The magnitude and associated factors of immune hemolytic anemia among human immunodeficiency virus infected adults attending University of Gondar comprehensive specialized hospital north west Ethiopia 2021 GC, cross sectional study design

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Abstract

Background
Immune hemolytic anemia commonly affects human immune deficiency infected individuals. Among anemic HIV patients in Africa, the burden of IHA due to autoantibody was ranged from 2.34 to 3.06 due to drug was 43.4%. IHA due to autoimmune is potentially a fatal complication of HIV which accompanies the greatest percent from acquired hemolytic anemia.

Objective
The main aim of this study was to determine the magnitude and associated factors of immune hemolytic anemia among human immunodeficiency virus infected adults at University of Gondar comprehensive specialized hospital north west Ethiopia from March to April 2021.

Methods
An institution-based cross-sectional study was conducted on 358 human immunodeficiency virus-infected adults selected by systematic random sampling at the University of Gondar comprehensive specialized hospital from March to April 2021. Data for socio-demography, dietary and clinical data were collected by structured pretested questionnaire. Five ml of venous blood was drawn from each participant and analyzed by Unicel DHX 800
hematology analyzer, blood film examination and antihuman globulin test were performed to diagnosis of immune hemolytic anemia. Data was entered into Epidata version 4.6 and analyzed by STATA version 14. Descriptive statistics were computed and firth penalized logistic regression was used to identify predictors. P value less than 0.005 interpreted as significant.

**Result**

The overall prevalence of immune hemolytic anemia was 2.8% (10 of 358 participants). Of these 5 were males and 7 were in the 31 to 50 year age group. Among individuals with immune hemolytic anemia, 40% mild and 60% moderate anemia. The factors that showed association were family history of anemia (AOR 8.30 at 95% CI 1.56, 44.12), not eating meat (AOR 7.39 at 95% CI 1.25, 45.0), and high viral load 6.94 at 95% CI (1.13, 42.6).

**Conclusion and recommendation**

Immune hemolytic anemia is less frequent condition in human immunodeficiency virus infected adults, and moderate anemia was common in this population. The prevalence was increased with a high viral load, a family history of anemia, and not eating meat. In these patients, early detection and treatment of immune hemolytic anemia is necessary.

**Introduction**

**Background**

In HIV infection, hematological parameters are mostly affected because of the viral effect on all lineages of blood cells and the immune system. The most typically affected hematological profiles are leukocytes, erythrocytes, and platelets [1]. Anemia is the most common hematological problem, affecting about 30% of asymptomatic and 75–80% of symptomatic HIV infections. From severely anemic individuals around 53% of HIV-infected adults may be caught by death [2, 3]. The most frequent anemia in HIV patients is normocytic normochromic type [4].

Immune hemolytic anemia (IHA) is normocytic or macrocytic normochromic anemia that occurs due to antibodies formed against one or more antigenic constituents of the individual's tissues and results in the destruction of the erythrocyte. The main causes of IHA are primary or idiopathic and secondary by different underlying causes [5]. It can be classified as autoimmune hemolytic anemia (AIHA), alloimmune hemolytic anemia, and drug-induced hemolytic anemia (DIHA) [6]. Alloimmune hemolytic anemia occurs when antibodies are produced against red cells from another individuals, in transfusion, abortion and pregnancy [7]. AIHA results from the autoantibodies secondary to malignancies, autoimmune disorders and genetically [6]. It can be also classified as warm IHA, cold IHA and mixed IHA according to temperature of the reaction [5, 8].

In HIV patients IHA occurs due to Viral binding by HIV immune complexes to erythrocytes by one of three mechanisms. The mechanisms include the binding of complement-opsonized immune complexes via complement component receptor 1 (CR1), direct virus binding in a complement-dependent manner, but without the need for specific antibodies, and a third mechanism in which complement was not required at all. In absence of specific antibodies virus directly transported by erythrocyte surface during primary infection through duffy antigen [9, 10].
In the development of IHA by antigen-antibody complexes, the viral accessory protein negative factor plays a critical role in the pathogenesis of HIV-associated hematopoietic dysfunction. These factors affect the clonogenic potential of hematopoietic stem cells down modulates host cell receptors like a cluster of differentiation and major histocompatibility complex (MHC-1) molecules. It facilitates the transformation of infection into disease, increase viral infectivity, and increasing immunogenicity of the viral antigen mimic cell [1, 11, 12].

In addition to direct viral effect, it also occurs due to indirect effects of chronic generalized immune activation by HIV infection. These induce production of autoantibodies owing to the structural antigen similarity between the viral proteins and self-antigens [13]. The progressive decline of helper T-cell (CD4) caused by direct killing of infected cells that results from molecular mimicry of alloantigen with self, antiretroviral therapy (ART) drugs and opportunistic pathogens [14].

The autoimmune manifestations of HIV infection that cause IHA can be increased cytotoxic cell activity, increased expression of autoantigens, and alteration of erythrocyte surface antigen by a virus, and a cross-reaction between antibody induced by an infectious agent against erythrocyte surface antigen [15–18]. The factors that contribute to IHA includes increased viral load, tuberculosis, poor nutrition [19], family history of hemolytic anemia, neoplasia, unmatched blood transfusion, infection, and ART drugs [5, 20]. There is a variation of burden depending on the stage of HIV disease, sex, age, pregnancy status, history of abortion, and adherence to ART [21].

Immune hemolytic anemia in HIV-positive patients is a serious complication that occurs mostly in advanced age and stage of acquired AIDS especially in female patients. In Africa among anemic HIV patients, the burden of IHA due to autoantibody was ranged from 2.34 to 3.06% [17, 22], and due to DIHA was 43.4% [23]. It causes fever, jaundice, dark-colored urine, weakness, dizziness, confusion, hepatosplenomegaly, tachycardia and heart murmur [24]. Its consideration is important if patients experience severe to moderate anemia with low CD4 count [17].

Immune hemolytic anemia causes highly severe form of anemia. The most commonly reported age groups were middle-aged adult patients. Patients with IHA had lower mean CD4, Hb, RBC count, positive direct antiglobulin test (DAT), higher immature reticulocyte fraction, and mean reticulocyte percent than the non-anemic patients [25]. But DAT negative doesn’t mean there is no IHA because it may not be positive in the case of leukemia patients, immunosuppressed individuals, and low proteins [26]. In other way, a positive DAT is not in all cases resulted from IHA, because overt hemolytic anemia and aplastic anemia with hemolysis might be positive [27].

