BENGHAZI UNIVERSITY – FACULTY OF MEDICINE
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Hematological Abnormalities in HIV Infected Children at Infectious Disease Center Benghazi – Libya

التغيرات الدموية عند الاطفال المصابين بمرض نقص المناعة المكتسبة بمركز الامراض السارية- بنغازي

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IN THE NAME OF GOD

THE MOST COMPASSIONATE, THE MOST MERCIFUL

TO MY MOTHER, BROTHER FATHI, HUSBAND,

MY DAUGHTERS AND SONS...

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A B B R E V I A T I O N

AIDS: Acquired immune deficiency syndrome.

CMV: Cytomegalo virus.

TOXO: Toxoplasmosis.

CDC: Center of disease control.

ART: Antiretro virus therapy.

HAART: Highly active anti-retroviral treatment.

JUNAIDS: The joint united nation Programme of HIV\AIDS.

PEG-IFN: Pegylated interferon.

RBV: Ribavirin.

TIP-HUS: Thrombtic thrombocytopenia purpura-hemolytic- uremic syndrome.

TPC: Thrombocytopenia.

1-S U M M A R Y

Hematological abnormalities are common manifestations of advanced HIV-infection, cytopenias of all major blood cell lines were increasingly recognized among HIV infected patients. And appears to increase with progressive immunosuppression. Hematological problems in HIV infected children have never been studied in Libya. This study was thus undertaken to assess the hematological manifestation HIV infected Libyan children

Aim: To review hematological abnormalities including (anemia, neutropenia, lymphopenia, thrombocytopenia) and to evaluate the influence of co- infection with HCV, CMV, Toxoplasmosis and stage of the disease (CDC classification) in these abnormality in HIV infected children at Infectious Disease Center in Benghazi- Libya.

Patients and Methods: One hundred HIV infected children, aged 13.5- 16 years were enrolled in a case series study. Data includes: gender, date of birth, nationality, residence and investigations:(complete blood count as well as serology for HCV, CMV, Toxoplasmosis), and stage of the disease (CDC classification) which was done at time of their diagnosis as well as during their follow up until 2- 2012.

Results and Conclusions: The 1st 100 children's charts as they organized in the register were included. Their age ranged from 13.5-16 years (with a mean age of 14.1 $\pm$ 1). 55% were male. 87% of them from Benghazi, 98% were Libyan.

Fifty three patients (53%) had cytopenia at time of diagnosis. 33 from them had anemia, 10 lymphopenia. From total cases who had hematological abnormality either at time of diagnosis or develop it during course of their disease. Fifty nine (59) of them had anemia (33) at time of diagnosis and (26) developed anemia subsequently:(33+26) , 49(4+45) leukopenia, 48:-(2+46) neutropenia, 40: (10+30) lymphopenia, 38(4+34) thrombocytopenia.

Approximately 65% of cytopenic patients were in middle and severe stage of the disease. Severity of cytopenia ranged from mild to severe. Persistence cytopenia were developing in 31% 21%, 20%, 10%, and 7% of anemic, thrombocytopenic, leukopenic, neutropenic and lymphopenic patients respectively. Anemia is more in females 33/59, other cytopenia more in males but there is no significance association between gender and cytopenia with P =0.57.

CMV were present in 95%, 89%, 88%, 85% of neutropenic, anemic,lymphopenic and thrombocytopenic patients respectively.

HCV affecting 56%, 52%, 47%, and 43% of neutropenic, anemic, thrombocytopenic and lymphopenic patients respectively.

Approximately 10% had toxoplasmosis, but there is no significance association between these co infections and stage of the disease and development of cytopenia with P > 0.05. Fife patients were died from different causes all of them had pancytopenia at time of death.

Conclusion:-Hematological abnormalities were common among Libyan HIV infected children. Anemia was the commonest hematological abnormality followed by leuckopenia and neutropenia. Hematological abnormalities increase with the progression of the disease. CMV co infection was the commonest followed by HCV.

Recommendation:-Further studies should include all the HIV children registered in the center. Further studies for each type of cytopenia individually are required; to investigate the causes, the disease burden and therapy received.

(2) **I N T R O D U C T I O N**

HIV has its roots in Africa and represents zoontic infection from Chimpanzee to human. The initial infection has occurred near 1930 in sub-Saharan; it was spread by rapid transportation.

Early cases started in 1981. In 1982 were named as Acquired immune deficiency syndrome (AIDS) by Center of Disease Control (CDC). In 1983 the virus was discovered and isolated from patients with persistent lymphadenopathy.

