Normocytic Norm Chromic Anemia Is Most Common Type Anemia HIV Infected Patients

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Abstract
Aims & Objectives - Establish care guidelines for HIV infected person and altered haematopoiesis resulting in anaemia. Review the pathogenesis of the haematological manifestations of human immunodeficiency virus (HIV). Identify the haematological manifestations (Peripheral smear) of altered haematopoiesis resulting from HIV infection.

Material & Methods - Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol and by universal precaution as per the guideline of National aids control organization (NACO, India). A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by Automated blood cell counter analyzer of all the patient on antiretroviral therapy. All cell count indices including WBC count with differential and platelet count, was further confirmed by manual oil immersion smear study method. Peripheral smears study was done with field A and B stain and leishman stain.

Conclusion - Males are more affected than female, highest prevalence of HIV infection is found in both sex 30-41 years of age group. Anaemia is most common haematological parameters of HIV positive cases. Normocytic normochromic anaemia is most common type of anaemia because HIV is a chronic disease. Second most common finding is microcytic hypochromic anaemia in our study due to poor nutrition and viral effect on fe metabolism. Third most common finding is macrocytic anaemia probably due to Zidovudine therapy. Normocytic normochromic anaemia is more common in male but microcytic hypochromic anaemia is more common in female and microcytic anaemia is near equal in both sex.
MATERIAL & METHODS

Study area and design- The present study was conducted at the Department of Pathology MGM Medical College associated with M.Y. Hospital Indore, M.P. The study was designed as an observational hospital based study over a period of time from 2010 to 2012 years.

Ethical consideration-Detailed general, systemic examination along with complete details of patient and informed consent was obtained from all study participant do from ART Center of M.Y. Hospital Indore during the time of registration at center.

Patients selection criteria-The study targeted medically diagnosed HIV positive cases with the help of ELISA technique and confirmed by western blot under the guideline of National aids control organization (NACO, India) over period of time from 2010 to 2012.

All studied 300 cases registered at ART Center and on HAART between the age of 5 to 69 years who are schedule to visit the hospital at regular intervals of time for routine medical review was studied.

Laboratory investigations- Blood was collected in a sterile EDTA containing tube and processed following our established laboratory protocol and by universal precaution as per the guideline of National aids control organization (NACO, India).

A complete blood counting including HB%, PCV, Red cell indices, platelet count and total white cell count and differential was done by Automated blood cell counter analyzer of all the patient on antiretroviral therapy. The all cell count indices including WBC count with differential and platelet count, was further confirmed by manual oil immersion smear study method.

Peripheral smears study was done with field A and B stain and leishman stain.

COMPLETE BLOOD COUNT (CBC) AND PERIPHERAL SMEAR.

Materials:

1. Purple vacutainer tube or capillary collector (EDTA)
2. Slides and blue capillary tube
3. Needle or lancet
4. Vacutainer holder
5. Alcohol swab
6. Cotton balls
7. Absorbent materials
8. Slide case

Procedure:

1. Specimen is collected into EDTA (purple) vacutainer. (5 or 7ml volume)
   - Blood smears must be made from freshly collected specimen and must be prepared within four hours of collection.

   Preparation of peripheral blood smear

   Step 1. A small drop of venous blood is placed on a glass microscope slide, using a glass capillary pipette.

   Step 2. A spreader slide is positioned at 45° angle and slowly drawn toward the drop of blood.

   Step 3. The spreader slide is brought in contact with the drop of blood and is being drawn away.

   Step 4. The spreader slide is further pulled out, leaving a thin layer of blood behind.

   Step 5. The blood smear is nearly complete.

   Step 6. End result will be a glass slide with a well-formed blood film. After drying for about 10 minutes, the slide is fixed in methanol & stained with field A and B stain.

   A well-made peripheral smear is thick at the frosted end and becomes progressively thinner.
toward the opposite end. The “zone of morphology” (area of optimal thickness for light microscopic examination) should be at least 2 cm in length. The smear should occupy the central area of the slide and be margin-free at the edges.

**Hematological examination**

Hematological examination including HB%, PCV, Red cell indices, platelet count and total white cell count with differential count should be done on peripheral smears stained with field A and B stain.

Following **Base line investigation** were done for all 300 patients.

1. Hemoglobin in grams/dl—(Cyanmethaemoglobin method of automated blood cell counter analyzer) and further confirmation by Sahli’s manual method in case of suspicious readings.

2. RBC counting and RBC indices parameters MCV, MCH, MCHC, PCV & RDW - automated cell counter analyzer

3. RBC morphology study under oil immersion manual stained smear study method

4. Total and differential leukocyte count - automated cell counter analyzer & confirmed by oil immersion manual stained smear study method

5. Platelets counts - automated cell counter analyzer & confirmed by oil immersion manual stained smear study method

Other counting parameters and morphological changes done under automated cell counter analyzer & confirmed by manual oil immersion smear study method.