The diagnosis of IHA depends on the presence of laboratory findings supporting hemolysis such as increase of serum lactate dehydrogenase, haptoglobin, and unconjugated bilirubin in addition to DAT. The peripheral blood smear changes that are used as an indicator for hemolysis include reticulocytosis, shtistocytosis, bite cells, and spherocytosis [8, 17].

The attention to IHA in PLHIV is less than expected globally, particularly in Ethiopia, due to limited studies considering IHA among PLWHVs. Some studies that were done concerned with IHA due to autoimmunity even if they did not use immature reticulocyte fraction for differentiation of other cause of hemolysis from immune-mediated ones. The IHA burden is not well studied as its effect on people’s health, especially HIV patients and there was a knowledge gap between ART clinicians and other health professionals [17]. Therefore the aim of this study was to determine the magnitude and associated factors of anemia in HIV infected adults attending UOGCSH, North West Ethiopia.
Method and materials
Study design and period
An institutional based cross-sectional study was conducted to determine the magnitude of IHA and associated factors among HIV infected adults attending UOCSH North West Ethiopia from March to April 2021.

Population

Source population. All HIV-infected adult individuals attending ART clinic in UOCSH, North West Ethiopia.

Study population. All HIV-infected adult individuals attending ART clinic at UOCSH, during a time of data collection can be used as the study population.

Inclusion criteria and exclusion criteria

Inclusion criteria. All HIV infected individuals who were greater than or equal to 15 years or older, who had a confirmed HIV infection upon follow-up at the UOGCSH, and who had a clinical data and laboratory data’s such as viral load and CD4 counts in record within last six month of data collection time were included in study.

Exclusion criteria. Individuals who had been seriously ill and unable to respond and give blood specimens were excluded from the study.

Sample size calculation and sampling technique

The sample size for this study was calculated using the single population proportion formula, since no study done was on IHA we used 50% proportion with 95% confidence interval and 5% marginal error, and finally using population reduction formula since the total population was less than 10,000 then the sample size obtained was 358.

Sampling technique

A systematic random sampling technique was used to select study participants. The average number of HIV patients attending ART follow up every day and who gave a blood sample for viral load and CD4⁺ T-cell count concurrently were twenty five. During the two-month data collection period, 1100 PLWHIV were expected to visit the hospital for viral load and CD4⁺ T-cell count follow-up. The sampling interval (K) value was calculated by dividing the total number of HIV/AIDS patients during our study period by the sample size (1100/358 = 3). Then lottery method was used to select the first participant of three then taken by interval of three. The study subjects selected by every three individuals who are attending ART clinic of UOGCSH (Fig 1).

Operational definition of variables

Anemia is condition at which adjusted Hb amount is less than 12 g/dl for female and less than 13 g/dl for male [2].

DAT positive: when agglutination is observed either after immediate centrifugation or after centrifugation that followed room temperature incubation of red cell suspension with antihuman globin reagent (83).

Reticulocytosis—reticulocyte count greater than 2.5% or 158 x 10⁹/L [28].

Reticulocytopenia—reticulocyte count less than 0.5% or 18x10⁹/L [28].
IHA: is defined by a normocytic normochromic anemia or macrocytic anemia, which has hemolysis evidence in blood film such as spherocytosis or schistocytes, nucleated RBC, a reticulocyte count greater than 2.5% or immature reticulocyte fraction greater than 0.53 with positive DAT [29–32].

Data collection tools and methods

Sociodemographic and clinical data collection. The data was collected by semi-structured questionnaire and collection was performed by trained expert nurses. The questionnaire had three parts including sociodemographic, clinical, and nutritional data which were related to IHA. The questionnaire was translated into the Amharic language. For sociodemographic data such as age, sex, residence, marital status, education, and religion were collected via face-to-face interview with study subjects. The clinical data’s such as history of abortion, CD4 result, viral load, neoplastic disease, opportunistic infection, history autoimmune disease and medication was extracted manually from participants’ records. For pregnancy, the female participants who were in age between 15–49 years old were screened by laboratory test of pregnancy and family history anemia was also requested by face to face interview (Annexes I, II in S1 File).

Sample collection procedures and hematological analysis

Blood collection procedures. About 5ml of blood was collected with sterile syringe and needle by expert medical laboratory technologist into study participant code number labeled EDTA anticoagulant test tube. The collected blood sample was delivered to hematology laboratory for analysis of hematological parameters, DAT, and blood film preparation. The blood was transported to the hematology laboratory within 1 to 2 hours and the analysis to be performed. From the collected blood sample, hematological analysis was performed, then blood
film were prepared from the remnant sample. Finally, DAT was performed on the rest blood sample (Annex V in S1 File).

**Hematological analysis.** The hematological analysis was performed on a blood sample in an EDTA anti-coagulated test tube to confirm the presence of IHA by following standard operating procedures. Hb measurement, reticulocyte count, immature reticulocyte fraction, RBC count, and RBC indices such as MCV, MCH, and MCHC were performed by using an automated hematology analyzer (Unicel DxC800, Danaher Corporation, Beckman Coulter, United States of America (USA)). Unicel DxC800 provides RBC count, reticulocyte count, immature reticulocyte fraction, and nucleated RBC on whole blood by impedance principle and leukocyte 5-part differential (Diff) and platelet by flow cytometry or light scattering principle [30, 32]. The blood sample is suspended in diluent and passes through the apparatus causing direct current resistance. Change in blood cell size is detected as the electrical pulse and blood cell count is measured by counting pulse (Annex V in S1 File).

**Blood film examination.** After hematological parameter performed, the remaining EDTA blood was used for blood film examination. A thin blood smear was prepared by wedge method by putting a drop of blood on the slide about 1-2cm from the end of the slide and making smear by another smooth-edged slide as spreader at an approximate angle of 30˚ on three fourth (¾) of the length of the slide. The prepared smear was air dried by placing the smear film side up on a staining rack. The dried smear was covered with filtered undiluted wright stain, left for 1 minute, washed then dried, and examined by using oil immersion (100x objective) on the microscope. The morphology was examined by a trained technologist for the presence of features of hemolysis. In blood film the presence of schistocytes, spherocyte, bite cells, and nucleated RBC were evidence of hemolysis and in the meantime for cross-checking for morphology with analyzer result was performed (Annex V in S1 File).

