CASE REPORT

Implications of increased haemoglobin A₂ values in HIV positive women in the antenatal clinic

J Howard, J S Henthorn, S Murphy, S C Davies

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Universal antenatal haemoglobinopathy screening in this hospital has identified several women with increased haemoglobin A₂ values, but without hypochromic microcytic red cell indices. This report describes two cases where there is evidence that the raised haemoglobin A₂ value is not caused by heterozygous β thalassaemia, but rather results from these patients being human immunodeficiency virus (HIV) positive and on antiretroviral therapy. This will have important implications as universal antenatal haemoglobinopathy screening becomes more widespread, and as the number of HIV positive women of childbearing age increases.

In adults, 96–98% of haemoglobin is haemoglobin A, with the remaining haemoglobin consisting of haemoglobin A₂ (1.5–3.5%) and haemoglobin F. Haemoglobin A₂ (HbA₂) is typically raised to 4–6% in heterozygous β thalassaemia, where there is an underproduction of normal β chains. This is of diagnostic importance in antenatal screening for haemoglobinopathies. However, raised HbA₂ values have been reported in other situations including in normal individuals,¹ unstable haemoglobins, hyperthyroidism, megaloblastic anaemia,² and in human immunodeficiency virus (HIV) infected patients on antiretroviral therapy.³ Of these conditions, only heterozygous β thalassaemia is associated with hypochromic microcytic red cell indices.

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Selective antenatal screening for haemoglobinopathy programmes generally rely on red cell indices. HbA₂ values are only measured if the pregnant woman has hypochromic microcytic red cell indices, and women with both appropriate red cell indices and a raised HbA₂ value are presumed to have heterozygous β thalassaemia trait. Universal haemoglobinopathy antenatal screening was introduced in our hospital in 1986. Since then, all pregnant women who are referred to our antenatal service have blood taken at about 14 weeks of gestation for full blood count and high performance liquid chromatography. This last test is performed irrespective of red cell indices using a Biorad variant II (Biorad, Hercules, California, USA). This identifies all of the common clinically relevant haemoglobin variants. Any abnormal results are confirmed by isoelectric focusing and haemoglobin electrophoresis, as appropriate. Women with HbA₂ values over 3.9% are diagnosed as having β thalassaemia trait and the haemoglobinopathy counsellors are informed. They arrange to counsel the woman and to test her partner.

This has led to the identification of several women who do not have hypochromic, microcytic red cell indices, but have a raised HbA₂ value and were referred to the haemoglobinopathy counsellors as if they had heterozygous β thalassaemia. Some of the patients with a normal or raised mean cell volume (MCV) and raised HbA₂ value have subsequently been identified as being HIV infected and on antiretroviral therapy. In two of these patients, there is evidence to suggest that their raised HbA₂ value is related to their medication and not to heterozygous β thalassaemia.

CASE REPORT

Patient 1

Patient 1 is a 35 year old woman who was diagnosed as being HIV positive in Spain in 1990. She has asymptomatic HIV disease and is coinfecting with hepatitis C. Her liver function tests are consistently normal and she is hepatitis C RNA negative. She has been on antiretroviral medication since 1992. She first presented to our hospital in December 1999 when she was on stavudine, didanosine, and nevirapine. At this time, she had a haemoglobin value of 137 g/litre, with an MCV of 96.3 fl and a mean cell haemoglobin (MCH) of 31.7 pg. She had a CD4 count of 630×10^6 /litre and a viral load of < 400 RNA copies/ml. Since then, her HIV viral load has been non-detectable and her CD4 count has been higher than 400×10^6 /litre. In 2000, her drug regimen was changed to zidovudine, lamivudine, and efavirenz. She was still on this medication in September 2002, when she presented at eight weeks pregnant. At this point, efavirenz was switched to nelfinavir because of the teratogenicity of efavirenz. She remains on zidovudine, lamivudine, and nelfinavir to this date.

On booking in antenatal clinic in September 2002, her full blood count showed a haemoglobin of 124 g/litre, MCV of 107.3 fl, and MCH of 36.3 pg. Her HbA value was 95.7% and HbA₂ was 4.3%. In view of her raised HbA₂ value, she was referred to the haemoglobinopathy counsellors and counselled as if she had heterozygous β thalassaemia. Her macrocytosis was attributed to her antiretroviral therapy. Her partner (also HIV infected but not on antiretrovirals) was tested and found to have a haemoglobin of 118 g/litre, MCV of 62 fl, and MCH of 20.1 pg. His HbA value was 87.2% and HbA₂ was 6.3%, implying that he too had heterozygous β thalassaemia. Because there was doubt about the patient's thalassaemia carrier status, and about the risk to the fetus, samples from the patient and her partner were referred for genetic analysis. It was confirmed that her partner had heterozygous β thalassaemia (IVS2-1 (G-A) β zero). The patient's entire β globin gene locus was sequenced and no β thalassaemia mutation was identified. Therefore, it is very unlikely that she is a carrier of β thalassaemia.

Abbreviations: Hb, haemoglobin; HIV, human immunodeficiency virus; MCH, mean cell haemoglobin; MCV, mean cell volume

The patient and her partner were counselled accordingly and she was delivered of twins in April 2003. Both children have normal full blood counts and neonatal haemoglobinopathy screening.