All the viruses discovered were named as human immune deficiency virus (HIV) by international committee on the taxonomy of viruses in 1986.
There are two types HIV I and HIV II. Various subtypes of HIV have been found in specific geographic area and in specific high risk group. Both viruses have the same mode of transmission and same opportunistic infection but type II appear to progress more slowly.

The pathogenesis of HIV is basically struggle between HIV replication and immune system response of the patient via cell mediated and immune mediated reaction.

The HIV viral burden directly or indirectly mediated by CD4+ T cell destruction, there is destruction in mature CD4+, CD4 progenitor cell in bone marrow, thymus, peripheral lymphoid tissue and CD4 in nervous system the result of this destruction is failure of T.cell production and eventually immune suppression.

The natural host immune response play role in CD4 depletion mainly through cytotoxic T cells, antibodies, natural killer autoimmune response, anergy, suprantigen mediated action of T cells and programmed cell death apoptosis.(1) HIV/AIDS is global pandemic, in 2012 more than 34 million people have HIV worldwide 3.4 million are less than 15y. There are 2.5 million new

infections among the new infections, 420,000 were among children younger than 15 years. . There are nearly 2 million deaths from aids in 2012 dawn from 3.1 million in 2001(2)

Shortly after the first description of the acquired immunodeficiency syndrome (AIDS), cytopenias of all major blood cell lines were increasingly recognized in patients with HIV infection, in one early series of patients with AIDS, anemia was noted in approximately 70 percent, lymphopenia in 70 percent, neutropenia in 50 percent, and thrombocytopenia in 40 percent.

The incidence of the various cytopenias correlates directly with the degree of immunosuppression. However, isolated abnormalities, particularly thrombocytopenia, may be encountered as the initial presentation of human immunodeficiency virus (HIV) infection. As a result, HIV infection should be considered in the assessment of patients presenting with any type of cytopenia (3).

Most HIV-1 infected individuals live in limited resource constrained countries; there is little information about the frequency of hematological abnormality such as anemia, neutropenia, and thrombocytopenia among individuals with advanced HIV-1 disease. In Libya very few studies have been conducted about HIV in general, and no single study regarding hematological abnormality.

This study was conducted in the Infectious Disease Center in Benghazi- the second city in Libya in the north east.

In this centre 429 patients are registered, 237 were less than 16 at the time of the study. Patients enrolled in this centre were infected in Benghazi Children Hospital during the period 1997-1998.

It was the biggest epidemic HIV infection in children reported in history. The exact reason behind this epidemic is still under investigation.

The aim of this study was to review the hematological abnormalities including various cytopenia of all major blood cell lines (anemia, neutropenia , lymphopenia and thrombocytopenia) and to evaluate the influence of contributing factors such as demographic characteristic , co infection with HCV, CMV and Toxoplasmosis in one hundred HIV infected children. Their correlations with the stages of the disease (according to CDC classification) were also analyzed.

(3) L I TE R AT U R E R E V I E W

3.1. HEMATOLOGICAL CHANGES IN HIV

HIV attack body's immune system it destroys all the T-cell lymphocytes and it also appears to be a major contributor to abnormalities in hematopoiesis. Retroviral infection has been clearly demonstrated in CD4+ lymphocytes, monocytes, and follicular dendritic cells, and CD34+ bone marrow progenitor cells. Antibodies to circulating red blood cells, platelets, and granulocytes may represent alterations in autoimmunity or nonspecific HIV-induced B-cell stimulation; they correlate with development of peripheral blood cytopenias (4, 5).

The CDC classification of HIV disease proposed to be used as a guide to clinical and therapeutic action in the management of HIV infected patients more than 13years. It is depended on immune state based on (CD4 count) and clinical symptoms.

- CD4categories:-
  1-CD4 more than 500cells/mm3.
  2-CD4 more than 200-499.
  3-CD4 less than 200.

- Clinical symptoms categories
  1-N asymptomatic.
  2- A mild.
  3- B advanced or moderate.
  4- C severe. (6)

Table 1: CDC classification of HIV disease stages

CD4 Category	Clinical Category			
	N	**A**	**B**	**C**
1	N1	A1	B1	C1
2	N2	A2	B2	C2
3	N3	A3	B3	C3

3.2. STUDIES DONE ON HEMATOLOGICAL ABNORMALITIES:-

- *A study done in 1987* revealed abnormal hematological findings in well-characterized cohorts of patients with AIDS, AIDS-related complex (ARC) and asymptomatic patients.

