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Multiple Episodes of TB Coinciding With Emerging Drug Resistance Mutations: 14 Years Follow up of an Indian Patient With Clade C Infection

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ABSTRACT

India has the second highest number of AIDS cases in the world but there is paucity of data on drug resistance mutations. There are some specific mutations that occur in patients harboring clade C virus. Tuberculosis is a common confection in these patients. We describe a male patient aged 38 years, with heterosexually acquired clade C who reported 12 years ago with high viral load and a CD 4 cell count of 28/mm^3. He posed a formidable challenge to the physician when only two drugs were available. His hemoglobin dropped to 4 gms% after zidovudine administration, developed 4 episodes of TB of different organs, 2 episodes of candida, single episode of pneumocystis pneumonia and repeated treatment failure over a period of 11years. He had a total of 17 mutations; 8 at 7 NRTI sites, 4 at 3 NNRTI sites and 5 at 4 PI sites, uncommon combination of TAMI and other typical clade C mutations and was resistant to most NRTI and NNRTI drugs. He was finally put on a five drug regimen including four protease inhibitors in 2008 and is doing well. He had to spend personally more than 2.3 million Indian Rupees (50000 US dollars) in 10 years to fight the onslaught of rapidly mutating virulent virus. In a resource constrained setting one may be forced to resort to a suboptimal medication in a desperate attempt to save the patient as in this case. We were able to prolong life but at a price as he was resistant to most NRTI and NNRTI drugs. There is a dire need for a cost effective, simple method to identify drug resistance mutations in resource poor countries for a rational approach to therapy. It high lights the economic burden in era of HAART and future strategies required to curtail resistance mutations. This case also gives an insight into the pitfalls of treatment of tuberculosis in patients suffering from AIDS. In our experience splenic tuberculosis in HIV positive individuals could be considered as an AIDS defining disease.

INTRODUCTION

The HIV virus has evolved into several variants, called HIV-A through HIV-H, with HIV-A and HIV-C being the most common in Africa while in India clade C predominates1. India has the second largest number of HIV cases in the world. Many patients on Highly Active Antiretroviral Therapy (HAART) eventually acquire resistance to several drugs posing a serious challenge to the physician. To compare drug resistance rates across geographic regions, the World Health Organization recommended adoption of a consensus genotypic definition of transmitted HIV-1 drug resistance [2] Valezquez et al [3] documented that the new drug-resistant proteases were up to 1000 times more resistant to protease inhibitors and recommended use of existing therapies aggressively to suppress or delay the emergence of resistant mutations.

A standard list of mutations to characterize the epidemiology of drug resistance[4] can facilitate meta-analyses of surveillance data collected by different groups at different times. As the number of patients on HAART increase in India, we will continue to witness a large number of cases with drug resistance mutations. We report here a case of HIV with clade C, diagnosed in 1997 who showed several mutations with multiple episodes of tuberculosis but eventually had undetectable viral load on new drug combinations

PATIENT AND METHODS

A 30 year HIV positive educated male was referred to the department in 1997. He had lost 5 kilos and his CD 4 count was 48/mm^3. He also had sputum positive pulmonary tuberculosis (TB) there fore put on a four drug regimen of antitubercular drugs (ATT). His viral load was 25000 RNA copies/ml and needed highly active antiretroviral therapy (HAART) but at the time only zidovudine and lamivudine were available in the market. After 2 months, his hemoglobin dropped to 4 gms% (table1) and AZT was replaced with stavudine. Subsequently, nevirapine became available in 2001 which was added but he started to deteriorate in 2003, developed lyodystrophy, low CD4 count and high viral load, pneumocystis pneumonia (PCP) and oral Candida. For

which he was treated effectively. Aspiration of a hypo echoic splenic lesion on ultrasonography revealed AFB positivity. He also developed consolidation in right lung and TB of cervical lymph nodes. He responded initially to ATT but low grade fever continued and fresh nodes appeared. TB was particularly difficult to treat during Dec 2002 to February 2003 when various permutation and combination had to be tried including streptomycin, ethambutol, pyrazinamide, isonaizide rifampicin, amicacin, ciprofloxacin and clarythromycin to cover atypical mycobacterium. He also showed features of immune reconstitution syndrome (IRS) in March 2003 which responded to a course of prednisolone. On two occasions, the CD4 cells were>300/mm^3 yet the viral load assay revealed> 100,000 copies/ml.