**Coombs test (DAT).** Three percent of washed blood suspension was used for direct ant globin test (DAT) for detection of coated antibody on the surface of red cells that results in immune hemolysis. Coombs test (DAT) was performed based on the principle of a hem agglutination test. Two Drops of the anti-globulin reagent added to two drops of the three percent of red cell suspension into the test tube. Polyspecific anti-human globulin antiIgG-C3d acts as a link between the antibodies and complement coating of neighboring RBC and induces agglutination. The test tube would be immediately centrifuged after thorough mixing and finally reading for presence of agglutination was examined microscopically then reported as positive or negative for DAT (Annex V in S1 File).

**Immune hemolytic anemia (IHA).** Finally, Immune hemolytic anemia was diagnosed from the result of hematological parameters, blood smear, and coombs test results. It was defined as low Hb, normocytic or macrocytic red cell, feature of hemolysis on blood film such as bur cells, schistocytes, spherocytes, reticulocytes, high immature reticulocyte fraction, and positive for direct anti-human globulin test. The test was finally confirmed for the presence of IHA by a laboratory technologist.

**Quality management of laboratory tests and data**

**Quality assurance for sociodemographic data.** Before data collection, training was given to the data collectors to ensure the reliability and validity of data to reduce technical and observation bias. The questionnaires were tested (pretest) on randomly selected patients from the study site for reliability and validity before it was used for actual data collection. To check language translation information quality the translated questionnaire was reviewed by three individuals and retranslated back to the English language from Amharic. The validity of the information was checked again.
**Quality control for hematology analyzer.** Quality control for working equipment and reagents was ensured using standard controls as well as standard operating procedures. For Unicel DHX800 hematology analyzer normal background reading was checked daily and the performance was checked by low, normal, and high controls. The result of each test was properly recorded (annex v in S1 File).

**Quality control for coombs test.** The quality control was done for DAT, by using Rh-positive blood sample coated with an anti-D for positive control and negative control by Rh-negative blood were used. Then the result of both control was properly recorded (annex v in S1 File).

**Quality control for microscopy and wright stain reagent.** The preventive maintenance was performed for the microscope to prevent the entrance of abnormal artifacts in the morphological examination. The microscope was cleaned daily for quality examination of blood smear morphology and reticulocyte count. A microscopic smear review was performed to check functionality of microscope, quality of slide and staining by using previously examined and confirmed slides. To make quality staining, the solution was filtered before staining the smear. The quality control for wright staining solution was performed by using a patient sample with a normal MCV, MCH, MCHC and total white blood cell count (annex v in S1 File).

**Data management and analysis.** Data entry was entered into Epi data version 4.6 (Epi-data, Inc. Redwood City, CA, United States) and analysis was done by using STATA (Software for statistics and data science) statistical software version 14 developed by StataCorp for data. Every day the collected data was checked for completeness and accuracy by the principal investigator. During the entry of data, it was cross-checked to assure the right data was entered and cleaned for accuracy. Descriptive statistics such as frequency, charts, tables, and percentages were used to summarize the data. The firth penalized logistic regression model was fitted to determine the associations of independent variables with outcome variables. For measure of association for variable was analyzed by bivariable firth penalized logistic regression model and those variables which had P value 0.2 were included in multivariable firth penalized logistic regression model to control the confounding factors. Then multivariable firth panelized logistic regression was computed for selected variables and the significance of association was determined and interpreted. Both Crude odds ratio (COR) and adjusted odds ratio (AOR) with their corresponding 95% confidence interval (CI) were used to see the strength of association between dependent and independent. A p-value <0.05 in multivariable firth penalized logistic regression model was considered statistically significant. The results were presented in words and tables. Based on the study result, conclusions and recommendations were done.

**Dissemination of results.** The study result would be submitted to Department of Clinical Hematology and immunohematology the School of BMLS and CMHS, UOG, and also the results would be submitted to the study site. The abstract would be submitted to local concerning bodies Such as EMLA and libraries. The result would be communicated with the research community through a presentation on conferences and publication on peer-reviewed reputable journals to communicate with the international community.

**Result**

**Sociodemographic characters**

The total number of participants in this study was 358. Of the total participants, 216 (62.1%) of them were females and the median age was 38 (interquartile range 33 to 45) years. Among the study participants 313 (87.43%) were Orthodox Christian, and 285 (79.61%) were urban residents. Of all participants 187(52.87%) were married followed by divorced, 90(25.14%) were government employee and 115(32.12%) had secondary school education level (Table 1).
Clinical characteristics of the study participants

From the study participants, 117 (32.68%) of them had a history of comorbidity of HIV and opportunistic infections including bacterium tuberculosis, 33 (9.22%), had a family history of anemia and 66 (18.44%) had history of an autoimmune disease. Of all study participants, 24 (6.70%) of study participants had a history of neoplastic disease, and 278 (77.65%) were on stage one of disease (AIDS). Among female study participants 2 (0.93%) had history of pregnancy but none of them had abortion history in last four months (Table 2). Among study participants, 52.79% (189) drink coffee at least once a day, 94.13% (337) of them use (consume) meat in diet and 280 (78.21%) use green vegetables daily (Table 2).

Hematological and immunological profiles

Among the study participants, 101 (28.21%), 53 (14.8%), 37 (10.34%) and 10 (2.8%) had anemia, thrombocytopenia, leukopenia and pancytopenia, respectively. The study participants mean Hb level was 13.17 (95% CI 12.99, 13.36) g/dl, mean immature reticulocyte count was 0.37 (95% CI 0.36, 0.38) and mean relative reticulocyte count was 0.96% (95% CI 0.90% to 1.01%). Based on RBC morphology and red cell indices of anemic individuals, 62 (61.38%), 21 (20.8%), 18 (17.82%) had normocytic normochromic, microcytic hypochromic, and macrocytic anemia respectively. Of those anemic study participants 1 (0.99%), 21 (20.80%), 79 (78.21%) had severe, moderate, and mild anemia respectively. Among anemic study
participants 18 (17.82%) had spherocyte and 24 (23.76%) had schistocytes and bite cells on peripheral morphology. From the total parameters IHA anemia can be defined based on decreased hemoglobin level with normocytic or macrocytic RBC, evidence of hemolysis increased reticulocyte count or immature reticulocyte fraction and positive for DAT. The individuals who met this criteria were 10 (2.8%) (Table 3).

