IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 23, Issue 12 Ser. 2 (December. 2024), PP 65-72
www.iosrjournals.org

Haematological Changes After Six Months of Anti-Retroviral Therapy

Dr. C. M. Shamim Kabir[1], Prof. Dr. Md. Abul Kalam Azad[2], Dr. Afroza Alam[3], Dr. Khaled Mahbub Murshed[4], Dr. Md. Nafis Areefin[5], Lt. Col. Mst. Tohmina Aktar[6]

[1]*Resident Physician, Department of Medicine, Kuwait-Bangladesh Friendship Government Hospital, Uttara, Dhaka, Bangladesh.*
[2]*Professor and Chairman, Department of Internal Medicine, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh*
[3]*Associate Professor, Department of Palliative Medicine, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh*
[4]*Assistant Professor, Department of Medicine, Bangabandhu Sheikh Mujib Medical University, Shahbag, Dhaka, Bangladesh*
[5]*Registrar, Department of Cardiology, Shaheed Ziaur Rahman Medical College Hospital, Bogura, Bangladesh*
[6]*OIC, OPD Wing, Combined Military Hospital, Dhaka Cantonment, Dhaka, Bangladesh*
Corresponding Author: *Dr. C. M. Shamim Kabir, Resident Physician, Department of Medicine, Kuwait-Bangladesh Friendship Government Hospital, Uttara, Dhaka, Bangladesh.*

Abstract:
Background: *This present study was undertaken to find out the haematological abnormalities of HIV positive individuals and changes taken place on anti-retroviral therapy (ART). The haematological changes associated with six months of antiretroviral therapy (ART) are key indicators of both the effectiveness and potential side effects of the treatment. ART can influence various components of the blood, often improving some blood parameters as HIV viral load decreases and immune function recovers, while other aspects might worsen due to drug toxicity or interactions.* ***Objective****: The aim of the study was to evaluate the haematological changes after six months of anti-retroviral therapy.* ***Methods****: This cross-sectional analytical study included a total of 154 HIV positive patients attended at the ART centre of Bangabandhu Sheikh Mujib Medical University (BSMMU) Hospital. HIV patients who had complete haematological parameters before commencement of ART and had completed at least 6 months of ART were investigated again within 7-12 months. The haematological parameters derived at the end-point of the study were compared with those of their pre-ART figures and the changes observed were noted down. Statistical analyses of the results were be obtained by using window-based Microsoft Excel and Statistical Packages for Social Sciences (SPSS-24).* ***Results:*** *The study revealed that HIV infected patients were predominantly middle-aged and young comprising > 70% of the patients with mean age of the patients being 35.5±9.5 years (range: 20-60 years). A male preponderance was observed in the study with male-to-female ratio being 3:1. The study subjects were mostly (40%) secondary level educated and majority (80%) belonged to middle class. Nearly half (43%) had history of staying or travelling abroad. History of blood transfusion was found in 6.5% patients. Majority was heterosexual (84.4%) and accustomed to practicing vaginal sex (83.2%). A few were used to having anal sex (11%) or both vaginal and anal sex (4.5%). Majority (92.2%) of patients received first-line ART. The most frequent abnormality observed in the present study was anaemia (53.8%) which reduced (29.2%) significantly after a mean treatment period of 9.5±1.9 months. The red cell indices like Hct, MCV, MCH and MCHC were also low at initiation of therapy, but changed to normality after treatment.* ***Conclusion:*** *HIV infected individuals are predominantly male, middle aged and young. The most common haematological abnormality is anaemia which significantly reduced in percentage after treatment with ART. The duration and types of ART does not have significant impact on differences in changes of haematological parameters.*

Keywords: *Haematological abnormalities, HIV, Anti-retroviral therapy (ART).*

Date of Submission: 05.12.2024 Date of Acceptance: 25.12.2024

I. Introduction

Acquired Immunodeficiency Syndrome (AIDS) was first recognized in 1981 and Human Immunodeficiency Virus (HIV) was identified in 1983. Since then, is continues to be a public health problem.

