.

Ideally, there would be a cost effective way in which to detect the effect of HAART on mitochondria prior to the development of clinical toxicity. Studies in adults and children have looked at the use of peripheral blood mononuclear cells to detect lower concentrations of mitochondrial DNA [5, 8, 19–24], mitochondrial RNA [5, 20, 25], and mitochondrial proteins involved in oxidative phosphorylation [22, 25, 26], as well as serial venous lactate levels [8, 27] as possible screening tests. Results have been conflicting as to the clinical utility of these tests; furthermore, in the developing world, the cost of routine laboratory work can be prohibitive for many families, or the technology to run the tests may not be readily available.

Crain et al. demonstrated that two sets of clinical criteria, the Enquête Périnatale Française (EPF) and the Mitochondrial Disease Classification (MDC), may be useful in screening HIV positive children receiving HAART for mitochondrial toxicity. Each tool is comprised of multiple clinical symptoms and diagnoses, a combination of which when present results in a positive score. They demonstrated that exposure to lamivudine and/or stavudine was independent risk factors for a positive score for both criteria sets. In addition, they showed that mortality was increased in those children who scored positive on either the EPF or the MDC, but was highest for those who scored positive on both criteria sets. However, no specific testing, such as biopsy of the affected tissue, was done to confirm mitochondrial toxicity in these patients [28].

The main objective of our study was to perform a retrospective chart review of those perinatally infected HIV positive children being treated for their disease at the Korle Bu Teaching Hospital in Accra, Ghana in order to assess whether the EPF or MDC could be applied in this resource-poor setting as a potential screening tool for possible mitochondrial toxicity. The primary goal was to determine whether we could replicate the HAART specific findings of Crain et al., using only the clinical assessments and documentation already in place. Secondary goals were to note the medications in use that had a higher correlation with a positive score, to identify those specific clinical criteria that might be more prevalent in this population, and to determine whether the EPF or MDC could identify a difference in the prevalence of mitochondrial toxicity between HAART naïve and HAART experienced patients.

2. Materials and Methods

2.1. Study Population. This was a single center retrospective chart review at the Pediatric HIV/AIDS Care program at Korle Bu Teaching Hospital in Accra, Ghana. The study population consisted of all confirmed HIV-infected children, both HAART naïve and HAART experienced, since 2004, the year that HAART became available at Korle Bu. Pediatric charts of HIV-infected children aged from 0 to 13 years were reviewed. The time period covered began February 2004 and ended April 2011. Charts were excluded from the review if no documentation of a confirmed HIV diagnosis could be found. Confirmatory testing accepted included viral RNA copy for

TABLE 1: Modified mitochondrial disease criteria and Enquête Périnatale Française.

MDC
Muscular symptoms
(i) Progressive external ophthalmoplegia
(ii) Ptosis, facies myopathica
(iii) Exercise intolerance
(iv) Reduced muscle power or muscular hypotonia <6 months
(v) Acute rhabdomyolysis
(vi) Abnormal EMG*
CNS symptoms
(i) Delayed or absent psychomotor development or mental retardation
(ii) Loss of acquired skills
(iii) Stroke like episodes
(iv) Seizures
(v) Migraine
(vi) Myoclonus or myoclonic epilepsy
(vii) Cortical blindness
(viii) Pyramidal tract involvement (increased muscle tone, opisthotonus, increased DTR, and upward Babinski)
(ix) Extrapyramidal involvement (athetosis, dystonia, and involuntary movement)
(x) Brainstem involvement
(xi) Cerebellar involvement (ataxia, intention tremor, and dysdiadochokinesis)
Multisystem symptoms
(i) Sideroblastic anemia
(ii) Pancytopenia
(iii) Hepatic dysfunction
(iv) FTT
(v) Pancreatic dysfunction
(vi) Intestinal pseudo-obstruction
(vii) >3 weeks chronic diarrhea
(viii) Short stature (< -2 SD or <3rd%)
(ix) Delayed puberty
(x) DM I or II
(xi) Hypoparathyroidism
(xii) Central DI
(xiii) Hypertrophic or dilated cardiomyopathy
(xiv) Conduction block
(xv) Proximal tubular dysfunction
(xvi) FSGS
(xvii) Cataracts
(xviii) Retinopathy
(xix) Optic atrophy
(xx) Sensorineural hearing loss
(xxi) Exacerbation of any of the above symptoms with minor illness
(xxii) Sudden unexplained infant death in family history

TABLE 1: Continued.