### Prevalence of immune hemolytic anemia

The overall prevalence of IHA (who met criteria for IHA) in this study was 2.80%. (95% CI 1.07, 4.50). The prevalence of IHA among HIV in patients the 15–30 years age group was

### Table 2. Clinical characters of study participants for the magnitude of IHA and associated factors among HIV infected adults at UOGCSH from March to April 2021.

| Variables                                    | Category      | Frequency (N) | Percentage (%) |
|----------------------------------------------|---------------|---------------|----------------|
| Pregnancy (n = 216)                          | Yes           | 7             | 3.27           |
|                                              | No            | 209           | 96.73          |
| History of birth in last four month (n = 216) | Yes           | 2             | 0.93           |
|                                              | No            | 214           | 99.07          |
| History of transfusion (n = 358)             | Yes           | 34            | 9.50           |
|                                              | No            | 324           | 90.50          |
| History of opportunistic infection including TB (n = 358) | Yes | 117 | 32.68 |
|                                              | No            | 241           | 67.32          |
| Family history of anemia (n = 358)           | Yes           | 33            | 9.22           |
|                                              | No            | 250           | 69.83          |
|                                              | Unknown       | 75            | 20.95          |
| Stage of AIDS (disease) (n = 358)            | Stage 1       | 278           | 77.65          |
|                                              | Stage 2       | 60            | 16.76          |
|                                              | Stage 3 and 4 | 20            | 5.59           |
| History of auto immune disease               | Yes           | 66            | 18.44          |
|                                              | No            | 292           | 18.44          |
| History of neoplastic disease (n = 358)      | Yes           | 24            | 6.70           |
|                                              | No            | 334           | 93.30          |
| HAART- regimen                               | AZT-containing | 81       | 22.63          |
|                                              | Non AZT-      | 277           | 77.37          |
| History of other medication                  | Yes           | 153           | 42.2           |
|                                              | No            | 205           | 57.8           |
| Other medication (n = 205)                   | ant-TB        | 107           | 69.93          |
|                                              | antibiotics   | 20            | 13.07          |
|                                              | other medication | 26       | 16.99          |
| HAART duration                               | On 3 months and bellow | 89       | 24.86          |
|                                              | On 6-months   | 245           | 68.44          |
|                                              | more than 6 month | 24        | 6.7            |
| Drink coffee per day                         | Less often per day | 169       | 47.21          |
|                                              | At least once a day | 189       | 52.79          |
| Weekly use (consume) meat (N = 358)*         | Less often per week | 261       | 77.44          |
|                                              | At least once a week | 76        | 21.23          |
|                                              | never         | 21            | 5.86           |
|                                              | At least once a day | 78        | 21.79          |
| Daily use green leafy vegetables             | Less often per day | 280       | 78.21          |

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Table 3. Hematological and immunological profiles of study participants for magnitude of IHA in HIV infected adults at UOGCSH during March to April 2021 (N = 358).

| Hematological and immunological profiles | Category | Frequency (N) | Percent (%) |
|-----------------------------------------|----------|---------------|-------------|
| RBC Male (n = 142)                      | < 4.26 \times 10^{12}/L | 45 | 12.6 |
|                                         | 4.26–6.68 \times 10^{12}/L | 94 | 26.25 |
|                                         | > 6.68 \times 10^{12}/L | 3 | 0.84 |
| Female (n = 216)                        | < 4.02 \times 10^{12}/L | 36 | 10.05 |
|                                         | 4.02–6.15 \times 10^{12}/L | 178 | 49.72 |
|                                         | > 6.15 \times 10^{12}/L | 2 | 0.56 |
| Hemoglobin Male (n = 142)               | <13 g/dL | 41 | 11.45 |
|                                         | 13–18.76 g/dL | 96 | 26.81 |
|                                         | >18.76 g/dL | 5 | 1.4 |
| Female (n = 216)                        | <12 g/dL | 60 | 16.8 |
|                                         | 12–16.7 g/dL | 151 | 42.18 |
|                                         | >16.7 g/dL | 5 | 1.4 |
| MCV                                     | <85(fL) | 24 | 6.7 |
|                                         | 85–100 (fL) | 273 | 76.26 |
|                                         | >100(fL) | 61 | 17.04 |
| MCH Male (n = 142)                      | <26.6(pg.) | 14 | 3.63 |
|                                         | 26.6–33.3(pg.) | 102 | 28.5 |
|                                         | >33.3(pg.) | 26 | 7.26 |
| Female (n = 216)                        | 25.8(pg.) | 8 | 2.24 |
|                                         | 25.8–32.8 (pg.) | 156 | 43.6 |
|                                         | >32.8(pg.) | 52 | 14.50 |
| Leukocyte                               | <3.24 \times 10^{9}/L | 37 | 10.34 |
|                                         | 3.24–10.5 x 10^{9}/L | 309 | 86.31 |
|                                         | >10.5 x10^{9}/L | 12 | 3.35 |
| Platelets Male (n = 142)                | <164X 10^{3}/L | 17 | 4.75 |
|                                         | 164–403X 10^{3}/L | 116 | 32.24 |
|                                         | >6.15X 10^{3}/L | 9 | 2.51 |
| Female (n = 216)                        | <202.5 X 10^{9}/L | 36 | 10.05 |
|                                         | 202.5–444.5 X 10^{9}/L | 174 | 48.6 |
|                                         | >274 X 10^{9}/L | 6 | 1.4 |
| Reticulocyte count (n = 101)            | <0.5% | 12 | 11.88 |
|                                         | 0.5–2.5% | 75 | 74.25 |
|                                         | >2.5% | 14 | 13.86 |
| Immature reticulocyte fraction (IRF) (n = 101) | < 0.3 | 16 | 15.84 |
|                                         | 0.3–0.53 | 74 | 73.30 |
|                                         | > 0. 53 | 11 | 10.89 |
| Direct Coombs test (DAT) (n = 101)      | Positive | 19 | 18.81 |
|                                         | Negative | 82 | 81.19 |
| Viral load                              | \leq 1000 | 336 | 93.85 |
|                                         | >1000 | 22 | 6.15 |
| Cluster of differentiation 4 (CD4)       | <200 | 75 | 20.95 |
|                                         | \geq 200 | 283 | 79.05 |

**NB:** Reticulocyte, immature reticulocyte fraction, DAT were tested for anemic individuals

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4.22% (n = 3), However, there was no IHA found in the age group greater than 50. Among individuals who had IHA, 6 (60%) of them had moderate, and the rest of them had mild anemia. However, there was no severe anemia case in a patient who had IHA in this study.