Patient 2

Patient 2 is a 32 year old woman whose first known haematological investigations were in 1996 when she had a full blood count showing a haemoglobin of 90 g/litre, MCV of 64.6 fl, and MCH of 20.4 pg. The HbA₂ value was 1.6% and zinc protoporphyrin was 195 (normal range, < 56 µm/MHb), implying a diagnosis of iron deficiency. In July 1999, she was diagnosed with AIDS when she was admitted to this hospital with pneumonia and weight loss and found to be HIV positive. She was started on antiretroviral therapy and in March 2000 was on zidovudine, lamivudine, and nelfinavir. At this time, she became severely anaemic and zidovudine was changed to stavudine.

She subsequently became pregnant and booking in blood tests in June 2000 showed a haemoglobin of 121 g/litre, MCV of 110.2 fl, and MCH of 36.8 pg. Her HbA₂ value at this time was 3.9% and we were suspicious that this was the result of her antiretroviral medication. However, we could not exclude the possibility that the raised HbA₂ was the result of heterozygous β thalassaemia, and she was referred to the haemoglobinopathy counsellors. She and her partner were counselled and his blood was subsequently tested. He was found to have HbA and HbA₂ values within the normal range, so no further investigations were performed. In January 2002, her nelfinavir was changed to nevirapine to aid adherence. A subsequent full blood count in April 2002 during her second pregnancy showed a haemoglobin of 112 g/litre, MCV of 92.3 fl, and MCH of 33.4 pg, with a HbA₂ value of 2.9%. This discrepancy in HbA₂ values led to further consideration of the case.

DISCUSSION

Patient 1 was found to have a raised HbA₂ value while on zidovudine, but subsequent genetic analysis showed no evidence of heterozygous β thalassaemia. Zidovudine has previously been reported to cause raised HbA₂ values, so that this drug is probably the cause of the raised HbA₂ values seen in this patient. She had only been on nelfinavir for a few days before the raised HbA₂ was detected, so this is unlikely to be the cause. Patient 2 had a normal HbA₂ value (1.6%) before starting antiretroviral therapy. On antenatal screening, the HbA₂ value was found to be 3.9%, raising the possibility that she may have heterozygous β thalassaemia, and leading to referral for haemoglobinopathy counselling. She was iron deficient at the time of the initial HbA₂ measurement, but it is very unlikely that correction of the iron deficiency could raise the HbA₂ value from 1.6% to 3.9%. Zidovudine had been stopped 10 weeks before the raised HbA₂ value was detected; however, because the approximate half life of erythrocytes is 120 days, zidovudine was possibly also the cause in this patient. After a longer period off zidovudine medication her HbA₂ value had returned to normal. Alternatively, nelfinavir may have been the cause of the raised HbA₂ in this patient because she was taking this drug when the raised HbA₂ was detected, but had stopped it three months before the normal HbA₂ was recorded.

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Take home messages

- We describe two patients with raised haemoglobin A₂ (HbA₂) values on antenatal screening, which were not caused by heterozygous β thalassaemia, but were the result of them being human immunodeficiency virus (HIV) positive and on antiretroviral therapy
- This will have important implications as universal antenatal haemoglobinopathy screening becomes more widespread, and as the number of HIV positive women of childbearing age increases
- We suggest that an estimate of HbA₂ should be taken in all HIV infected women of child bearing age, before they are started on antiretroviral drugs, to allow a diagnosis of heterozygous β thalassaemia to be made or refuted

The nucleoside reverse transcriptase inhibitor antiretroviral drugs zidovudine and stavudine are known to cause a rise in MCV and MCH. The rise caused and sustained by zidovudine tends to be greater than that seen with stavudine.^{4,5} It is thought that these drugs impair DNA synthesis, resulting in delayed nuclear maturation in a similar way to which DNA replication and cell division are affected in megaloblastic anaemia. In this last situation, more haemoglobin synthesis occurs in less mature erythroid precursors, and the synthesis of d chains is relatively greater in less mature cells; therefore, the increased synthesis of d chains leads to higher HbA₂ values.² This may also be the mechanism by which antiretroviral drugs cause raised HbA₂ values.

The diagnosis of heterozygous β thalassaemia is of key importance in the antenatal setting because it leads to haemoglobinopathy counselling, partner screening, and possibly extensive haematological and molecular analyses of patient samples. With the imminent onset of universal antenatal haemoglobinopathy screening in the UK for areas of high and moderate prevalence, and with the increasing numbers of HIV infected women of child bearing age who are being treated with antiretroviral drugs, the incidence of the finding of a raised HbA₂ value in this population will probably increase. The clinician is then faced with a dilemma because without previous normal HbA₂ values, or molecular investigation, it is impossible to exclude a diagnosis of heterozygous β thalassaemia. The finding of macrocytosis does not allow heterozygous β thalassaemia to be excluded because antiretroviral therapy can cause macrocytosis in this group of patients.

We advise that in all HIV infected women of child bearing age, an estimate of HbA₂ should be taken before they are started on antiretroviral drugs, to allow a diagnosis of heterozygous β thalassaemia to be made or refuted. This should be clearly documented in their notes. Then, if they subsequently become pregnant, the raised HbA₂ value will not lead to an inaccurate diagnosis of heterozygous β thalassaemia, and unnecessary genetic counselling and genetic analysis.

Authors' affiliations

J Howard, J S Henthorn, S C Davies, Department of Haematology, Central Middlesex Hospital, Acton Lane, Park Royal, London NW10 7NS, UK

S Murphy, Department of Genito-Urinary Medicine, Central Middlesex Hospital

The patients gave their permission for these details to be published.

Correspondence to: Dr J Howard, Department of Haematology, Central Middlesex Hospital, Acton Lane, Park Royal, London NW10 7NS, UK; Jo.Howard@nwlh.nhs.uk

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