**DISCUSSION**

<table>
<thead>
<tr>
<th>Type of Anaemia</th>
<th>Total Cases (n=300)</th>
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<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Normocytic Normochromic Anaemia</td>
<td>172</td>
</tr>
<tr>
<td>Microcytic Hypochromic Anaemia</td>
<td>78</td>
</tr>
<tr>
<td>Macrocytic Anaemia</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Type of Anaemia</th>
<th>Female</th>
<th>% (n=112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocytic Normochromic Anaemia</td>
<td>58</td>
<td>51.78%</td>
</tr>
<tr>
<td>Microcytic Hypochromic Anaemia</td>
<td>35</td>
<td>31.25%</td>
</tr>
<tr>
<td>Macrocytic Anaemia</td>
<td>19</td>
<td>16.97%</td>
</tr>
</tbody>
</table>
Anaemia in Male

<table>
<thead>
<tr>
<th>Type of Anaemia</th>
<th>Male</th>
<th>%  (n=188)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normocytic Normochromic Anaemia</td>
<td>114</td>
<td>60.64%</td>
</tr>
<tr>
<td>Microcytic Hypochromic Anaemia</td>
<td>43</td>
<td>22.88%</td>
</tr>
<tr>
<td>Macrocytic Anaemia</td>
<td>31</td>
<td>16.48%</td>
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</tbody>
</table>

Out of the 300 study cases of normocytic hypochromic anaemia is the most commonly affected hematological parameter.

Data analysis - Distribution Hematological Parameter- Anaemia

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Female</th>
<th>Male</th>
</tr>
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<tr>
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</table>

- Data analysis in following hematological parameters with the difference of sex distribution under the Extended Mantel-Haenszel test for trend of chi-Squares test.

- Chi-sq. test X2 Value =0.873 [DF = 1] 2-sided P = 0.350

- For trend in a given direction: P = 0.175

CONCLUSION

Males are more affected than female, highest prevalence of HIV infection is found in both sex 30-41 years of age group. Anaemia is most common haematological parameters of HIV positive cases. Normocytic normochromic anaemia is most common type of anaemia because HIV is a chronic disease. Second most common finding is microcytic hypochromic anaemia in our study due to poor nutrition and viral effect on iron metabolism. Third most common finding is macrocytic anaemia probably due to Zidovudinetherapy. Normocytic normochromic anaemia is more common in male but microcytic hypochromic anaemia is more common in female and macrocytic anaemia is near equal in both sex. Anaemia in patients with HIV infection who are not undergoing antiretroviral therapy with zidovudine is typically normochromic normocytic, although a mild degree of anisocytosis and poikilocytosis is common. Macrocytosis occurs in
the majority of patients treated with zidovudine. Schistocytes are prominent in the setting of thrombotic thrombocytopenic purpura, which may complicate HIV infection, as described in later section.

In addition to being complication of the HIV infection itself, anaemia is a frequent side effect of antiretroviral drugs particularly zidovudine, as well as drugs used to treat the or prevent opportunistic infections, including dapsone, primaquine, rimethoprim/sulfamethoxazole, and ganciclovir.

Depressed erythropoiesis in AIDS has been suggested by a low or inappropriately normal reticulocyte count, and in HIV-infected patients the reticulocyte amount cannot be used as a reliable indicator of either hemolysis or bleeding. Similar to the anaemia of chronic disease, it is likely that inflammatory cytokines that play a role in suppressing erythropoiesis in patients of HIV infection. Tumor necrosis factor and interleukin-1 have been suppress erythropoiesis in vitro, and both of these cytokines can be increased in HIV-infected patients. In addition, the finding on bone-marrow examination of normal to increased numbers of erythroid progenitor cells, along with a variable degree of dyserythropoiesis, has indicated that ineffective erythropoiesis may be an additional contributing factor.

Anaemia is the most common haematological abnormality found in children and adult with HIV infection. Indeed, anaemia was the initial manifestation of HIV infection in about 10% of children in a recent study in Italy. The importance of finding and treating anaemia in adult with HIV infection is underscored by data from their study showing anaemia to be an independent prognostic factor of mortality in children with HIV infection. The prognostic significance of anaemia at baseline is statistically significant in multiple retrospective studies in adults in the United States and Europe both in the pre-highly active antiretroviral therapy (HAART) and HAART eras.

The etiology of anaemia in adult with HIV infection is multifactorial, and managing anaemia can involve a variety of modalities. HIV infection and its direct effects on HSCs and stromal elements can lead to anaemia. Opportunistic infection and myelosuppressive drugs might also cause anaemia.

Another well-known cause of anaemia is pure red cell aplasia, caused by infection with parvovirus B19, and should be considered with HIV infection that have isolated anaemia. Other marrow-suppressive infections such as CMV and MAC often affect the white cell lineage, first leading to neutropenia rather than anaemia. Anaemia of chronic infection as caused by these agents is normocytic.

Myelosuppressive drugs such as zidovudine and trimethoprim-sulfamethoxazole can also lead to anaemia. The anaemia associated with zidovudine treatment is macrocytic, and indeed the red cells may be macrocytic even without anaemia. Some practitioners use this finding to assess adherence to zidovudine therapy.

Finally, anaemia can be a result of red cell destruction, haemolysis, as opposed to an aberration of production. Clinically significant haemolysis in patients with HIV infection is rare.

So a common approach involves classifying anaemia into macrocytic, normocytic, or (most commonly) microcytic, on the basis of mean cell volume. Normocytic anaemia is most common with HIV infection. In, one can often find this
development as a result of the chronic disease state.

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