Research Article

A Comparison of Hemoglobin A\textsubscript{2} Levels in Untreated and Treated Groups of HIV Patients on ART Including Zidovudine

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Objective. To assess the level of haemoglobin A\textsubscript{2} in HIV patients on antiretroviral therapy (ART) including zidovudine with untreated HIV patients. Material and Methods. The study was a case control study. A total of 185 patients were included in the study; the case group included 125 HIV patients who were on antiretroviral therapy (ART) including zidovudine and 60 were in the control group who were not receiving ART. The high-performance liquid chromatography (HPLC) was done and hemoglobin A\textsubscript{2} level was observed; value more than 3.5% was considered significant. The Hb A\textsubscript{2} percentages of HIV patients were compared with those of control using an unpaired \(t\)-test. Results. The mean of Hb A\textsubscript{2} in case group was 3.462% (SD 0.675) and in control group it was 2.815% (SD 0.246). The higher Hb A\textsubscript{2} value was seen in insignificant number of treated patients than control group (\(P<0.0001\)). Conclusion. The clinicians, pathologists, haematologists, and genetic counsellors should be aware of effects of nutritional anaemia and ART on Hb A\textsubscript{2} to reduce the chances of misdiagnosis of \(\beta\)-thalassaemia especially in developing countries and for centres for antenatal screening.

1. Introduction

Cooley and Lee were the first to describe the diminished rate of synthesis of one or more globin chains or its part, which consequently results in reduced rate of synthesis of the haemoglobin. Whipple and Bradford in 1936 gave the term “thalassaemia” [1]. \(\beta\)-thalassaemia is commonly seen in Mediterranean region, Indian subcontinent, South-east Asia, and African ancestry [1]. For \(\beta\)-thalassaemia, screening of populations was done either by measuring the haemoglobin A\textsubscript{2} percentage or by red cell indices in patients with MCV or MCH below a certain cut-off point [1]. The haemoglobin A\textsubscript{2} is 4%-5% in most cases of heterozygous \(\beta^0\) or severe \(\beta^+\) thalassaemia and 3.6–4.2% in heterozygous mild \(\beta^+\) thalassaemia [1]. The raised Hb A\textsubscript{2} values are seen in unstable haemoglobins, hyperthyroidism, and megaloblastic anaemia, and human-immunodeficiency-virus- (HIV-) infected patients on antiretroviral therapy, while hypochromic microcytic red cell picture is seen only in heterozygous \(\beta\)-thalassaemia [2]. History, clinical details, and coexisting condition help to rule out the causes of increase in Hb A\textsubscript{2}, which can avoid unnecessary investigation and reduce the financial burden of institutional setting in developing countries. Awareness of the fact that there are increases in Hb A\textsubscript{2} due to antiretroviral drugs among clinicians and pathologists should be must. Spiga et al. reported that zidovudine inhibits \(\beta\)-globin gene expression in human erythroid progenitor cells [3]. HIV-infected patients receiving highly active ARV therapy (HAART) or ZDV are associated with elevation of Hb A\textsubscript{2} value [2–5]. Galacteros et al. observed that HIV-infected patients at stage IV of disease have moderate elevation of Hb A\textsubscript{2} level and beta-thalassemia-like unbalanced biosynthetic globin ratio [6].

2. Materials and Methods

This study was a case control study. A total of 185 patients were included in the study, who were recruited from the ART center of the DDU Hospital. The case group included 125 HIV patients who were on antiretroviral therapy (ART)
Table 1: Results.

|                | Case                     | Control                  |
|----------------|--------------------------|--------------------------|
| Number of patients | 125 (75 M/50 F)          | 60                       |
| Mean MCV        | 96.88 fl                 | 82 fl                    |
| MCV > 100 fl    | 54.4% (68/125)           | Nil                      |
| Mean RDW        | 20.9                     | 14.6                     |
| RDW †           | 60.8% (76/125)           | 16.67% (10/60)           |
| Serum ferritin  | Normal                   | Normal                   |
| Folic acid      | Normal                   | Normal                   |
| Vitamin B12     | Normal                   | Normal                   |
| Mean Hb A2      | 3.462% (SD 0.675)        | 2.815% (SD 0.246)        |
| <3 Hb A2        | 11.2% (14/125)           | 70% (42/60)              |
| 3–3.5 Hb A2     | 56.8% (71/125)           | 30% (18/60)              |
| >3.5 Hb A2      | 37.33% (28/75) male;     | None                     |
|                 | 24% (12/50) female       |                          |
| Osmotic fragility (OF) | Normal                 | Normal                   |

