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INCIDENCE AND ANALYSIS OF ZIDOVUDINE INDUCED ANAEMIA IN HIV INFECTED PATIENTS IN WESTERN INDIA.

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ABSTRACT

Background: Zidovudine (AZT) a nucleoside reverse transcriptase inhibitor is the first line antiretroviral regimen in India, and was the first break through in AIDS therapy in 1990. It is known to be associated with life threatening toxicity like anaemia. **Objective:** The study had been designed to determine the prevalence of AZT induced anaemia in HIV infected patients initiated on AZT containing antiretroviral therapy regimen in western India. **Methods:** This retrospective study was carried out in ART Centres, of western India (Gujarat). HIV infected patients registered at ART Centres were treated according to guideline of National AIDS Control Organization (NACO).1269 patients with haemoglobin (Hb) >8 g/dl were prescribed AZT based antiretroviral therapy regimens. Patients who developed anaemia (<8g/dl) due to other causes of anaemia were excluded from the study. Even attempt had been made to correlate of baseline characteristic (age, gender, starting haemoglobin level, patients weight, CD4 count and WHO clinical stages) with risk of developing anaemia. **Results:** One hundred eighty one (14.2%) patients on AZT regimen developed anaemia(<8 g/dl). Out of this 78 patients(6.15%) developed sever anaemia (<6.5 g/dl). Females were more prone to develop anaemia. No correlation had been found between development of anaemia and age, weight,

WHO clinical stage and CD4 counts. **Interpretation & conclusion:** High incidence of AZT induced anaemia were found in the TB/HIV co-infections, BMI < 18 kg/m^2 and MCV <80 fL in the present study. In these risk group patients regular monitoring of Hb should be carried out in such patients, particularly even in women on AZT based antiretroviral regimens.

KEYWORDS: HIV, CD4, Anaemia, Zidovudine, Antiretroviral therapy, HAART.

INTRODUCTION

Over the last few years, an impressive scale-up of antiretroviral therapy (ART) has been seen in low and middle income countries (LMIC), with 6,650,000 patients on treatment in 2010.[1] Most of the patients in these countries currently use stavudine (D4T)-containing regimens, followed by zidovudine (AZT)-based treatment.[1] One of the key clinical and operational challenges is the management of treatment-related drug toxicity. Whereas mitochondrial toxicity is the major concern with D4T, AZT use is often complicated by the occurrence of – sometimes severe – anemia.[2] Recent World Health Organization (WHO) guidelines have recommended to phase-out the use of D4T, in favor of tenofovir or AZT.[3] Consequently, millions of HIV-infected individuals on ART for prolonged time periods will replace D4T with AZT in the near future. However, studies on the incidence and determinants of anemia in LMIC in such patients are currently scarce. A number of key questions remain to be addressed.

Availability of free antiretroviral drugs to HIV infected persons in India and other countries has provided a new lease of life to these patients. However, resource constrains limit the national programme to use nucleoside reverse transcriptase inhibitor (NRTIs) like zidovudine (AZT) or stavudine (d4T) with lmivudine (3TC) and non nucleoside transcriptase inhibitors (NNRTI) like nevirepine and efavirenz for highly active anti-retroviral therapy (HAART).

Due to mitochondrial toxicity woth long term use of stavudine (d4T), it is no longer recommended [4] and zidovudine (AZT) is the preferred NRTI in National AIDS Control Organisation (NACO) sponsored ART centres in India.[5] The prevalence of zidovudine induced anaemia vary widely (5.42- 9.62%), in studies from different part of the world.[6,7] One recent study from Southern Odisha, India mentioned very high incidence of 14.6 per cent in their findings.[8]

First, there is some evidence that the risk of anemia is particularly high in patients with low body weight. In Peru, discontinuation rate of AZT-containing regimen due to toxicity in the first 120 days increased dramatically with lower baseline weight (< 60 kg) among antiretroviral-naïve patients starting ART.[9] The authors suggested that a weight-based approach for AZT dosing should be considered to reduce the occurrence of anemia. Such findings could be particularly relevant for regions like South-East Asia, where most HIV-infected patients have a body weight clearly below 60 kg. A report from a small study in Thailand demonstrated a relationship between lower body weight and lower AZT clearance, associated with more frequent side effects (gastrointestinal intolerance and anemia) [10] and current Thai guidelines recommend a dose ranging from 200 to 300 mg twice a day.[11] AZT has been found to exhibit cytotoxicity to the erythroid precursor cells in the bone marrow *in vitro* in a dose-dependent manner. This toxicity could possibly be more pronounced in individuals with a low body weight, due to higher AZT levels.[12] However, these findings are not yet generally accepted, and the current WHO guidelines still recommend the standard dose of AZT 300 mg twice a day for adult patients.[3]