MDC
Metabolic labs*
(i) Elevated lactate >2000 umol/L on 3 occasions
(ii) Elevated L/P ratio >18
(iii) Alanine >450 umol/L
(iv) CSF lactate >1800 umol/L
(v) CSF protein
(vi) CSF alanine
(vii) Elevated urine amino acids or lactate
(viii) Urine ethylmalonic acid or 3-methylglutaconic acid or dicarbonic acids
(ix) Abnormal muscle bx,
(x) Abnormal brain MRI
EPF
Major criteria
(i) Nonfebrile seizures
(ii) Febrile seizures (>2 episodes or 1 episode in child <6 months)
(iii) Peripheral neuropathy
(iv) Acquired microcephaly
(v) Cranial nerve paresis
(vi) Impaired cognitive development (>1y)
(vii) Cerebellar dysfunction and ataxia
(viii) Motor disabilities, paraparesis, spasticity
(ix) Abnormalities on MRI or CT scan*
(x) Pancreatitis
(xi) Cardiomyopathy
(xii) Myopathy
(xiii) Decrease in visual acuity, retinopathy
(xiv) Abnormal ocular motor function
(xv) Nystagmus
(xvi) Deafness
(xvii) Unexplained death
Minor criteria
(i) Febrile seizures
(ii) Isolated changes in muscle tone (hypo or hyper)
(iii) Behavioral disturbances and hyperactivity disorder
(iv) Moderate cognitive delay
(v) Increase in transaminase levels
(vi) Persistent anemia, neutropenia, or thrombopenia
(vii) Tubular defect (renal)

*Criteria not used in this study.

children under the age of 18 months, or positive ELISA and Western Blot in a child over the age of 18 months.

2.2. Chart Review. Each chart was manually reviewed in order to try to identify those children who met the specific criteria outlined in these two case definitions. The same

individual reviewed all charts included in this study. Charts were pulled from the central chart room at the Korle Bu Pediatric HIV Clinic in groups of twenty. Once documentation of a positive HIV diagnosis was identified, the chart number was recorded. Demographic data was reviewed first for each chart, followed by MDC criteria, followed by EPF criteria. For each data set, the entire chart was reread looking for the specific information; thus each chart was read from cover to cover approximately three times in total. The charts were provided by the World Health Organization and were specific for pediatric HIV patients. The format included both checklists of questions reviewed at each visit, as well as space for free-texting by the physician, though the latter was utilized less frequently. Positive criteria were recorded regardless of HAART status at the time the symptom or diagnosis was recorded.

Additional data collected included current age in months as of April 2011, date of birth, sex, age at HIV diagnosis, exposure to prenatal ART, WHO stage, date of HAART initiation, medications used and total length of time on each medication up to the last documented visit, number of HAART regimen changes, and adherence to medication regimens. The WHO stages recorded were those noted at the last visit on record in the chart prior to April 2011. HAART usage was at any time point from February 2004 through April 2011. Any exposure to individual medications was totaled by number of months spent on that medication. Exposure to HAART was defined as any amount of time on HAART during the study period. Adherence was self-reported by the child or the parent and recorded in the chart as “taking medications as prescribed” or in some cases, the physician noted the number of missed medication doses in the last week or month.

2.3. Definition of Clinical Tools. Two clinical case definitions of mitochondrial disease were used: the Mitochondrial Disease Criteria (MDC) and the Enquête Périnatale Française (EPF) criteria (Table 1).

The MDC was developed to stratify those children in the general pediatric population whose combination of signs and symptoms might be due to mitochondrial dysfunction [18]. The criteria of the MDC are divided into four categories: muscular, central nervous system, multisystem, and metabolic labs. Over 40 diagnoses are included, with points assigned based upon the number of systems involved with 0-1 points being unlikely mitochondrial disease, 2-4 points as possibly, 5-7 points as probably, and 8-12 points as definitely. The maximum number of points that may be accumulated without including the metabolic labs category is five; thus, for the purposes of our *clinical* study the highest risk category that a child could attain was “probable mitochondrial disease.”