The phenomenon of HIV/AIDS is best viewed as a pandemic affecting almost all countries of the world. [1] The first case of HIV/AIDS in Bangladesh was documented in 1989. [2] Global AIDS Monitoring and UNAIDS 2019 estimated that during 2018, 1600 (all ages) new HIV infection was found in Bangladesh, among them <100 (0-14 years), <500 (women of 15+ years) and 1000 (men of 15+ years). Total number of people living with HIV (PLHIV) in Bangladesh is 14000 (all ages), out of them <500 (0-14 years), 4800 (women of 15+ years) and 8700 (men of 15+ years). HIV incidence per 1000 population is 0.01 and HIV prevalence (15-49 years) is <0.1. There was total 580 (all ages) AIDS related death in 2018. [3]

HIV can spread through the exchange of a range of body fluids, including blood, breast milk, sperm and vaginal secretions, from infected people. During pregnancy and delivery, HIV can be passed from a mother to her child. [4] HIV is an enveloped virus that predominantly affects the immune system by targeting T-lymphocytes. It replicates by exploiting the deoxyribonucleic acid of CD4+ T cells, decreasing their numbers and putting the patient at risk of opportunistic infections over months to years, finally leading to death. HIV is classified into HIV-1 and HIV-2 with HIV-1 being the predominant cause for AIDS worldwide. When HIV enters the body, it spreads quickly to cells and tissues, insidiously destroying the lymphnode's architecture and prompting the immune system to soar a defense against it via CD4+ and CD8+ T cells, which are then killed by the virus, allowing free HIV replication and eventually full-blown AIDS. [5]

The World Health Organization (WHO) has classified AIDS into four stages based on symptoms, clinical signs, and opportunistic infections, starting with Stage 1, which is asymptomatic, then Stage II, which is mildly symptomatic, Stage III, which is moderately symptomatic, and Stage IV, which is HIV wasting syndrome. [5] Based on the stage of infection, HIV may have different symptoms. Despite the fact that persons living with HIV are most infectious in the first few months following infection, many do not feel they are infected until later. People may have no symptoms or an influenza-like sickness, such as fever, headache, rash, or sore throat, in the first few weeks following infection. They may develop other signs and symptoms when the virus impairs their immune system, including as swollen lymph nodes, weight loss, fever, diarrhoea, and cough. They could acquire serious illnesses like tuberculosis (TB), cryptococcal meningitis, severe bacterial infections, and malignancies like lymphomas and Kaposi's sarcoma if they don't get treatment. [4]

Haematological abnormalities commonly found in HIV-infected individuals are anaemia, granulocyte disorders, thrombocytopenia, lymphomas, coagulopathies and vascular malignancies. Although these abnormalities are detected in the majority of cases in the middle or advanced stages of HIV infection, but anaemia and thrombocytopenia may occur in early stages of HIV infection. The origin of haematological disorders in HIV infection remains incompletely understood, but has been attributed to several factors causing dysfunctional haematopoiesis in bone marrow. These include severe nutritional stress in advanced stages of HIV infection, marrow suppression by invading opportunistic infections or neoplasm, changes associated with chronic disease and toxic effects of anti-retroviral compounds. The possibility of HIV infecting the haematopoietic precursor cells directly and inhibiting their differentiation and development into mature cells was an attractive hypothesis for the origin of HIV-related dysfunctional haematopoiesis, but remains, to a large extent, an incompletely understood phenomenon. [6]

Anaemia in AIDS depends primarily on CD4 count and increased viral load resulting in reduced RBC (Red blood cell) production or increased RBC destruction or defective RBC. [5] The risk of bacterial infections in HIV- infected cases with absolute neutrophil count below 1 x 10^9/L and 0.5 x 10^9/L increases to > 2-fold and 8-fold respectively. [7] Similarly, the threat of opportunistic infections, such as pneumocystis carinii, cytomegalovirus, mycobacterium avium and candida thrush is high when CD4+ T cell count < 200 cells/microliter. In HIV cases, thrombocytopenia is common (40%) and accounts for first sign in 10% of the cases. High risk of developing HIV-related idiopathic thrombocytopenic purpura and thrombotic thrombocytopenic purpura (TTP) is associated with thrombocytopenia. [5] To prevent disease progression, a particular determination of haematological parameters is needed to initiate and monitor early therapy.