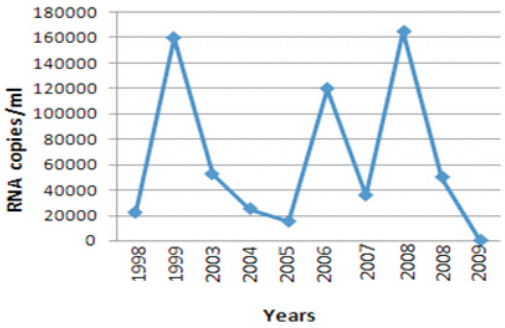

Fig. 1: Sequential viral load in the patient

Table No.1: Drugs given to the patient from time to time

Drug used	99 TB@	00	01	10.02	12.02 TB$	11.03 TB*IRIS	11.03	04	10.05	6.06	3.2008 TB&
ATT	++			PCP+ candida	++	++				Candid VL+++	++
AZT	++	Omit									
3TC	++	++	++					++/#-			
D4T		++	++	++	++	++	++	Omit			++
NVP	NA	NA	++	++	Omit						
DDI				++	++	++	++	++	++	++	
EFV					++	++					
NLFr							++	++	++	++	
ABC						NA		--/++	++	++	
TDF											++
SQVr											++
LPVr											++
RTNr											++

Caption: TB@=pulmonary TB, **TB$=** splenic aspirate+ for AFB, **TB***=Lymph node positive, **IRIS**=immune reconstitution syndrome, **TB&**=TB cervical lymph nodes. AZT had to be omitted because of severe anemia, nevirapine withdrawn because of TB, stavudine withdrawn because of facial lypodystrophy, Lamivudine restarted because of partial resistance only. **V++** = high viral load. **PCP**= pneumocystis pneumonia.

In 2003 he was switched over to a combination of stavudine, didanosine and nelfinavir. Antiretroviral therapy was given according to availability, affordability, development to toxic effects and emergence of mutations detected in 2004 and 2008.

He developed facial dystrophy and stavudine was withdrawn and replaced with abacavir. He continued to remain stable for 2 years but developed high viral load and oral Candidiasis in June 2006. He remained well after treatment of opportunistic infection (O.I) till March 2008 when he developed lymph node TB one more time (figure1, table 2). Genotype analysis revealed fresh mutations as depicted below:

Mutation studies in 2004: (courtsey, Dr. S Gartner, Johns Hopkins University) revealed the following:

D67N, K70R, G190S, and T215F. The patient had K101H; which is associated with resistance to NNRTI usage. He had K219E.

No typical protease resistance mutations or heterozygosity at position 88 or 90 was seen but an L to M change at codon 89 was observed; (it was all methionine); at that time, significance of L89M was not clearly defined.

Mutation studies in 2008: conducted by Acunovalife, Bangalore, revealed high level of resistance to abacavir, didanisine, dalavirdine efacirenz, lamivudine, neviapine and emetricitabine.

Intermediate resistance was reported to stavudine, tenofovir and zidovudine. Details of mutation are shown in Table No. 2 (mutations in bold are the ones observed in 2004; rest indicate new mutations or a new substitution):

This patient had a total of 17 mutations: 8 at 7 nucleoside reverse transcriptase (NRTI) sites, 4 at 3 non nucleoside reverse transcriptase (NNRTI) sites and 5 mutations at 4 protease sites. He was put on tenofovir, saquinavir, ritonavir lopinavir and stavudine. His viral load dropped to 62000 copies/ml and finally to less than 200 copies/ml and virus was undetectable in his blood from 2009 onwards.

Table No.2: Cumulative mutations in the patient

NRTI	NNRTI	PI
D67N	**K101H**	D30N
	Y181C	
K70R/G	**G190S**/A	N88D
Y115F		**L89M**
Y118l		L89V
M184V		
T215F		T74S
K219E		

July 2009 NACO had not yet started providing free ART when he needed HAART; when he needed the second line drugs, NACO was providing only first line drugs and he needed third drugs in 2008(Table No.1). Thus if he had not been able to afford drugs on his own, he would not have survived the onslaught of this rapidly mutating virulent virus. He had to spend more than 2 million Rupees (50000 US dollars) during 13 years After addition of lopinavir, his CD4 count increased to 570.cm ml and virus was undetectable in his blood and remained undetectable till June 2011.