Factors associated with immune hemolytic anemia
To determine the association between the IHA and independent variable bi-variable and multi-variable firth penalized logistic regression model was used. Based on the analysis variables with p value less than 0.2 in bivariable firth penalized logistic regression model included in multivariable analysis. Accordingly, vegetarianism (do not using meat in their diet), high viral load and family history of anemia showed significant association with IHA (Table 4).

Discussion
Human immuno deficiency virus infection triggers anemia, which is most likely caused by HIV infection of stromal cells and hematopoietic stem cells. In HIV infection, the commonly affected hematological parameters are leukocyte, erythrocyte, and platelets due to viral effect on all lineages of blood cells and immune system [1]. Of all hematological abnormalities anemia is common among HIV patients. The pathophysiology of anemia includes decreased production, increased destruction, and increased loss due to hemorrhage [3]. IHA is a type of anemia caused by immune mediated destruction of RBC by antibodies against erythrocyte antigens. It is characterized by normocytic normochromic or macrocytic anemia with hemolysis evidence in -blood film, reticulocytosis or high immature reticulocyte fraction and positive for DAT [29–31].

The overall prevalence of IHA among HIV-positive adults was 2.80% (95% CI 1.08%, 4.50%), The prevalence was in agreement with studies conducted in Addis Ababa (2.34%) [17] and Benin (Nigeria) (3.06%) [22]. However, it is higher than that of study done in Lagos (Nigeria) (0%) (38). The difference might be resulted from the variation in the defining IHA. The study in Lagos (Nigeria) was the defined IHA by using a reticulocyte count, hemoglobin level and coombs test only, without using immature reticulocyte count. But in this study, immature reticulocyte fraction was used for diagnosis of IHA. Reticulocytopenia is common in HIV patients, this causes misdiagnosis of IHA in HIV patients. The immature reticulocyte fraction is the best parameter for diagnosis of IHA in HIV patients, because it increases in case of IHA regardless of HIV status [33, 34].

According to this study, among individuals who had IHA, 60% of them had moderate and 40% of them had mild anemia. This finding did not agree with the study in Addis Abeba [17]. The study indicated 22.2% of them had severe and 33.3% of them had moderate anemia. This variation might be due to the advancement of ART medication from AZT-based regimen especially navirapine to new advanced regimens with reduced adverse effect such as dolutegravir based regimen. Even though the mechanism is not fully elucidated, IHA occurs as part of drug rash with eosinophilia and systemic symptoms syndrome in the presence of drugs (navirapine). The drug dependent antibody mediated hemolysis appears within two weeks after initiation of the drug, whereas the patient presents with rapidly progressing IHA. But dolutegravir did not cause anemia and dolutegravir-containing regimen demonstrated a high virologic efficacy. This might protects patients from developing severe anemia [35, 36].

The determinant factors which shown significant association with IHA were family history of anemia (AOR 8.30 at 95% CI 1.56, 44.12), vegetarian life style (not consuming meat) (AOR 7.39, CI, 95% 1.13, 42.6), and high viral load (AOR of 6.94 at 95CI % (1.13, 42.6).
Table 4. Bivariable and multivariable firth penalized logistic regression model analysis of factors associated with IHA among HIV infected adults from March to April 2021 (N = 358).

| Variables                          | Category       | IHA       | COR at CI (95%) | AOR at CI (95%) | p-value  |
|------------------------------------|----------------|-----------|----------------|----------------|----------|
|                                    |                | Yes       | No             |                |          |
| Age                                | 15–30          | 3 (4.22)  | 68 (95.78)     | 1              |          |
|                                    | 31–50          | 7 (2.86)  | 238 (97.14)    | 0.61 (0.16, 2.25) |          |
|                                    | 51–65          | 0 (0.00)  | 42 (100)       |                |          |
| Residence                          | Urban          | 5 (1.75)  | 280 (98.25)    | 1              |          |
|                                    | Rural          | 5 (6.85)  | 68 (93.15)     | 4.11 (1.27,14.4) | 2.58 (0.59,11.20) | 0.84 |
| Gender                             | Male           | 5 (3.52)  | 137 (96.48)    | 1              |          |
|                                    | Female         | 5 (2.32)  | 211 (97.68)    | 0.65 (0.19, 2.16) |          |
| Education                          | No formal education | 3 (3.30)   | 90 (96.70)    | 0.95 (0.23,3.98) |          |
|                                    | Primary School | 3 (3.22)  | 87 (96.78)     | 0.99 (0.23,4.11) |          |
|                                    | Secondary school | 4 (3.48)  | 111 (96.52)   |                |          |
|                                    | University/college | 0 (0.00)  | 60 (100.00)  |                |          |
| History of opportunistic infection | Yes            | 4 (3.54)  | 113 (96.46)   | 1.43 (0.42, 4.87) |          |
|                                    | No             | 6 (2.49)  | 235 (97.51)    | 1              |          |
| Medication                         | Ant-TB         | 4 (3.74)  | 103 (96.26)    | 1.56 (0.44, 5.0) |          |
|                                    | Other medication | 1(2.17)  | 45 (97.87) | 1.22 (0.19, 7.69) |          |
|                                    | Never          | 5 (2.38)  | 205 (97.62)    | 1              |          |
| History of autoimmune disease      | Yes            | 3(4.54)   | 63 (95.45)     | 0.47(0.13,1.74) |          |
|                                    | No             | 7(2.4)    | 285(97.60)     | 1              |          |
| Meat consumption                   | At least per week | 2 (2.6) | 73 (97.4) | 1          |          |
|                                    | Less often / weak | 5 (1.90) | 257 (98.1) | 0.62(13,2.86) | 0.98(0.19, 5.0) |          |
|                                    | Never          | 3(14.28)  | 185 (95.71)    | 5.56(1.1,30.23) | 8.18(1.60,41.74) | 0.01 |
| Daily use green leafy vegetables   | Less often per day | 8 (2.86) | 272 (97.14) | 1.04(25, 4.4) |          |
|                                    | At least per day | 2 (2.56) | 76 (97.44) | 1          |          |
| Coffee drinking                    | Less often per day | 8 (2.98) | 161 (97.02) | 3.95(0.95,4.87) | 2.29(0.51, 10.19) | 0.38 |
|                                    | At least per day | 2 (1.06) | 187 (98.94)    | 1              |          |
| Tea drinking                       | Less often per day | 4 (1.80) | 218 (98.20) | 0.40(0.12,1.39) | 0.51(0.12, 2.17) | 0.22 |
|                                    | At least per day | 6 (4.48) | 128 (95.52)    | 1              |          |
| CD4                                | <200           | 4 (5.30)  | 71 (94.70)     | 2.62(0.77,8.93) | 0.89(0.15, 5.01) | 0.93 |
|                                    | ≥ 200          | 6 (2.12)  | 277 (97.88)    | 1              |          |
| Stage of disease                   | Stage 1        | 6 (2.16)  | 272 (97.84)    | 1              |          |
|                                    | Stage 2        | 3 (5.00)  | 57 (95.00)     | 2.53(0.74,8.38) | 1.48 (0.37, 5.93) | 0.2 |
|                                    | Stage III and IV | 1 (5.00) | 19 (95.00) | 3.22(0.51, 20.18) | 1.73 (0.08, 39.31) | 0.16 |
| Viral load                         | >1000          | 5 (6.60)  | 71 (93.40)     | 19 (5.25, 67.4) | 6.94(1.13, 42.6) | 0.04 |
|                                    | ≤ 1000         | 5 (1.77)  | 277 (98.23)    | 1              |          |
| HAART regimen                      | AZT-containing | 4 (4.94)  | 77 (95.06)     | 2.40(0.70,8.17) | 1.44 (0.37, 5.61) | 0.61 |
|                                    | Non-AZT        | 6 (2.17)  | 271 (97.83)    | 1              |          |
| Family history of anemia           | Yes            | 3 (9.09)  | 30 (91.01)     | 5.12(1.29, 20) | 8.3(1.56,44.12) | 0.01 |
|                                    | No             | 5(2)      | 245(98)        | 1              |          |
|                                    | I do not know  | 2 (2.6)   | 73 (97.4)      | 1.51(33, 6.92) | 1.790(375, 8.55) | 0.45 |