II. Methodology

This was a cross-sectional analytical study. The study was conducted under Department of Internal Medicine Bangabandhu Sheikh Mujib Medical University, Dhaka, Bangladesh among one hundred and fifty-four HIV positive patients attending at ART center from 01 April 2019 to 31 October 2021. Patients were included as per inclusion and exclusion criteria. Co-morbid conditions were excluded mostly by self-reporting and clinically relevant investigations. Study purpose was explained to the study subjects and informed written consent was taken. All demographic characters like age, sex, address, education level, occupation, marital and socio-economic status were documented in a structured form after patient's registration. History related to risk factors and sexual pattern asked face-to-face to the patients and documented in the data sheet. Duration and types of ART and complete

blood count reports were collected from individual patients file and documented in the data sheet. Duration of ART was calculated manually in days and later on converted into months. This study had minimum chance of physical risk during 5 (five) ml of venous blood sample collection for complete blood count and peripheral blood film. Blood samples were collected at ART centre using universal precautions in EDTA tube and sent to Haematology Department, Bangabandhu Sheikh Mujib Medical University. Estimations of complete blood count were carried out by SYSMEX 6-Part (Model XN 2000) and Pentra ABX-120DX Automated Haematology Analyzer and checked manually. Measurement of erythrocyte sedimentation rate (ESR) was carried out by Westergreen method. Reports were collected on day-to-day basis and entered in Microsoft Office 2010 Excel worksheet. After taking consent and matching eligibility criteria, data were collected from patients on variables of interest using the predesigned structured questionnaire by interview, observation. Statistical analyses of the results were be obtained by using window-based Microsoft Excel and Statistical Packages for Social Sciences (SPSS-24).

III. Result

Table-1: Age distribution of the study population (n=154)

Age (years)	N=154	%
≤30	53	34.4
31-40	57	37.0
>40	44	28.6
Gender Distribution		
Male	117	76.0
Female	37	24.0

Table-1 shows age distribution of the study population, it was observed that (34.4%) patients were belonged to age ≤30years, (37.0%) patients were in age group of 31-40 years and (28.6%) patients were belonged to age >40years. Table shows gender distribution of the study population, it was observed that majority 117 (76.0%) patients were male and 37 (24.0%) were female.

Table -2: Distribution of patients by their risk factors and sexual characteristics (n= 154)

Risk factors	N=154	%
History of staying or travelling abroad	66	42.9
Family history of HIV	24	15.6
History of blood transfusion	10	6.5
History of surgery	06	3.9
Sexual behavior		
Sexuality		
Heterosexual	130	84.4
Homosexual	18	11.7
Bisexual	06	3.9
Pattern of sex		
Vaginal	128	83.2
Anal	17	11.0
Vaginal + Anal	07	4.5
Vaginal + Anal + Oral	02	1.3

Some histories related to risk factor of HIV infection are illustrated in Table 2. History of staying or travelling abroad found in 42.9% patients, 15.6% had family history of HIV, 6.5% had history of blood transfusion and 3.99% had previous history of surgery. Probing into the sexual behavior of the patients revealed that majority was heterosexual (84.4%), accustomed to practicing vaginal sex (83.2%). However, a few were used to having anal sex (11.0%) or both vaginal and anal sex (4.5%).

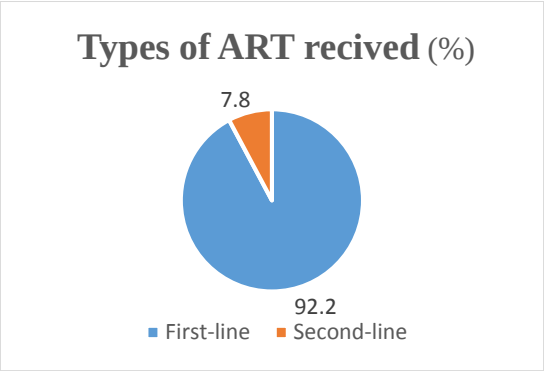

Figure-1: Distribution of the study patients by types of ART recived during study period (n=154)

Out of 154 study patients, 142 (92.2%) received first-line ART. On the other hand, second-line ART was received by 12 (7.8%) patients.