The EPF criteria have been used primarily to evaluate *HIV negative infants* who were exposed to HIV and ART in utero [29, 30]. The EPF contains major and minor criteria. The child is considered to have possible mitochondrial disease if he or she fulfills one major criterion on any clinic visit, or two minor criteria on two separate visits. Although the EPF

TABLE 2: Demographics.

	HAART Experienced (<i>n</i> = 331) <i>n</i> (%)	HAART Naive (<i>n</i> = 72) <i>n</i> (%)	<i>P</i> value
Sex:			0.25
Female	159 (48.0)	40 (55.5)	
Male	172 (52.0)	32 (44.4)	
Average age (months)	108.1 ± 41.4	97.3 ± 40.7	0.05*
Average age at diagnosis (months)	55.7 ± 37.5	63.6 ± 39.1	0.09
Congenital transmission	320 (99.1)	70 (98.6)	0.55
Exposure to prenatal ART:	6 (1.8)	6 (1.5)	0.60
Current WHO stage:			<0.0001*
I	27 (8.2)	24 (34.3)	
II	56 (17.0)	22 (31.4)	
III	154 (46.7)	13 (18.6)	
IV	93 (28.2)	11 (15.7)	

*Statistical significance.

criteria are nearly all clinical, it does include abnormalities on CT or MRI of the brain as well as routine laboratory abnormalities, such as persistent anemia, thrombocytopenia, or leukopenia, and elevated transaminases. These studies were not routinely available for every chart reviewed; however, when the information was noted, it was included in the data collection.

2.4. Statistics. Fisher's Exact Test was used to determine significance regarding MDC or EPF positive scores for each cohort. McNemar's Test was used to establish correlation of the two clinical tools. Logistic regression models were applied to the medications and individual criteria to determine risk related to positive score.

3. Results

Of the 403 children with documented HIV infection, 331/403 (82%) were HAART experienced as of April 2011. Older children ($P = 0.05$) and children with higher WHO stages as recorded at their most recent visit ($P < 0.0001$) were more likely to be on HAART. Otherwise, HAART experienced and HAART naive groups were similar in all aspects evaluated (Table 2).

The MDC did not demonstrate a difference between HAART experienced and HAART naive cohorts with regards to possible mitochondrial toxicity ($P = 0.81$). In addition, only one child from each cohort scored in the "probable mitochondrial disease" category (Table 3). The EPF also did not demonstrate a significant difference in mitochondrial toxicity between the HAART experienced and HAART naive cohorts; however, the P value approached significance at 0.10 (Table 3). Therefore, the remainder of our analysis focused on the EPF as we were interested to see if any associations might be worth further investigation. Male sex ($P = 0.012$),

TABLE 3: MDC and EPF total scores.

	HAART Experienced (<i>n</i> = 331) <i>n</i> (%)	HAART Naive (<i>n</i> = 72) <i>n</i> (%)	<i>P</i> value
MDC categories			
Metabolic disease unlikely (0-1 point)	220 (66.5)	52 (69.4)	
Metabolic disease possible (2-4 points)	110 (33.3)	22 (29.23)	0.81
Metabolic disease probable (5-7 points)	1 (0.3)	1 (1.4)	
EPF categories			
Positive	53 (16.0)	6 (8.3)	
Negative	278 (83.9)	66 (91.6)	0.10

TABLE 4: Risk factors for EPF positive score.

	EPF Positive (<i>n</i> = 59) <i>n</i> (%)	EPF Negative (<i>n</i> = 344) <i>n</i> (%)	<i>P</i> value
Male	38 (64.4)	166 (48.3)	0.012*
Average age (months)	97.7 ± 45.6	107.6 ± 40.5	0.088**
Average age at diagnosis (months)	46.3 ± 40.0	59.1 ± 37.0	0.002*
Total exposure time to HAART (months) [†]	49.1 ± 24.4	47.3 ± 26.0	0.469
Age at HAART initiation (months) [†]	55.5 ± 43.1	71.2 ± 37.0	0.003*
Current WHO stage			<0.0001*
I	4 (6.8)	47 (13.8)	
II	5 (8.4)	73 (21.4)	
III	21 (35.6)	146 (42.8)	
IV	29 (49.2)	75 (22.0)	
Number of HAART regimen switches [†]	3 (5.67)	18 (6.48)	0.532
Nonadherent [†]	10 (18.87)	84 (30.22)	0.093**

*Statistical significance ($P < 0.05$).