At P value <0.05
AZT: Ziduvidine, CI: Confidence Interval, HAART: Highly active antiretroviral therapy AOR: Adjusted odd ratio, COR: Crude odd ratio, IHA: Immune Hemolytic Anemia

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In this study, individuals whose families had a history of anemia were 8.30 times more likely to develop IHA than their counterparts (AOR 8.30, 95% CI 1.56, 44.12). This might be due to the presence of study participants with family history of IHA. IHA might be caused by a fundamental defect in the immune system that inhibits the immune system from establishing a proper homeostatic mechanism. This disorder appears to be passed down in families which block erythrocyte immune homeostasis. The patients with hereditary spherocytosis, who had naturally occurring autoantibodies directed against different membrane proteins. This antibody makes the reaction with the surface antigen of erythrocyte and results in immune mediated hemolytic anemia [37, 38].

According to finding of this study, vegetarians or peoples who did not eat meat were 7.39 times (AOR 7.39, CI, 95% 1.13, 42.6) at risk of developing IHA than individuals who eat meat in their diet. This study agrees study done in Shalla, [Ethiopia] [39], Vietnam [40], and Pakistan [41], which reported that anemia was higher among individuals who did not use meat and animal products. Lack of meat in diet results in vitamin B12 deficiency which causes impaired immune system activity such as decreased lymphocyte especially CD8, natural killer cells, lymphokine activated killer cells and an increase in the CD4/CD8 ratio [42, 43]. The impaired levels of the immune activity is associated with higher risk of HIV disease progression and increased viral replication. The increased virus causes viral protein induced immune activation which might result in IHA in HIV patients [44].

In this study, individuals whose viral load greater than 1000 copies/μl were 6.94 times more likely to develop IHA than individuals whose viral load less than 1000 copies/μl (AOR of 6.94 at 95CI % (1.13, 42.6)). The higher viral load is indicative of poor suppression of viral quantity. This might be occurred due to the positive correlation between plasma HIV ribonucleic acid levels and both CD4+ T-cell activation and CD8+ T-cell activation levels [45]. Virus induces IHA by HIV binding with erythrocytes, then causes immune activation, dysregulation of T and B cells, immune intolerance and expression of auto antigens similar to virus [9, 10]. The structural antigen similarity between HIV proteins and RBC antigens can induce autoantibody production. Moreover, the presence of viral negative factor protein induces autoimmune responses by cross-reaction of specific viral antigens with self-proteins through the stimulation of auto-reactive T cells [45]. The circulating autoantibodies to RBC and host red cell with increased immunogenicity finally these results antibody-mediated hemolysis or IHA [11, 45, 46].

**Strength and limitation**

**Strength of the study**

In this study hematological analysis, such as reticulocyte count, mean reticulocyte volume and immature reticulocyte fraction were performed by automation. This study also attempted to describe associated factors of IHA in addition to prevalence.

**Limitation**

The first limitation of this study was a cross-sectional nature of its design, it did not allow us to observe causality in the relationship between IHA and its associated factors, as it is temporal association. The other limitation was, this study was not included DAT negative IHA which requires latest technology such as gel technology and molecular methods. The serum lactate dehydrogenase, haptoglobin, and unconjugated bilirubin were not tested for additional evidence of hemolysis.
Conclusion and recommendation

Conclusion

According to the findings of this cross-sectional study IHA in HIV patient is rare public health problem. This finding revealed that IHA was significantly associated with vegetarianism, family history of anemia and high plasma viral load.

Recommendations

The ART clinicians were recommended to focus on viral load to monitor disease progress and give attention for IHA. IHA screening test has to be done specifically before blood cell transfusion in HIV patients. We recommend HIV patients to use meat in their diet to protect themselves from vitamin B12 deficiency induced IHA. Individuals who had family history of anemia should be screened for IHA. Additionally, we recommend that additional study to be done by using sensitive and specific advanced technology products like flow cytometry and advanced molecular tests which also help to quantify amount of RBC bounded antibodies to know the probability for hemolysis. It is better for researcher in hematology area, give attention to set the reference interval of immature reticulocyte fraction and mean reticulocyte volume. Even though it is less frequent IHA diagnosis needs prior identification to minimize severity and burden of disease because it may result in fatal condition. We suggest policy makers to develop guideline for HIV patient by considering IHA and work to make screening tests for IHA to be available in every ART clinic across the country.