Table-4: Overall changes is selected haematological parameters before and after 7-12 months of ART (n=154)

Haematological parameters	Groups		p-value
	Before ART	After ART	
Haemoglobin (gm/dl)	12.6±1.9	13.3±1.6	0.0005
ESR (mm in 1st hr)	37.9±16.5	17.3±11.0	<0.0001
TC of WBC (/cmm)	6759±1991	7045±1729	0.1793
N (%)	59.2±11.7	55.0 ±12.0	0.0020
L (%)	31.6±10.0	35.2±10.4	0.1389
Platelet count (10^9/L)	258±74	190±77	0.0002
Hct (%)	38.3±5.6	41.7±4.9	<0.0001
MCV (fl)	84.6±7.4	95.0±10.8	<0.0001
MCH (pg)	27.8±2.7	30.3±3.9	<0.0001
MCHC (gm/L)	31.9±1.5	32.8±1.4	<0.0001

The level of haemoglobin increased significantly from 12.6 gm/dl at baseline to 13.3 gm/dl after a mean treatment period of 9.5 ± 1.9 months (range: 7-12 months) ($p < 0.0005$), ESR decreased drastically from 37.9 ± 16.5 mm in 1st hr to 17.3 ± 11.0 mm in 1st hr at the end-point of study ($p<0.0001$). The total count of WBC did not show significant response. However, neutrophil decreased and platelet count increased significantly at the end-point of the study ($p=0.0020$ and $p=0.0002$ respectively). All the red-cell indices like Hct, MCV, MCH and MCHC responded well from them before ART figures to the end-point of the study ($p<0.0001$)

Table-5: Changes in selected haematological parameters before and after ART for 7-8 months (n=50)

Haematological parameters	Groups		p-value
	Before ART	After ART	
Haemoglobin (gm/dl)	12.3±2.1	13.2±1.5	0.0154
ESR (mm in 1st hr)	33.5±17.7	14.3±6.5	<0.0001
TC of WBC (/cmm)	6934±2125	7530±2225	0.1739
N (%)	58.5±12.4	55.5±12.6	0.2330
L (%)	31.2±11.5	34.5±10.6	0.1389
Platelet count (10^9/L)	265±83	287±67	0.1479
Hct (%)	37.3±6.3	41.3±4.6	0.0005
MCV (fl)	84.3±8.1	93.5±10.0	<0.0001
MCH (pg)	27.7±2.7	29.8±3.7	0.0016
MCHC (gm/L)	31.8±1.3	32.9±1.4	0.0001

The table 5 shows the changes in haematological parameters before and after 7-8 months of ART. The level of haemoglobin improved significantly from 12.3 gm/dl to 13.2 gm/dl ($p=0.0154$), while ESR decreased abruptly from 33.5±17.7 mm in 1st he to 14.3±9.1 mm in 1st hr at the end-point of study ($p <0.0001$). Neutrophil decreased and lymphocyte increased to some extent, although the difference was statistically not significant ($p=0.2330$ and p 0.1389 respectively). The haematocrit (Hct), MCV, MCH and MCHC changed significantly from them before ART figures to the end-point of the study ($p = 0.0005$, $p<0.0001$, $p=0.0016$ and $p=0.0001$ respectively).

Table-6: Changes in selected haematological parameters before and after ART or 9-10 months (n=50)

Haematological parameters	Groups		p-value
	Before ART	After ART	
Haemoglobin (gm/dl)	12.7±1.9	13.4±1.7	0.0491
ESR (mm in 1st hr)	40.0±13.9	19.6±17.2	<0.0001
TC of WBC (/cmm)	6540±2158	7190±1606	0.0907
N (%)	60.6±11.6	55.4 ±11.7	0.0279
L (%)	30.6±10.2	34.7±10.5	0.0505
Platelet count (10^9/L)	262±69	278±81	0.2903
Hct (%)	38.3±5.4	41.6±4.8	0.0017
MCV (fl)	84.9±8.3	96.5±9.3	<0.0001
MCH (pg)	28.0±3.2	30.9±3.5	<0.0001
MCHC (gm/L)	31.9±1.6	33.1±1.3	0.0001