Another controversy relates to the effect of prior ART use before AZT initiation. Some studies have suggested that prior exposure to ART before starting AZT is protective against AZT-induced anemia[13]–[15] and that longer duration of ART use prior to starting AZT is associated with a reduced risk of anemia.[16] Possibly, this toxicity could be exacerbated by ongoing HIV-1 infection or immune activation early after starting ART.[17, 18] However, the reported association was not confirmed in other studies.[19]

Despite the recent WHO recommendation, some poor countries continue to use D4T-based regimens as the preferential first line treatment due to its good short-term tolerance, the availability of a fixed-dose combination and especially the low cost compared to other regimens. In line with the Cambodian national guideline, [20, 21] D4T is still used within the first line regimen in Sihanouk Hospital Center of HOPE (SHCH), a tertiary hospital in the capital. However, by seven years of follow-up, D4T was discontinued in around 48% of patients starting ART with D4T-based regimen due to the D4T-intolerance[22] and AZT was usually used as alternative. Based on carefully collected program data over a period of seven years, we report the incidence and risk factors of AZT-induced anemia within one year after substituting AZT for D4T in adult patients on ART in Cambodia. The main purpose of this

study was to determine how the risk of anemia after AZT initiation varies across patient characteristics like body weight and duration of prior ART use.

METHODS AND MATERIALS

This retrospective study had been conducted on HIV infected patients who had attended the National AIDS Control Organisation(NACO), India, sponsored ART Centres of Gujarat. Information had been collected from the records. HIV infection had been confirmed using three sets of diagnostic tests, either rapid test or ELISA each using different antigens according to National Guidelines.[5] Demographic details, weight, WHO clinical stage[23], baseline investigations and CD4 counts of HIV positive patients were recorded. Treatment was initiated as per national guideline in India, according to which fixed dose combination of two NRTIs (zidovudine/stavudine + lamivudine) and one NNRTI (nevirepine/efavirenz) is recommended.[5] Zidovudine was prescribed if the initial haemoglobin was found more than 8 g/dl and stavudine based regimen was started if the initial haemoglobin was found to be <8 g/dl.

All patients aged more than 16 years who received zidovudine combination were included in this present study. This all patients were followed for a minimum period of 12 months. Haemoglobin estimation was done at the start of antiretroviral therapy, at day 15 and monthly during the first 6 months, thereafter haemoglobin estimation was done every two monthly. Fall in haemoglobin levels <8 g per cent in a patient on zidovudine therapy and subsequent increase in haemoglobin in haemoglobin levels on stopping therapy was considered as zidovudine induced anaemia. All the patients who developed anaemia were shifted to stavudine based therapy.

Presence of opportunistic infections, particularly intestinal infestation s and other conditions which could lead to anaemia, were ruled out. History of intake of drugs such as NSAIDs were recorded, menstrual disturbances in women and haemorrhoids were also noted. Patients with history of haemorrhoids underwent rectal examination. Upper gastrointestinal endoscopy was also done in the patients with gastrointestinal bleeding and difficulty in deglutination. Stool examination for occult blood, ova and cyst was also performed.

Besides haemoglobin estimation, patients in the present study underwent peripheral smear examination, reticulocyte counts, serum iron binding capacity, serum iron estimation to rule out other haemoglobin disorders. Vitamin B_{12} and folate levels were done in the patients who

could afford it. Patients with evidence of anaemia from other causes were not included in AZT anaemia group.

Prevalence of zidovudine induced anaemia, and its occurrence in relation to baseline weight, age, gender, haemoglobin levels, CD4 counts and WHO clinical stages were also determined. The prevalence rate of anaemia was defined as the number of patients with haemoglonbin levels below 8 per cent recorded in any of the scheduled or unscheduled visit during follow up per 100 person – year of at risk follow up.

Baseline factors at AZT commencement included gender and age, body mass index (BMI), CD4 cell count, mean corpuscular volume (MCV), Hb, and ART regimen. Tuberculosis (TB) after ART initiation but before the development of anaemia was considered as a potential risk factor.

