REVIEW

Reverse transcriptase and protease inhibitor resistant mutations in art treatment naïve and treated HIV-1 infected children in India

A Short Review

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Abstract:

Introduction of first line and second line antiretroviral therapy has dramatically improved the quality of life and survival of the HIV-1 infected individuals. Extension of this therapy in children has similar effect. However the emergence of drug selected resistance has hampered the response to the therapy. A database of prevalence of drug resistance mutations in the Indian children both ART naïve and treated will help in deciding the appropriate regimen for the individual patient as well as formulating the policies regarding the composition of drugs included in the fixed dose combinations and its periodic review by analysis of the information that is made available from time to time. This will enable us to utilize our limited resources in most prudent way.

Keywords:

Drug Resistance, Mutations, Reverse transcriptase, and Protease.

Introduction:

According to UNAIDS Global AIDS Update 2016[(1)] around 36.7 million people (all ages) are living with HIV, out of which 2.1 million are new HIV infections. An estimated 1.8 million Children are infected with HIV worldwide. National AIDS Control Organisation (NACO) reports that around 21.17 lakh people are infected with HIV in India. Out of these 6.54% are children (<15 years)[(2)].

Children can have an infection with HIV via mother to child transmission, infected blood and blood products and through sexual assault. Most of the paediatric HIV infections are due to mother to child transmission. The infection can be transmitted from mother to her child during pregnancy, labour, delivery or breastfeeding. Most commonly it occurs during peripartum period[(3)].

Introduction of Antiretroviral Therapy (ART) has dramatically increased survival and quality of life of a patient having HIV[(4)]. ART has been effective in both reducing viral load as well as in

increasing the CD4 counts of HIV infected individual. However the long term response to ART is hampered by the emergence of drug resistant mutations in the viral genome[5]. The viral genome replication by viral reverse transcriptase enzyme is highly error prone leading to genetic diversity of the viral pool. Individual treated with ART puts selection pressure on virus with the emergence of drug resistant strains over a period of time. Children can acquire drug resistant strain from mother via vertical transmission[6]. Single dose Nevirapine used for prevention of mother to child transmission has also been demonstrated to be responsible for Non Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drug mutations[7].

As ART is being given to more and more individuals and with the emergence of drug resistant mutations for the first line therapy it is imperative to have knowledge of prevalence of mutations a population harbours so that the treatment can be customized accordingly.

HIV-1 Reverse Transcriptase Mutations:

The study conducted by Soundararajan et al.[8] in 48 drug naïve south Indian children showed no significant drug resistant mutation in reverse transcriptase gene. However more than half of the sequences had important amino acid substitutions in codons 35, 36, 39, 48, 60, 121,135, 162, 173,177, 200, 207, 211, 214, and 245. In about 30% of sequences Threonine was substituted by Glutamic Acid at position 39 and 45% sequences had Aspartic acid in position 39.

An analysis of genotyping of 12 virological failure ART treated Children in St. Johns, Bangalore reveal that 11 out of 12 had clinically relevant drug associated mutation in the reverse transcriptase gene[9]. The most frequent Nucleoside Reverse Transcriptase Inhibitor (NRTI) mutation was M184V associated with resistance to Lamivudine, Abacavir and Emtricitabine. Other common NRTI mutations were M41L and T214Y/F/I. Both these mutations confer intermediate level cross resistance to Tenofovir. K103N/R, Y181C and G190A were the most frequent NNRTI mutations with associated resistance to Nevirapine, Efavirine and intermediate to high cross resistance to Etravirine.

A clinical brief by Shah et al.[10] reported two cases of ART treated children in B.J Wadia Hospital, showing both TAMS-1 (M41L, T215Y/F, L210W) and TAMS-2 (D67N) mutations. These mutations are cross resistant to all NRTI's and are selected for by Stavudine or Zidovudine.

A study conducted in Pune, India reported drug resistant mutation for NNRTI in 7.4% ART naïve children[11]. They identify A98G and K103N mutation in the two separate sequences. A98G mutation confers low level resistance to NNRTI's and

K103N confer high level resistance to Efavirine and Nevirapine.

A genotyping analysis of ART naïve and treated children in AIIMS, New Delhi reveal that drug resistant RT mutation was present in 30% of ART naïve and 36% of ART treated children respectively[12]. RT mutations conferring resistance to NRTI drugs were identified at positions 65,67,74,77,151,184,215,219. Mutations in the RT gene that confer resistance to NNRTI drugs were detected at amino acid positions 101, 106, 179, 190, and 227. One ART naïve patient had both K101E and G190A mutations which confer high level resistance to Nevirapine and Efavirine.

NNRTI mutations after administration of single dose Nevirapine in both mother and child has been reported previously[13]. In India a feasibility study conducted at National AIDS Research Institute (NARI), Pune had observed that 10.5% of children had low levels mutation for NNRTI after 48 hours of administration of single dose Nevirapine and 46.15% of children had high level of NNRTI resistant mutation after 2 months of single dose Nevirapine prophylaxis[7]. Although this study failed to find K103N mutation, one of the common NNRTI mutation, presence of low to high level resistant mutation even after single dose of Nevirapine is highly significant.