The table 6 shows the changes in haematological parameters before and after 9-10 months of ART. The level of haemoglobin responded well (p = 0.0491) and ESR decreased appreciably from 40.0 ± 13.9 mm in 1 hr to 19.6 ± 17.2 mm in 1st hr at the end-point of study (p < 0.0001). The total count of WBC did not show significant response. However, neutrophil decreased insignificantly at the end-point of the study (p=0.0279). All the red-cell indices like Hct, MCV, MCH and MCHC improved significantly from them before ART figures to the end-point of the study (p=0.0017, p<0.0001, p<0.0001 and p = 0.0001 respectively).

Table-7: Changes in selected haematological parameters before and after ART for 11-12 months (n=54)

Haematological parameters	Groups		p-value
	Before ART	After ART	
Haemoglobin (gm/dl)	12.8±1.7	13.5±1.6	0.0297
ESR (mm in 1st hr)	40.0±17.1	18.0±4.7	<0.0001
TC of WBC (/cmm)	6798±1699	6462±1040	0.2179
N (%)	58.4±11.0	54.7±11.5	0.0905
L (%)	32.6±9.6	36.5±10.2	0.0432
Platelet count (10^9/L)	247±68	302±81	0.0002
Hct (%)	39.3±5.0	42.3±5.2	0.0028
MCV (fl)	84.6±5.6	95.0±12.7	<0.0001
MCH (pg)	27.8±2.2	30.2±4.4	0.0005
MCHC (gm/L)	31.8±1.6	32.6±1.5	0.0085

The table 7 depicts the changes in haematological parameters before and after 11- 12 months of treatment with ART. The level of haemoglobin increased significantly from 12.8 gm/dl to 13.5 gm/dl (p=0.0297). The ESR decreased well from 40.0±17.1 mm in 1st hr to 18.0 ± 4.7 mm in 1st hr at the end-point of study (p = <0.0001). Lymphocyte increased significantly during the same period of time (p=0.0432) Platelet count also increased significantly (p=0.0002). All the red-cell indices like Het, MCV, MCH and MCHC improved significantly from them before ART figures to the end-point of the study (p=0.0028, p < 0.0001, p = 0.0005 and p = 0.0085 respectively).

Table-8: Differences in changes in selected haematological parameters among three groups of patients with different duration of treatment

Haematological parameters	Duration of treatment (months)			p-value
	7-8 (n=50)	9-10 (n=50)	11-12 (n=54)	
Haemoglobin (gm/dl)	0.90±0.36	0.70±0.35	0.70±0.31	0.8921
ESR (mm in 1st hr)	-19.20±2.66	-20.40±3.12	-22.00±2.41	0.7648
TC of WBC (/cmm)	596.0±435.11	650±380.42	-336±271.00	0.8049
N (%)	-3.00±2.50	-5.20±2.33	-3.70±2.16	0.7966
L (%)	3.30±2.12	4.10±2.07	3.90±1.90	0.9596
Platelet count (10^9/L)	22.00±15.08	16.00±15.04	55.00±14.39	0.1319
Hct (%)	4.00±1.10	3.30±1.02	3.00±0.98	0.7812
MCV (fl)	9.20±1.82	11.60±1.76	10.40±1.88	0.6571
MCH (pg)	2.10±0.64	2.90±0.67	2.40±0.66	0.6924
MCHC (gm/L)	1.10±0.27	1.20±0.29	0.80±0.29	0.5788

Comparison of changes in haematological parameters from pre-ART to end-point of the study among three groups of patients with different durations of treatment are shown in table 8. The differences in changes in all the parameters were insignificant (p>0.05).