Declarations

Ethical approval and consent to participate

Ethical considerations. The study was carried out after receiving ethical approval from the University of Gondar college of medicine and health sciences (CMHS), school of biomedical and laboratory science research, and ethical review committee (Reference number SBLS/2750). All activity in this research work was based on Helsinki declaration. Furthermore, support and permission letter were secured from UOGSCH. In addition, following an explanation of the purpose, the benefits and the possible risks of the study, written informed consent was taken from a parent/legal guardian and assent was sought from children before commencement of the study. It was made clear that participation in the study were purely on a voluntarily basis and refusal was possible. To ensure confidentiality of data, study participants were coded by using unique codes, and only authorized persons were accessing the collected data. The study participant’s with abnormal findings were linked to the physicians who are working at the ART clinic for proper patient care.

Availability of data and materials

All relevant data are available within the manuscript. In case of need, the data that support the findings of this study are available from the corresponding author on reasonable request.

Supporting information

S1 File.

(DOCX)
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References

1. Kirchhoff F, Silvestri G: Is Nef the elusive cause of HIV-associated hematopoietic dysfunction? The Journal of clinical investigation 2008, 118(5):1622–1625. https://doi.org/10.1172/JCI35487 PMID: 18431512

2. Geneva S, Organization WH: Haemoglobin Concentrations for the Diagnosis of Anaemia and Assessment of Severity. Vitamin and Mineral Nutrition Information System. Document Reference WHO. In.: NMH/NHD/MMN/11.1. http://www.who.int/entity/vmnis/indicators/haemoglobin...; 2011.

3. Huibers MH, Bates I, McKew S, Allain TJ, Coupland SE, Phiri C, et al: Severe anaemia complicating HIV in Malawi: Multiple co-existing aetiologies are associated with high mortality. PLoS One 2020, 15(2):e0218695. https://doi.org/10.1371/journal.pone.0218695 PMID: 32097440

4. Parinitha S, Kulkarni M: Haematological changes in HIV infection with correlation to CD4 cell count. The Australasian medical journal 2012, 5(3):157. https://doi.org/10.4066/AMJ.20121008 PMID: 22952560

5. Axelsson JA, LoBuglio AF: Immune Hemolytic anemia. Med Clin North Am 1980, 64(4):597–606. https://doi.org/10.1016/s0025-7125(16)31583-8 PMID: 6995725

6. Saif MW: HIV-associated autoimmune hemolytic anemia: an update. AIDS patient care and STDs 2001, 15(4):217–224. https://doi.org/10.1089/10872910151133783 PMID: 11359864

7. Hughes-Jones NC, Wickramasinghe SN, Hatton C: Lecture notes haematology: John Wiley & Sons; 2008.

8. Barcellini W, Fattizzo B: Clinical applications of hemolytic markers in the differential diagnosis and management of hemolytic anemia. Disease Markers 2015, 2015. https://doi.org/10.1155/2015/635670 PMID: 26819490
9. Horakova E, Gasser O, Sadallah S, Inal JM, Bourgeois G, Ziekau I, et al: Complement mediates the binding of HIV to erythrocytes. The Journal of Immunology 2004, 173(6):4236–4241. https://doi.org/10.4049/jimmunol.173.6.4236 PMID: 15356175

10. Hess C, Klimenti T, Schlappbach L, Del Zenero V, Sadallah S, Horakova E, et al.: Association of a pool of HIV-1 with erythrocytes in vivo: a cohort study. The Lancet 2002, 359(9325):2230–2234. https://doi.org/10.1016/S0140-6736(02)09291-7 PMID: 12103286

11. Joseph AM, Kumar M, Mitra D: Nef: “necessary and enforcing factor” in HIV infection. Current HIV research 2005, 3(1):87–94. https://doi.org/10.2174/1570162052773013 PMID: 15638726

12. Khosla J, Yeh A, Spitzer T, Dey BR: Hematopoietic stem cell transplant-associated thrombotic microangiopathy: current paradigm and novel therapies. Bone Marrow Transplantation 2018, 53:129–137. https://doi.org/10.1038/bmt.2017.207 PMID: 28967899

13. Martinez V, Diemert M-C, Braibant M, Potard V, Charuel J-L, Barin F, et al: Anticardiolipin antibodies in HIV infection are independently associated with antibodies to the membrane proximal external region of gp41 and with cell-associated HIV DNA and immune activation. Clinical Infectious Diseases 2009, 48(1):123–132. https://doi.org/10.1086/595013 PMID: 19035778

14. Vishnuprathap D: Typing of hemolytic anemias: Role of basic diagnostic screening panel. Tirunelveli Medical College, Tirunelveli; 2016.

15. Harmening DM: Modern blood banking & transfusion practices: FA Davis; 2018.

16. Kreuzer K-A, Rockstroh J: Pathogenesis and pathophysiology of anemia in HIV infection. Annals of hematology 1997, 75(5–6):179–187. https://doi.org/10.1007/s002770050340 PMID: 9433373

17. Ibrahim J, Taye M, Defar M: Prevalence of Autoimmune Hemolytic Anemia in Human Immunodeficiency Virus–Infected Anemic Adults: A Cross-Sectional Study at Tikur Anbessa Specialized Teaching Hospital From June 5, 2015, to September 10, 2015, Addis Ababa, Ethiopia. American Journal of Clinical Pathology 2018, 150:S108.

18. Neil SJ, McKnight A, Gustafsson K, Weiss RA: HIV-1 incorporates ABO histo-blood group antigens that sensitize virions to complement-mediated inactivation. Blood 2005, 105(12):4693–4699. https://doi.org/10.1182/blood-2004-11-4267 PMID: 15728127

19. Duffy C, Kenga DB, Gebretsadik T, Maussé FE, Manjate A, Zaqueu E, et al: Multiple Concurrent Illnesses Associated with Anemia in HIV-Infected and HIV-Exposed Uninfected Children Aged 6–59 Months, Hospitalized in Mozambique. The American journal of tropical medicine and hygiene 2020, 102(3):605–612. https://doi.org/10.4269/ajtmh.19-0424 PMID: 31933456

20. Ako S, Njunda L, Akum E, Benjamin P, Assob J: Hematological Related Disorders and Transfusion of HIV Patients on Highly Active Antiretroviral Therapy (HAART) in the South West Region of Cameroon: Hematological Monitory Parameters for HIV Follow-Up. J HIV Retrovirus 2018, 4(1):5.

21. January CT, Wann LS, Alpert JS, Calkins H, Cigarroa JE, Cleveland JC, et al: 2014 AHA/ACC/HRS guideline for the management of patients with atrial fibrillation: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines and the Heart Rhythm Society. Journal of the American College of Cardiology 2014, 64(21):e1–e76.