including zidovudine, nevirapine, and lamivudine for the last three years and 60 untreated newly diagnosed patients were in the control group who were not receiving ART. All these patients were tested with rapid ELISA method for HIV and V.D.R.L for syphilis. In case group, 75 patients were male and 50 were female. The complete clinical data, haemogram, osmotic fragility (OF) test, and biochemical investigations were done. All the patients were tested for HIV. The red cell indices, red cell distribution width (RDW), and OF test were done for the evaluation of thalassaemia. High-performance liquid chromatography (HPLC) was done and hemoglobin A2 level was observed; value more than 3.5% was considered significant. The Hb A2 percentages of HIV patients were compared with those of control using an unpaired t-test. HbE trait, history of alcohol, and abnormal liver function test were the exclusion criteria for the study.

3. Results

The red cells indices show mean MCV 96.88 fl, and 54.4% of (68/125) patients had MCV > 100 fl. The rest of the patients had MCV within normal range. In control group, all patients have MCV within normal range with mean value of MCV 82 fl. RDW is increased in 60.8% (76/125) of patients with mean RDW 20.9. Serum ferritin, folic acid levels, and vitamin B12 level were within normal limits. The mean of Hb A2 in HIV patients on treatment was 3.462% (SD 0.675) and control has a mean Hb A2 of 2.815% (SD 0.246). The high Hb A2 was seen in significantly higher number of patients than controls (P < 0.0001). Osmotic fragility (OF) test of all patients and control was normal (Table 1).

4. Discussion

The β-thalassemia is one of the most common single gene disorders in India with an overall prevalence of 3–4%, and β-thalassemia carriers vary between 8 and 10% in certain communities like Sindhis, Muslims, Cutchi Bhanushalis, and some tribal groups [7]. The diagnosis of heterozygous β-thalassaemia is of key importance in the antenatal setting because it is helpful in concern to haemoglobinopathy counseling, partner screening, and possibly extensive haematological and molecular analyses of the patient samples [2]. Rao et al. observed that patients with megaloblastic anaemia have raised Hb A2 (P < 0.001) as compared to normal cases [8]. In the present study, vitamin B12 and folic acid levels were within normal limits in both study and control groups, but mean MCV in study group was 96.88 fl and 54.4% showed macrocytosis (MCV > 100 fl). The impair DNA synthesis in megaloblastic anaemia occurs due to delayed nuclear maturation, while zidovudine causes inhibition of nucleoside reverse transcriptase [9]; therefore, in both conditions, the increased synthesis of δ chains leads to higher Hb A2 values because more Hb synthesis occurs in less mature erythroid precursors, and the synthesis of δ chains is relatively greater in less mature cells [10]. Thyroid hormone affects the δ gene transcription and causes increase in both the percentage and absolute amount of Hb A2 [9]. In the present study, thyroid functions test was within normal limits in both study and control groups. Wilkinson et al. observed that incidence of increased Hb A2 value was significantly high in patients treated with zidovudine containing HAART than in those treated with non-zidovudine HAART and normal volunteers [4]. In the present study all patients on zidovudine containing ART had raised Hb A2 value than control group. 37.33% (28/75) of the males and 24% (12/50) of the females showed raised Hb A2. Pornprasert et al. also observed that ART increases the Hb A2 value and alters some haematological parameters that might affect the diagnosis of thalassaemia carriers, especially in the investigation of β-thalassaemia trait [11]. Kosalaraksa et al. observed that abnormal high % Hb A2 was found in more than a half of ZDV-exposed HIV-infected children; therefore, low MCV and MCH were important coparameters to reduce the misinterpretation of β-thalassaemia trait; however, the DNA analysis should be performed to confirm the diagnosis in such situation [12]. Howard et al. concluded that Hb A2 should be estimated before the initiation antiretroviral drugs in all HIV-infected women of child-bearing age to allow a diagnosis of heterozygous β-thalassaemia to be made and help to prevent the inaccurate diagnosis of heterozygous β-thalassaemia, and unnecessary genetic counselling and genetic analysis [2]. Pornprasert et al. also concluded that Hb A2 values should be measured in all HIV-1-infected couples before the initiation of antiretroviral drugs to rule out misdiagnosis of β-thalassaemia [13]. In the present study, all cases showed normal Hb A2 before starting ART. Few drugs have effect on various haemoglobins like butyrate, which induces fetal hemoglobin (HbF) synthesis in cultures of erythroid progenitors in man by beta oxidation by mitochondrial enzymes resulting in the formation of two acetate molecules from each molecule of butyrate [14]. The fatty acid analogue valproic acid (n-dipropylacetic acid) may increase the synthesis of fetal hemoglobin [15]. Macrocytosis was a striking result of hydroxy urea therapy and paralleled the increase in HbF synthesis [16]. In the present study, no history of intake of other drugs was present.
5. Conclusion