** ($P < 0.10$).

[†] Calculated using only those patients who are on HAART ($n = 331$, with 53 EPF Positive/278 EPF Negative).

higher WHO stage at the last recorded visit ($P < 0.0001$), younger age at HIV diagnosis ($P = 0.002$), and younger age of HAART initiation ($P = 0.003$) were all significantly associated with EPF positivity. Interestingly, self- or parent-reported *adherence* to medication ($P = 0.093$) and younger overall age as of April 2011 ($P = 0.088$) trended toward a significant association with EPF positive score (Table 4).

Medications in use at Korle Bu in order of most common to least common were lamivudine ($n = 331$), zidovudine ($n = 327$), efavirenz ($n = 250$), nevirapine ($n = 93$), abacavir ($n = 44$), kaletra ($n = 16$), stavudine ($n = 21$), didanosine ($n = 9$), and nelfinavir ($n = 3$). The most common HAART regimen in use was zidovudine plus lamivudine, and either efavirenz or nevirapine. Any exposure during the course

of HAART treatment to nevirapine (OR = 3.55, 95% CI = 1.99%–6.33%), abacavir (OR = 4.76, 95% CI = 2.39%–9.43%), or didanosine (OR = 4.93, 95% CI=1.29%–18.87%) appears to increase an individual's risk of a positive EPF score. Any exposure to efavirenz (OR = 0.50, 95% CI=0.28%–0.87%) appears to be protective. None of the clinical or laboratory criteria of those children with a positive EPF seem to occur more frequently in the HAART experienced or HAART naive patients ($P = 0.18$ – 1.00). Overall, the most common positive criteria in the 59 children with a positive EPF score regardless of HAART status include the following: moderate cognitive delay (entered into charts as developmental delay) (38.9%), persistent anemia (hemoglobin < 10 on more than one laboratory result) (38.0%), increased transaminases (ALT and AST > 40 on more than one laboratory result) (22.0%), decrease in visual acuity/retinopathy (16.9%), cardiomyopathy (15.2%), motor disabilities (13.5%), nonfebrile seizures (10.0%), impaired cognitive development (entered into chart as severe developmental delay or mental retardation) (8.4%), and changes in muscle tone (8.4%).

4. Discussion

To date, our study is the only one to have examined a clinical criteria set for possible HAART related mitochondrial toxicity in a resource-limited setting. We were unable to demonstrate a clear difference in either MDC or EPF positive scores between HAART experienced and HAART naive children; however, our findings highlight some important associations that are worth discussion and further research. In resource-limited settings, HIV positive children are only beginning to have access to the lifesaving antiretroviral medications. Unlike in the Western World where new, potentially less toxic, medications are constantly being exchanged for the old, these children will likely continue on early generation antiretrovirals for the foreseeable future. Monitoring for toxic effects is important, not only to document their occurrence, but also to ultimately prevent nonadherence leading to increased viral loads and HIV transmission. A clinical tool would be ideal, as it would be low cost, easy to implement, and universally accessible. Our study suggests that that the EPF may be worth further investigation as that tool.

We found that any exposure to nevirapine, abacavir, or didanosine, as well as a younger age of HAART initiation was significantly correlated with EPF positivity. In addition, adherence, meaning those children (or in some cases, their parents) self-reporting compliance with their medications, trended towards significant correlation with EPF positivity. These findings hint that perhaps there is an association between HAART exposure and a positive EPF score, and therefore possible mitochondrial toxicity, which was simply not illuminated in a retrospective study. In addition, the difference in EPF scores between HAART exposed and HAART naive patients did trend towards significance. Studying the EPF in a prospective model would help to determine whether this trend could be beneficial to patient management decisions.

There were clear limitations to our study design. One major problem that we encountered had to do with the way in which records were kept at our site. Charts were hand written, brief, and subjective. There was a lack of basic laboratory data, and even in those cases where there was an indication in the chart that laboratory studies had been obtained, often no results were recorded. There were instances when portions of charts were missing, or charts were accidentally taken by patients and never returned. All of these things contributed to a heterogeneous data set. In addition, the criteria sets themselves are composed of both objective and subjective components. For example, the EPF criteria include “impaired cognitive development” which may be described in the chart in a variety of indirect ways, alongside more objective findings such as “cranial nerve paresis.” During the retrospective review of the chart, we could only score criteria as positive if they were documented in a way that made the diagnosis, or suspected diagnosis, explicit. Because of this, it is possible that many diagnoses were underscored. This bias would be lessened in a prospective model in that the physician making the diagnosis would be the same physician marking the criteria as positive. Future studies would benefit from an objective tool, possibly a set form to be completed or, even better, a biologic marker of mitochondrial toxicity.