Table-9: Differences in changes in selected harmatological parameters between first-line and second-line ART (n=154)

Haematological parameters	Type of ART		p-value
	First-Line ART (n=142)	Second-Line ART (n=12)	
Haemoglobin (gm/dl)	0.70±0.19	0.30±0.09	0.5430
ESR (mm in 1st hr)	-20.50±1.62	-22.40±7.47	0.7507
TC of WBC (/cmm)	281±216	230±190	0.9457
N (%)	-3.80±1.36	-5.80±5.62	0.6867
L (%)	2.20±1.18	11.20±5.40	0.9790
Platelet count (/cmm)	36.00±8.78	-19.00±3.80	0.5757
Hct (%)	3.40±0.61	3.10±2.41	0.8922
MCV (fl)	10.60±1.05	8.90±5.30	0.6648
MCH (pg)	2.50±0.37	1.10±0.40	0.2764
MCHC (gm/L)	-1.00±0.16	-2.00±0.57	0.0839

Comparison of changes in haematological parameters from pre-ART to end-point of the study between first-line and second-line ART groups of patients are shown in table 9. The differences in changes in all the parameters were insignificant (p>0.05**).**

Table-10: Distribution of study patients on the basis of peripheral blood flim findings between 7-12 months of ART (n=154)

Peripheral blood flim findings	N=154	%
Non-specific findings	85	55.3
Macrocytic anaemia	17	11.0
Microcytic hypochromic anaemia	16	10.4
Normocytic normochromic anaemia	12	7.8
Eosinophilia	10	6.5
Neutrophilic leukocytosis	5	3.2
Thrombocytosis	3	1.9
Relative lymphocytosis	2	1.3
Thrombocytopenia	2	1.3
Leukopenia	2	1.3

Out of 154 study patients, more than half (55.3%) of the study participants peripheral blood film (PBF) showed non-specific findings. Macrocytic anaemia, microcytic hypochromic anaemia and normocytic normochromic anaemia was found in 11.0%, 10.4% and 7.8% respectively. Eosinophilia was found in 6.5% patients. Thrombocytosis and thrombocytopenia were found in 1.9% and 1.3% respectively.

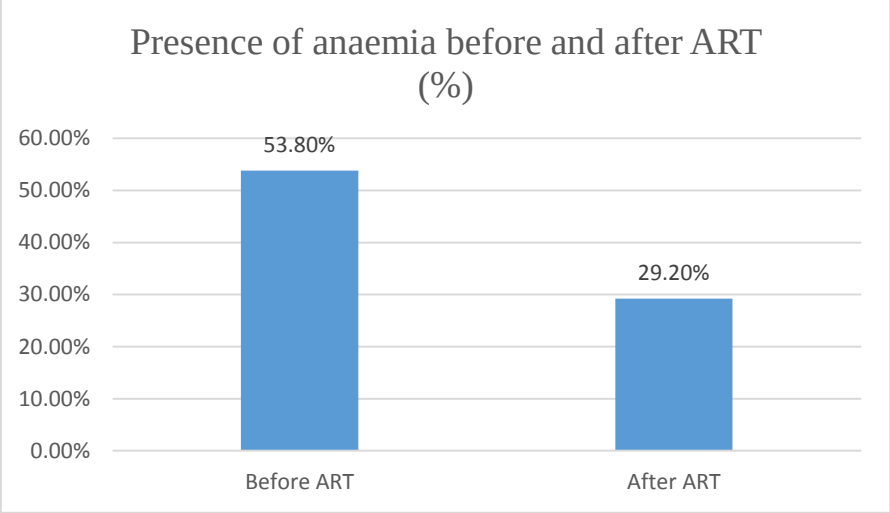

Figure 2: Showing presence of anaemia before and after ART

Figure 2 shows that 53.8% of the patients had anaemia before treatment with ART, which decreased drastically to 29.2% after 7-12 months of treatment (p=0.0005, p value was calculated from Fisher's exact test).