The follow up period, during which the patients was considered at risk, started on the day of the treatment initiation with zidovudine till the date of developing anaemia. Mean duration and level of haemoglobin during fall and recovery of haemoglobin levels were calculated.

RESULTS

Total 1692 patients were studied who were on HAART. AZT based HAART was started in 1290. Among all patients on AZT based HAART developing anaemia, six patients had evidences of intestinal infestation, two patients had haemorrhoids, nine patients had excessive menstrual bleeding, four patients had features suggestive of Fc deficiency anaemia, and all of them were excluded from the study. Estimation of serum folate and serum B_{12} levels could not be afforded by these patients. So finally we included 1269 (75 %) patients (Hb >8 gm/dl) in our study group. Among them 922 (72.73 %) were male, 347(27.27%) were female.

Among 1269 patients on AZT based regimen 181(14.2%) developed AZT induced anaemia (Hb <8gm/dl). In 128 patients (70.7 %) AZT induced anaemia occurred within 3 months of therapy and 175 (96.7 %) patients within six months of therapy.

Nature of anaemia was Normocytic-Normochromic in 47.7 % and Macrocytic in rest of the patients in peripheral smears. Mean decline in Hb was 5.8 $\pm$ 1.3 gm/dl. On stopping AZT therapy Hb level increased to a mean of 8.8 $\pm$ 1.6 gm/dl. Mean duration for fall of Hb was 3.66 $\pm$ 2.33 months. After substitution of stavudine mean duration for increase in Hb level was 1.46 $\pm$ 0.78 months.

Table 1: Incidence of AZT induced anaemia in relation to duration of therapy.

Months	n(%)	Cumulative n(%)
1/2	14(7.7)	14(7.7)
1	35(19.3)	49(27.1)
3	79(43.6)	128(70.7)
6	41(22.7)	169(93.4)
9	06(3.3)	175(96.7)
12	05(2.8)	180(99.5)
>12	01(0.6)	181(100)

Table II: Comparison of baseline characteristic of patients on zidovudine therapy who on follow up developed >grade 2 anaemia (groupT) to those who did not develop anaemia (group C).

Variables	Group T(n=181)	Group C(n=1088)
Age, years		
(Mean + SD)	32.23 ± 8.12	34.47 ± 8.46
Gender		
Male	49 (27.0)	790(72.6)
Female	132(72.9)	298(27.4)
BMI($Kg/m)^2$		
Mean + SD	17.6 ± 2.03	19.98 ± 2.41
Haemoglobin g (%)		
Mean + SD	10.37 ± 1.69	11.09 ± 1.67
Mean Corpuscular Volume(MCV)		
≥ 80 fL	90(50%)	781(71.79)
< 80 fL	91(50%)	307 (28.21)
WHO Clinical Stages		
I- II	42(23.2)	765(70.3)
III- IV	139(76.8)	323(29.7)
CD4 Counts /	114.34 + 89,5	117 + 96.6
<50	74(40.9)	327(30.0)
50-199	80(44.2)	561(51.6)
>200	18(09.9)	144(13.2)
Missing	9(4.9)	56(5.1)

Baseline characteristics of patients in group C and T, showed that there was increased risk of developing anaemia in the patients with lower haemoglobin levels (P <0.002). Females were more prone to develop anaemia.

DISCUSSION

Anaemia has been shown to be the most frequent hematological abnormality in HIV-infected patients globally.[1, 2] Even among those initiating antiretroviral therapy (ART), anaemia has been demonstrated to be a strong risk factor for disease progression and subsequent death.[1–5]

independent of CD4 count and viral load. In a large European cohort study, the presence of severe anaemia at ART initiation was associated with a 13-fold increased risk of death.[3] In sub-Saharan Africa, which has the largest burden of HIV in the world, anaemia is common as patients are more likely to be malnourished, have advanced immunosuppression, and have higher rates of comorbidities (especially tuberculosis and malaria) than those in high-income countries.[6, 7] Despite the public health importance of anaemia, prospective data from sub-Saharan Africa on its impact are limited. Recent reports suggest that hemoglobin levels improve with ART[2, 3, 8]; however, few studies have documented the evolution of hemoglobin levels among patients on ART in resource-limited settings, and whether the effects on hemoglobin levels vary by ART regimen. Given the number of patients on ART in this region, understanding the role of anaemia in HIV treatment is critical to developing strategies to improve survival and reduce morbidity on ART.