It may be that we were ultimately measuring something other than mitochondrial toxicity in this population. Malnutrition, low standard of living, and low parental education level could all contribute to many of the criteria met by this population of children, such as “anemia” and “moderate cognitive delay.” That being said, the hallmark of mitochondrial disease is multiorgan system involvement [31], a key point that the two scales utilize to try to tease out who has mitochondrial disease versus another disease process. Furthermore, other criteria that were commonly met, such as “cardiomyopathy” and “decreased visual acuity” are not as readily explained by poor living standards and are often regarded as red flags in children as possible indicators of mitochondrial disease [31]. Although some of these symptoms are also among those expected with HIV infection, the mechanisms are not always entirely clear; hence the interest in determining if there is a difference in the rate of occurrence of the clinical criteria put forth in the EPF between HIV infected children exposed to and naive to HAART in a prospective model.

Also important to note is that without a reliable, objective marker of mitochondrial toxicity with which to compare the clinical results, it will be difficult, even in a prospective model, to prove a true association between a positive score in either the MDC or EPF with actual mitochondrial toxicity. The positive predictive values of the EPF and MDC, especially when used without consistent laboratory and imaging data, are unknown. In the study that originally compiled the criteria set for the EPF, only 29/196 EPF positive children were considered to have symptomatology compatible with true mitochondrial dysfunction [29], thus giving the EPF a PPV of approximately only 14.7%. After scoring positive on the EPF, these children were evaluated by a physician who determined if there was another possible cause for their symptoms other than mitochondrial disease. In addition, if the symptoms

resolved without any intervention, the child was believed not to have mitochondrial disease. Interestingly, of the 29 thought to have true mitochondrial dysfunction, 7 went on to have a tissue confirmed diagnosis, 14 had laboratory data supporting the diagnosis, and 8 had not yet had diagnostic studies performed at the time of publication. Thus the application of this algorithm for those with positive EPF scores increases the PPV, something that we could utilize in a prospective study, but could not take advantage of in the retrospective study design. The diagnosis relies heavily upon the combination of clinical symptoms, laboratory and imaging abnormalities, as well as an invasive tissue biopsy as the gold standard; thus, highlighting the need for a clinical tool, especially in resource-limited settings, with the capability to identify those who would benefit most from these costly diagnostic procedures.

The only other group to have examined the MDC and EPF in the context of HAART related mitochondrial toxicity is Crain et al. In contrast to our findings, they reported that exposure to lamivudine and/or stavudine was independent risk factors for a positive score for both EPF and MDC criteria sets. They also had 16.4% of their study subjects, compared to our 0.4%, meeting criteria for mitochondrial toxicity on *both* the MDC and EPF. Major differences that likely account for these discrepancies between our studies include the design and the study population. Their study was designed as a prospective cohort study; ours was a retrospective chart review. Their study subjects were participants in the Pediatric AIDS Clinical Trials Group (PACTG). Although some protocols under this group were conducted in resource-poor settings, protocols 219 and 219C were only conducted at sites in the United States and Puerto Rico (ClinicalTrials.gov no. NCT00006304). Therefore, to date, our study is the only one to have examined a clinical criteria set for possible HAART related mitochondrial toxicity in a resource-limited setting.

5. Conclusions

Our study serves as a starting point for those interested in the evolution of HAART associated mitochondrial toxicity in resource-limited settings. We demonstrated an association between indicators of HAART exposure and EPF positive scores. The retrospective design of our study was not able to detect a significant difference with regards to possible mitochondrial toxicity between HAART exposed and HAART naïve children. However, the trend towards significance encourages us to think that the EPF is a good candidate for further study in a prospective model as a screening tool for possible mitochondrial toxicity. Such a tool could help guide the use of more costly laboratory testing, diagnostics, and even allocation of second line medications in resource-limited settings.

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