As it is a common observation that the resistance to Nevirapine develops after single dose or after interruption of therapy, Sehgal et al. investigated the K103N mutation which confer resistance to Nevirapine in 25 children in which 6 were ART naïve and rest 19 were on Nevirapine containing fixed dose combination therapy. K103N mutation was found in 56% of children including two drug naïve individuals[14].

The presence of Reverse Transcriptase mutations in ART naïve children should raise a general concern. This necessitates the genotyping of individual case before starting ART therapy. Same is applicable in a patient where change in therapy regimen or individual drug is being considered.

HIV-1 Protease Mutations:

With the introduction of 1st line ART, over a period of time treatment failure appears in score of individuals. Who are then shifted to 2nd line ART containing protease inhibitors. Baseline drug resistant mutation profile of protease inhibitors is largely unknown in the population. Resistant strain may also be vertically transmitted from mother to child if mother is receiving protease based regimen. Due to recent introduction of 2nd line ART very few studies have been conducted to look for protease inhibitor resistant mutations.

Soundararajan et al. found no major mutation in the protease gene of 48 ART naïve children[8]. However they observe that

more than half of the sequences had polymorphism at position 12,19,41,89 and 93. Frequent substitutions were seen at positions 15, 36, 63 and 69.

Shet et al. after doing genotyping of 80 children on first line of ART couldn't find any protease resistant mutation in any of the sample although they had detected significant mutations for reverse transcriptase gene[9].

Protease inhibitors selected major mutations has been observed by Toor et al. in a study conducted at PGIMER, Chandigarh. Both major and minor mutations were seen in patients with first line ART treatment failure[15]. One patient had L33F and I47T major mutation. L33F is selected by each of the Protease Inhibitors except Atazanavir, Indinavir and Saquinavir. In combination with other Protease Inhibitor resistant mutations L33F reduce the susceptibility to each of the Protease Inhibitors. I74V It associated with reduced susceptibility to each of the Protease Inhibitors except Saquinavir and Atazanavir. The same patient also had minor mutations M46G and G48E. Other minor mutations observed were L10I and T74S in one individual each.

A study conducted at AFMC, Pune involving 27 ART naïve children observe no major mutations in the protease gene. But 3 individual had single minor mutations namely L10I, A71T and T74S[11]. L10I is an accessory mutation, which either reduce Protease Inhibitor susceptibility or increase the replication of viruses containing Protease Inhibitor resistance mutations. A71T is a common accessory polymorphic mutation that increase replication and/or reduce Protease Inhibitor susceptibility in viruses. Another accessory mutation found was T74S which is polymorphic in non B-subtype viruses.

Shah et al. have reported two cases of first line treatment failure children having both major and minor mutations for the protease inhibitors[10]. The first case had major mutations at position 46 and 54 and minor mutations at positions 10,20,36,63. The genotyping points towards the high resistance to Nevirapine with possible resistance to Amprenavir. The second patient had major mutation D30N and minor mutations at positions 13,17,19,20,35,36,37,41,45,57,63,64,69,74 and 93 conferring high resistance to Nevirapine possible resistance to Ritonavir boosted Atazanavir (ATV/r).

The AIIMS, New Delhi study detected five children having single minor mutation for the protease gene in codon 10, 76 and 84[12]. Out of these five 2 children were on first line drugs and rest were ART naïve. The minor mutations were L10I/V, L76T and I84R/T. L10I/V are associated with resistance to most Protease Inhibitors when present with other mutations. L76T and I84T are highly unusual mutations at these positions.

With the introduction of second line therapy containing protease inhibitors for first line treatment failure children[16], it is advisable to check for the Protease Inhibitor selected mutations before starting the treatment.

HIV-1 Subtypes:

Subtype C is the most common subtype in India in adults as well as in paediatric age group[17]. Most of the genotyping studies done in India in paediatric age group reveals HIV-1 subtype C. However other subtypes have also started emerging in Indian paediatric population. Toor et al. reported two first line treatment failure children of subtype B harbouring polymorphism at different positions for protease gene[15]. Kumar et al. in their study had two protease isolate align with subtype A1 and one RT isolate align with subtype A1[11]. However the significant observation of subtype diversity came from study at AIIMS, New Delhi. The authors found that 30% of Reverse Transcriptase sequences are clustering with subtype B and 36% of protease gene sequences are aligning with subtype B[12]. they also had one ART naïve individual whose sequence align with subtype A. All these observations point towards the emergence of HIV-1 subtype diversity in Indian paediatric population.

Circulating Recombinant Forms (CRF's):

Till date no study has been able to detect circulating recombinant form of HIV in Indian paediatric population.

Future:

As more number of first line treatment failure patients are emerging, genotyping of individual patient helps in customizing therapy for that individual and avoiding the drugs mutations against which are already present. The importance of population data of drug resistant mutations cannot be more emphasized. It will help in formulating the combination of drugs to be included in the regimen and also fixing the recommended dosage. To be enable to do this more drug resistance mutation studies needed to be conducted and more classes of drugs required to be included in the research investigation.

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