DISCUSSION

Today while more than 25 antiretroviral drugs are in use the world over, more than 200 mutations associated with drug resistance have been identified. Ideal therapy should aim to suppress viremia completely but there are serious limitations because of drug resistance mutations. The picture is compounded by Clade differences in different geographic areas[5].

Thymidine analog mutations (TAMS) appear in 2 overlapping patterns i.e. type 1 and type 2 [6, 7] M184 V is the commonest mutation which developed late in 2005 in this patient. He had type 2 TAM mutations e.g. D67N, K70R, T215F and K219E. Although Schafer and Shapiro [3] commented that while the significance of type 1 TAMS was well characterized, that of type 2 TAMS was not. This case illustrates that TAM 2 mutation are also associated with treatment failure in clade C patients.

NRTI Mutation K70 R occurs in patients treated with thymidine analogs while K70 G occurs in patients treated with non thymidine analogs as happened in this patient in 2008. This patient also had K219E in 2004 which usually occurs with type 1TAMS, Similarly Y118l is an accessory mutation and occurs with type 1 TAM but this patient had a combination of type 2TAMs and Y118l mutation. This combination is now known to reduce susceptibility to NRTI[8.] Another study from Sweden[9] showed that the virological failing patients harbored 3-6 reverse transcriptase (RT) mutations, including the V118l mutation in 5/6 cases prior to Pl-ART or at viral rebound. Stepwise multiple regression for viral failure resulted in model with only the V118I mutation entering the model ($P<0.01$), which this patient had in 2008 study. Further, D69N, T215F and K219E are also associated with resistance to didanosine. Some drug resistance mutations occur commonly in the absence of drug selective pressure. K70R substitution does not reduce susceptibility to ZDV. In drug naïve subject our group also reported a high level of K70 R mutations in India [10.] In 2004, he had a wild type 184M but in 2005 he had M184V and lamivudine had to be discontinued.

NNRTI Mutations: This patient initially showed G190S and later in 2008 showed G190A which imparts a high level of resistance to nevirapine. This patient had K101H early in 2004 which is an uncommon mutation conferring resistance to NNRTI. At that time its significance was not that clear. Now we know that this by itself is sufficient to impart partial resistance to NNRTI. Loemba et al [11] also showed that Ethiopian immigrants in lsrael with clade C harbored K70R and G190A naturally associated with resistance to AZT whereas G190A substitution results in high-level resistance to nevirapine (NVP). We earlier documented that K103N is increased in Indian children with AIDS even on combination ART. Sequential use of nevirapine and efavirenz (in either order) is not recommended because of cross resistance between these drugs but was used when he developed TB. At our centre it is customary to use efavirenz in patients with TB and after therapy; they are switched back to nevirapine.

Protease Mutation were largely absent 5 years ago apart from L89M, with no detectable heterozygosity.Subsequently he developed mutations at codons 30,74,88 and a new mutation at 89 I.e. L89V which is associated with treatment failure[12]. In Israeli patients with subtype C infection, L89M mutation has been reported to be significantly higher than those with B[13]. Mutation T74S is known to occur in 8% of patients with clade C virus but rarely in other subtypes and is associated with decreased susceptibility to nelfinavir. This patient had a spurt of viral load while on nelfinavir. Mutations at 23,30, 46,48,84,88 and 90 are relative contraindication for nelfinavir usage. Thus this patient had 2 major mutation (D30N andN88D) and 2 more non polymorphic mutations (T74S and K89M). Velazquez *et al* [13] commented that these naturally occurring genetic variations can dull the effects of protease inhibitors in African strains and help them develop resistance to drugs more quickly. Each time he developed mutations, he showed OI, primarily TB of different organs and high viral load. The patient developed cancer of the gall bladder in October 2011 and rapidly succumbed to it in early January.

CONCLUSION

An Indian patient with Clade C who a total of 17 mutations with several uncommon mutations associated with treatment failure is described. He was resistant to most NRTI and NNRTI drugs, had 4 episodes of TB, two episodes of Candida and 1 episode of PCP and IRS each and needed frequent change in drug regimes. He finally responded to a regimen containing lopinavir and became free from virus. In a resource poor setting mutations can pose a great challenge to the physician. Further, there was an imperfect correlation between the viral load and CD4 cells; the main problem being the emerging mutations and flare of TB. In our experience splenic TB could be taken as an AIDS defining disease.

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