IV. Discussion

The study revealed that HIV infected patients were predominantly middle-aged (31- 40 years) and young (≤30 years old) comprising >70% of the patients with mean age of the patients being 35.5±9.5 years (range: 20-60 years). Mean age was 34.5±9.6 years among 250 HIV positive patients in a study by Parinitha and Kulkarni in India. [1] Mean age was 35.3±9.5 years in another study in Bangladesh by Rahman. [6] A male preponderance was evidenced in the study with male-to-female ratio being roughly 3:1. In India, a study by Kathuria reported the male-to- female ratio 1.5:1. [8] Another study in Bangladesh by Rahman reported male-to-female ratio roughly 2:1. [6] Nearly half (43%) had history of staying abroad or history of travelling to foreign countries, 15.6% had family history of HIV and 6.5% had past history of blood transfusion Majority was heterosexual (84.4%), which correlated well with the findings by Dhal in India. [5]

Majority (92.2%) of patients received first-line ART. However, 12 patients were switched to second-line ART due to virological, immunological or clinical failure. In the present study, there was significant improvement in haematological parameters is noted after 7- 12 months of treatment with ART. There was significant change in haemoglobin level in all patients who received ART for 7-8 months, 9-10 months and 11-12 months. Although the significant level of neutrophil, lymphocyte and platelet count varies in different groups and this might be due to the different factors such as the difference in the study population, sample size, study design and anti-retroviral drug formulations. While many medications used to treat HIV-related disorders are myelosuppressive, the use of zidovudine is the most common cause of severe cytopenia. [7, 9] After initiation of ART the mean value of red cell indices (Hct, MCV, MCH and MCHC) increased significantly in overall and as well as in 7-8 months, 9-10 months and 11-12 months group, which act as evidences of step up of haemoglobin level following ART. These findings are consistent with other studies. [10-12]

The most frequent abnormality observed in the present study was low haemoglobin level (anaemia) but this haemoglobin level significantly increased after 7-12 months of treatment with ART in most of the patients. Before treatment 53.8% of the patients had anaemia which decreased significantly in percentage to 29.2% after a mean treatment period of 9.5 ± 1.9 months. This is also evidenced by peripheral blood film findings. The red cell indices like Hct, MCV, MCH and MCHC were also low at initiation of therapy, but changed to normality after a mean treatment period of 9.5±1.9 months. These findings are almost consistent with the haematological abnormalities found in other similar studies conducted round the world.

A study from Nigeria reported that the prevalence of anaemia was 57.5% and 24.3% before and after highly active anti-retroviral therapy (HAART), respectively. [13] In 2021 Damtie in a study in Ethiopia showed that the prevalence of anaemia was 37.1% before initiation of HAART and 17.4% after initiation of HAART. [14] Their study also showed that leucopenia, neutropenia, lymphopenia, and thrombocytopenia was present at baseline and after initiation of HAART the prevalence of all these decreased significantly. A study from Gondar, Ethiopia reported the prevalence of anaemia as 29.7% before HAART and 11.7% after HAART. [10] Another study from

Addis Ababa, Ethiopia reported the prevalence as 41.9% and 11.4% before and after HAART initiation, respectively. [12] Other studies from Addis Ababa, Ethiopia reported the prevalence as 24.1% before HAART and 11.9% after HAART initiation. [15] In the present study more than half (55.3%) of the participants peripheral blood film showed non-specific findings, macrocytic anaemia was found predominantly (11%) followed by microcytic hypochromic anaemia (10.4%) and normocytic normochromic anaemia (7.8%). Studies by Dhal, Parinitha and Kulkarni showed occurrence of normocytic normochromic anaemia in 56% and 40.4% cases respectively, however these patients were without ART. [1, 5] Rahman reported the most common type of anaemia was microcytic hypochromic anaemia in 29.1% cases in patients on ART. [6]

In resource-poor countries like Bangladesh, haematological abnormalities in HIV patients continue to be a concern. The detection of haematological defects during anti- retroviral therapy provides the best possible care for HIV patients.

Limitations of the study

The present study was conducted in a very short period due to time constraints and funding limitations. The small sample size was also a limitation of the present study.

V. Conclusion

From the findings of the study, it can be concluded that HIV infected individuals are predominantly male, middle-aged and young. The most common haematological abnormality is anaemia which significantly reduced in percentage after a mean treatment period of nine and a half month with ART. The red cell indices like Hct. MCV, MCH and MCHC are found low at the initiation of therapy, but changed to normality with ART. The duration and types of ART does not have significant impact on differences in changes of haematological parameters.

VI. Recommendation

This study can serve as a pilot to much larger research involving multiple centers that can provide a nationwide picture, validate regression models proposed in this study for future use and emphasize points to ensure better management and adherence.

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