The major cause of anaemia is impaired erythropoiesis resulting from the release of inflammatory cytokines and decreased production of hematopoietic growth factors, coupled with malabsorption and impaired recycling of iron.[9, 10] Additionally, there are multiple other causes of anaemia, which include nutritional deficiencies (iron, cobalamine, or folate deficiency), malignant bone marrow infiltration, bone marrow infection, and hemolysis.[11–14] Among patients initiating antiretroviral therapy, the use of zidovudine containing regimens has been associated with the incidence of anaemia, and bone marrow toxicity has been postulated.[2,9, 10] Amongst patients in an urban HIV clinic in Uganda, severe anaemia improved with ART in the majority of patients. These findings suggested that baseline severe anaemia should not be used as a criterion for avoiding the use of zidovudine in patients initiating ART in resource-limited settings.[15, 16]

The most important risk factors for having early severe anaemia, in patients initiated on ART were a pre-existing diagnosis of TB, a low MCV, and baseline severe anaemia. In this resource-limited setting, AZT was not associated with an increased risk for early severe anaemia after highly active ART despite its known toxicity when used as a single agent in the pre-ART era. This finding has been previously described in the developed world.[17, 18]

In this prospective cohort study conducted in western India, we set out to determine the prevalence, incidence, and predictors of anaemia among patients initiating first line ART. We also sought to explore whether the degree of immunosuppression at ART initiation or the

initiating ART regimen (zidovudine versus other) impacted the magnitude of hemoglobin increase while on treatment.

CONCLUSION

Still, our study, taken together with other available data, shows that ART should not be withheld from patients with severe anaemia if regimens containing AZT are either the only ones available or are preferred for other reasons. Our data suggest that setting a lower limit Hb, specifically Hb ≤8 g/dL, as a determinant of whether AZT-containing regimens should be prescribed may not be warranted. Low BMI (<18 kg/m^2) and low MCV (<80fL) may be more useful in predicting which patients are at highest risk for AZT-induced anaemia. Finally, intensified TB screening of anaemic patients is warranted, as well as vigilance for TB after ART initiation.

REFERENCES

1. World Health Organization (2011). Global HIV/AIDS Response: Epidemic Update and health sector progress toward universal access. Progress Report, Geneva. Available: http://www.who.int/hiv/pub/progress_report2011/en/
2. Zhou J, Jaquet A, Bissagnene E, Musick B, Wools-Kaloustian K, et al. (2012) Short-term risk of anaemia following initiation of combination antiretroviral treatment in HIV-infected patients in countries in sub-Saharan Africa, Asia-Pacific, and central and South America. J Int AIDS Soc., 15: 5 doi:10.1186/1758-2652-15-5.
3. World Health Organization (2010) Antiretroviral therapy for HIV infection in adults and adolescents, Recommendations for a public health approach. Geneva, ed 2010 revision. Available:http://whqlibdoc.who.int/publications/2010/9789241599764_eng.pdf
4. World Health Organization. Scaling Up Antiretroviral Therapy in Renounce Limited Settings: Treatment Guideline for a Public Health Approach -2003. Revision available at: http://www.whoint / 3by5 /publications / guidelie /en /execsumm. Pdf accessed on December 10, 2008.
5. Operational Guidelines on ARTCentres, March 2007. Available at: http://www.nacoonline.org/ About_NACO/Policy_Guidelines/, accessed on December 28, 2008.
6. Kumarasamy N, Venkatesh KK et al, Spectrum of adverse events after generic HAART in Southern Indian HIV-infected patients. AIDS Patients Care STDS, 2008; 22: 337-44.