22. Olayemi A, Awodu O, Bazuaye G: Autoimmune haemolytic anemia in HIV patients. Annals of African Medicine 2008, 7(2):72–76.

23. Gonzalez C, Guzman L, Nocetti G: Drug-dependent antibodies with immune hemolytic anemia in AIDS patients. IMMUNOHEMATOLOGY-WASHINGTON DC-2003, 19(1):10–15. PMID: 15373540

24. Dhaliwal G, Cornett PA, Tierney LM Jr: Hemolytic anemia. American family physician 2004, 69(11):2599–2606. PMID: 15202694

25. Adewumi AA, Titilope AA, Osamuedem VA, Vincent OO, Akinsegun AA, Dapus OD, et al: Prevalence of HIV-related autoimmune haemolytic anaemia in Lagos, Nigeria. Nigerian medical journal: journal of the Nigeria Medical Association 2014, 55(1):63. https://doi.org/10.4103/0300-1652.128175 PMID: 24970973

26. Hill QA, Hill A, Berentsen S: Defining autoimmune hemolytic anemia: a systematic review of the terminology used for diagnosis and treatment. Blood advances 2019, 3(12):1897–1906. https://doi.org/10.1182/bloodadvances.2019000306 PMID: 31235526

27. Munker R, Hiller E, Glass J, Paquette R: Modern hematology: biology and clinical management, vol. 864: Springer Science & Business Media; 2007.

28. Cheesbrough M: District laboratory practice in tropical countries, part 2: Cambridge university press; 2005.

29. Jaime-Pérez JC, Aguilar-Calderón P, Salazar-Cavazos L, León G-D, Gómez-Almaguer D: Treatment of autoimmune hemolytic anemia: real world data from a reference center in Mexico. Blood research 2019, 54(2):131–136. https://doi.org/10.5045/bjr.2019.54.2.131 PMID: 31309092
30. Carr J, Geesaman S, Czader M: Performance evaluation of the new UniCel DxH800 coulter cellular analysis system in a large hospital setting. Laboratory Medicine 2012, 43(5):157–163.

31. Rastogi P, Bhatia P, Varma N: rational diagnostics. Indian Pediatr 2017, 54:395–401.

32. McNair E, Qureshi AM, Bally C: Performance evaluation of the Platelet networks® in the measurement of blood cell counts as compared to the Beckman Coulter Unicel DXH 800. The journal of extra-corporeal technology 2015, 47(2):113. PMID: 26405360

33. Telen MJ, Roberts KB, Bartlett JA: HIV-associated autoimmune hemolytic anemia: report of a case and review of the literature. JAIDS Journal of Acquired Immune Deficiency Syndromes 1990, 3(10):933–937. PMID: 2204697

34. Chang C-C, Kass L: Clinical significance of immature reticulocyte fraction determined by automated reticulocyte counting. American journal of clinical pathology 1997, 108(1):69–73. PMID: 9208980

35. Reghukumar A, Gurudas A, Kumar VK, Ravi R: Auto immune haemolytic anaemia as part of Nevirapine induced DRESS syndrome. BMC infectious diseases 2014, 14(3):1

36. L, Erb S, Furrer H, Cavassini M, Calmy A, Vernazza P, Günthard H, et al: Adverse events of raltegravir and dolutegravir. AIDS (London, England) 2017, 31(13):1853.

37. Zaninoni A, Fermo E, Vercellati C, Marcello AP, Barcellini W, Bianchi P: Congenital hemolytic anemias: is there a role for the immune system? Frontiers in Immunology 2020, 11.

38. Pirofsky B: Hereditary aspects of autoimmune hemolytic anemia; a retrospective analysis. Vox sanginis 1968, 14(5):334–347. https://doi.org/10.1111/j.1423-0410.1968.tb01723.x PMID: 5660845

39. Obse N, Mossie A, Gobena T: Magnitude of anemia and associated risk factors among pregnant women attending antenatal care in Shalla Woreda, West Arsi Zone, Oromia Region, Ethiopia. Ethiopian journal of health sciences 2013, 23(2):165–173. PMID: 23950633

40. Pasricha S-R, Caruana SR, Phuc TQ, Casey GJ, Jolley D, Kingsland S, et al: Anemia, iron deficiency, meat consumption, and hookworm infection in women of reproductive age in northwest Vietnam. The American journal of tropical medicine and hygiene 2008, 78(3):375–381. PMID: 18337329

41. Ali SA, Abbasi Z, Shahid B, Moin G, Hambidge KM, Krebs NF, et al: Prevalence and determinants of anemia among women of reproductive age in Thatta Pakistan: Findings from a cross-sectional study. PloS one 2020, 15(9):e0239320. https://doi.org/10.1371/journal.pone.0239320 PMID: 32970719

42. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al: Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. Clinical & Experimental Immunology 1999, 116(1):28–32. https://doi.org/10.1046/j.1365-2249.1999.00870.x PMID: 10209501

43. Tong TYN, Key TJ, Gaitskell K, Green TJ, Guo W, Sanders TA, et al: Hematological parameters and prevalence of anemia in white and British Indian vegetarians and nonvegetarians in the UK Biobank. The American Journal of Clinical Nutrition 2019, 110(2):461–472. https://doi.org/10.1093/ajcn/nqz072 PMID: 31190054

44. Ullum H, Leprì AC, Aladdin H, Katzenstein T, Victor J, Phillips AN, et al: Natural immunity and HIV disease progression. AIDS 1999, 13(5):557–563. https://doi.org/10.1097/00002030-199904100-00004 PMID: 10203380

45. Stratton R, Slapak G, Mahungu T, Loes SK-d: Autoimmunity and HIV. Current Opinion in Infectious Diseases 2009, 22(1):49–56. https://doi.org/10.1097/QCO.0b013e3283210006 PMID: 19532080

46. Tsiakalos A, Routsias JG, Kordossis T, Moutsopoulos HM, Tzioufas AG, Sipsas NV: Fine Epitope Specificity of Anti-erythropoietin Antibodies Reveals Molecular Mimicry With HIV-1 p17 Protein: A Pathogenetic Mechanism for HIV-1-Related Anemia. Journal of Infectious Diseases 2011, 204(6):902–911. https://doi.org/10.1093/infdis/jir433 PMID: 21848287