In India, HIV patients have wider access to ART to reduce the annual AIDS-related deaths, and all patients with increased Hb A₂ should be investigated for detailed history, identified high risk factor for HIV, and all haematological parameters including OF test, MCV, MCH, MCHC, and RDW. The clinicians, pathologists, haematologists, and genetic counselors should be aware of effects of nutritional anaemia and ART on Hb A₂ to reduce the chances of misdiagnosis of β-thalassaemia.

References

[1] B. J. Bain, *Haemoglobinopathy Diagnosis*, Blackwell Publishing, Malden, Mass, USA, 2nd edition, 2006.
[2] J. Howard, J. S. Henthorn, S. Murphy, and S. C. Davies, “Implications of increased haemoglobin A₂ values in HIV positive women in the antenatal clinic,” *Journal of Clinical Pathology*, vol. 58, no. 5, pp. 556–558, 2005.
[3] M. Spiga, D. A. Weidner, C. Trentesaux, R. D. Leboeuf, and J. Sommadossi, “Inhibition of β-globin gene expression by 3'-azido-3'-deoxythymidine in human erythroid progenitor cells,” *Antiviral Research*, vol. 44, no. 3, pp. 167–177, 1999.
[4] M. J. Wilkinson, B. J. Bain, L. Phelan, and A. Benzie, “Increased haemoglobin A₂ percentage in HIV infection: disease or treatment?” *AIDS*, vol. 21, no. 9, pp. 1207–1208, 2007.
[5] J.-P. Routy, M. Monte, R. Beaulieu, E. Toma, L. St.-Pierre, and M. Dumont, “Increase of hemoglobin A₂ in human immunodeficiency virus-1-infected patients treated with zidovudine,” *American Journal of Hematology*, vol. 43, no. 2, pp. 86–90, 1993.
[6] F. Galacteros, F. Amaudric, C. Prehu et al., “Acquired unbalanced hemoglobin chain synthesis during HIV infection,” *Comptes Rendus de l'Academie des Sciences. Series III*, vol. 316, no. 4, pp. 437–440, 1993.
[7] A. C. Gorakshakar and R. B. Colah, “Cascade screening for β-thalassemia: a practical approach for identifying and counseling carriers in India,” *Indian Journal of Community Medicine*, vol. 34, no. 4, pp. 354–356, 2009.
[8] S. Rao, R. Kar, S. K. Gupta, A. Chopra, and R. Saxena, “Spectrum of haemoglobinopathies diagnosed by cation exchange-HPLC & modulating effects of nutritional deficiency anaemias from north India,” *Indian Journal of Medical Research*, vol. 132, no. 11, pp. 513–519, 2010.
[9] G. Moyle, W. Sawyer, M. Law, J. Amin, and A. Hill, “Changes in hematologic parameters and efficacy of thymidine analogue-based, highly active antiretroviral therapy: a meta-analysis of six prospective, randomized, comparative studies,” *Clinical Therapeutics*, vol. 26, no. 1, pp. 92–97, 2004.
[10] M. H. Steinberg and J. G. Adams III, “Hemoglobin A₂: origin, evolution, and aftermath,” *Blood*, vol. 78, no. 9, pp. 2165–2177, 1991.
[11] S. Pornprasert, P. Leechanachai, V. Klinbuayaem et al., “Effect of hematological alterations on thalassaemia investigation in HIV-1-infected Thai patients receiving antiretroviral therapy,” *HIV Medicine*, vol. 9, no. 8, pp. 660–666, 2008.
[12] P. Kosalaraksa, S. Wiangnon, T. Bunupuradah et al., “High HbA₁c due to zidovudine exposure: implication for β-thalassaemia trait screening,” in *Proceedings of the 19th International AIDS Conference*, Abstract no. MOPE045.