7. Moh R, Danel C, Sorho S, Sauvageot D, Minga A et al. Haematological changes in adults receiving a zidovudine-containing HAART regimen in combination with cotrimoxazole in Cote d'Ivoire. Antiviral Therapy, 2005; 10: 615-24.
8. Dash KR, Meher LK, Hui PK, Behera SK, Nayak SN, (2015). High incidence of zidovudine induced anaemia in HIV infected patients in Southen Odisha. Ind J Hematol Blood Transfus, 31(2): 247-250.
9. Willig JH, Echevarria J, Westfall AO, Iglesias D, Henostroza G, et al. (2010) Durability of initial antiretroviral therapy in a resource-constrained setting and the potential need for zidovudine weight-based dosing. J Acquir Immune Defic Syndr 53: 215–221. doi: 10.1097/qai.0b013e3181bc0f10
10. Cressey TR, Leenasirimakul P, Jourdain G, Tawon Y, Sukrakanchana PO, et al. (2006) Intensive pharmacokinetics of zidovudine 200 mg twice daily in HIV-1-infected patients weighing less than 60 kg on highly active antiretroviral therapy. J Acquir Immune Defic Syndr 42: 387–389. doi: 10.1097/01.qai.0000221685.90681.1b
11. Sungkanuparph S, Techasathit W, Utaipiboon C, Chasombat S, Bhakeecheep S, et al. (2010) Thai national guidelines for antiretroviral therapy in HIV-1 infected adults and adolescents 2010. Asian Biomedicine 4: 515–528.
12. Norrby SR, Gotzsche PC, Melander H, Anderson P, Lifidevirta J, et al. (1992) Double blind dose-response study of zidovudine in AIDS and advanced HIV infection. BMJ 304: 13–17. doi: 10.1136/bmj.304.6818.13
13. Huffam SE, Srasuebkul P, Zhou J, Calmy A, Saphonn V, et al. (2007) Prior antiretroviral therapy experience protects against zidovudine-related anaemia. HIV Med, 8: 465–471. doi: 10.1111/j.1468-1293.2007.00498.x
14. Toeung PD, Pov S, Chel S, Huffam S, Khol V, et al.. (2007) Routine switch after 6 months from D4T to ZDV containing antiretroviral therapy, at an outpatient HIV clinic in Phnom Penh, Cmbodia. Fourth IAS Conference on HIV pathogenesis, treatment and pervention, July 22-25, 2007 Syney, Australia, WePeB059.
15. Agarwal D, Chakravarty J, Chaube L, Rai M, Agrawal NR, et al. (2010) High incidence of zidovudine induced anaemia in HIV infected patients in eastern India. Indian J Med Res., 132: 386–389.
16. Kumarasamy N, Venkatesh KK, Devaleenol B, Saghayam S, Manohar D, et al. (2009) Safe substitution to zidovudine among HIV-infected patients initiated on stavudine-containing highly active antiretroviral therapy from a resource-limited setting. Int J Infect Dis 13: e360–e364. doi: 10.1016/j.ijid.2008.11.030

17. Bastard JP, Soulie C, Fellahi S, Haim-Boukobza S, Simon A, et al. (2012) Circulating interleukin-6 levels correlate with residual HIV viraemia and markers of immune dysfunction in treatment-controlled HIV-infected patients. Antivir Ther, 17: 915–919. doi: 10.3851/imp2093
18. Lichtfuss GF, Cheng WJ, Farsakoglu Y, Paukovics G, Rajasuriar R, et al. (2012) Virologically suppressed HIV patients show activation of NK cells and persistent innate immune activation. J Immunol. 189: 1491–1499. doi: 10.4049/jimmunol.1200458
19. Isaakidis P, Raguenaud ME, Phe T, Khim SA, Kuoch S, et al. (2008) Evaluation of a systematic substitution of zidovudine for stavudine-based HAART in a program setting in rural Cambodia. J Acquir Immune Defic Syndr 49: 48–54. doi: 10.1097/qai.0b013e31817bec19
20. Cambodian Ministry of Health (2005) National Guidelines for the use of Antiretroviral Therapy in Adults and Adolescents, National Center for HIV/AIDS, Dermatology and STD, 2005 ed NCHADS.
21. Cambodian Ministry of Health (2012) National Guidelines for the use of Antiretroviral Therapy inn Adults and Adolescents, National Center for HIV/AIDS, Dermatology and STD, 2nd Revision.
22. Phan V, Thai S, Choun K, Lynen L, van Griensven J (2012) Incidence of treatment-limiting toxicity with stavudine-based antiretroviral therapy in Cambodia: a retrospective cohort study. PLoS One 7: e30647 doi: 10.1371/journal.pone.0030647.
23. WHO case Defination of HIV for Survillance and Revised Clinical Staging and Immunological Classification of HIV Related Diseases in Adult and Children. WHO. 2006. Available at: http://www.who.int/hiv/pub/guidelines/HIV staging 150307: pdf, accessed on November 18, 2008.