  Anemia, granulocytopenia and thrombocytopenia were found in increasing prevalence according to the severity of clinical disease associated with retroviral infection. Bone marrow aspirations and biopsies revealed frequent hypercellularity, dysplasia, plasmacytosis and lymphoid infiltrates. These marrow morphologic findings were strongly associated with anemia and granulocytopenia. Review of transfusion records of patients with HIV antibodies revealed a 21% prevalence of a positive direct coomb's test (7).
- *Another study done in 2000 in Newyork* the hematological profile of 100 symptomatic children infected with HIV (clinical AIDS) was evaluated. Anemia was found in 94%, leukopenia in 47%, thrombocytopenia in 33%, neutropenia was more severe in children with opportunistic infection (8).
- *Also a cross sectional study* done in 2006 of base line hematological parameters was under taken in 68 children in Nigeria also with confirmed HIV infection and the result was: anemia present in 77.9%, severe in 5.9% moderate in 32.3% and mild in 39.7%, leucopenia 6% neutropeina 17.5% and 2.5% thrombocytopenia. The severity of peripheral cytopenia is related to the disease burden (9).
- In the study presented in a conference on HIV pathogenesis, treatment and prevention in Rome Italy 2011, the Database and medical record of 274 HIV infected children attending clinics between September 2002 and March 2007 were reviewed.

  The children age ranged from 0-18yrs with median of 2.9 yrs and 50% were male. Analysis determined the prevalence of anemia among

80.7%, leukopenia in19%, thrombocytopenia 11.9%, which increase with disease progression (10).

- *In a study done in 2009 in south western Ethiopia* at Jima University specialized hospital, the hematological abnormalities among children on HAART was evaluated.

  This study was a cross sectional study. The number of HIV infected children was 64. The result of the study was as follow: Anemia 21.9%, thrombocytopenia 7.8%, neutropenia 4.7% severe anemia was found in 14.3%. This study also concluded that the hematological abnormalities were common problem among HIV infection especially in those on HAART treatment (11).

- *A randomized controlled study conducted in Asia, Africa and south America* in 2010. In this study the prevalence of hematological abnormalities among 1571 was studied, neutropenia had a frequency of 14%, anemia 12% and thrombocytopenia 7%.

  Frequency varied from country to country $p < 0.0001$; frequency of anemia was also related to gender and platelet count, for Neutropenia it correlates with CD4 and platelet count. Thrombocytopenia was also associated with gender and hepatitis B (12).

- *A study conducted in India* in 2011 on 50 HIV infected children. The study was a prospective cross sectional study; the children were followed up over a period of 6 months.

  These children underwent a baseline hematological analysis, clinic examination and factor such as age, sex, growth, opportunistic infection, and their associations with various hematological manifestations were studied.

  Anemia was found in 70%, 28% leukocytosis, 24% hymphopenia, 10% thrombocytopenia, 2% leucopenia with neutropenia. The study concluded that the hematological manifestations are common in HIV infected children in India and anemia is the commonest feature (13).

- An eight study on 200 newly diagnosed HIV Patients was also evaluated. In this study patients divided into 2 groups. The first group received treatment the second did not receive treatment .A third group was a control group. The results showed that increased PCV, Hb, WBC, and CD4 count in the group of patients who received anti retroviral treatment (14).

3.2.1 ANEMIA

Is the most common hematologic abnormality associated with HIV infection, affecting 60 to 80 percent of patients in late stage of the disease. The etiology of the anemia is most often multifactorial, reflecting the additive effects of infection, malignancy, malnutrition, and polypharmacy. Risk factors includes: MCV less than 80 fl, CD4 count less than 200 per micro/L, HIV-1viral load more than 50,000 per ml and use of AZT.

The presence of anemia is associated with increased morbidity and mortality in patients with HIV infection and mortality rates fall in those who recover from the anemia (15).

- *In a study* done in 1996 of patients receiving no myelosuppressive therapies, 8% of asymptomatic HIV-seropositive patients, 20% of those with symptomatic middle-stage HIV disease, and 71% of those with Centers for Disease Control (CDC)-defined AIDS were anemic (16)**.**
- *In a multicentre cohort study* in 2002 the investigation of HIV infected children revealed anemia in 18% of asymptomatic HIV-seropositive patients, in 50% of those with symptomatic middle stage HIV disease and in 75% of those with CDC-defined AIDS(17)**.**
- *Another study in Atlanta Georgia* they *review a 32,000* patients for incidence of the factors associated with and the effect on survival of anemia in HIV infected patients, they analyzed data from the longitudinal medical record reviews of HIV-infected patients who received medical care from January 1990 through August 1996 in clinics, hospitals, and private medical practices in nine United States

cities. They calculated the 1-year incidence of anemia (a hemoglobin level of<10 g/dl) ,the risk of death for patients who developed anemia compared with risk for patients who did not develop anemia; and the risk of death for those who did not recover from anemia compared with the risk for those who did recover. The 1-year incidence of anemia was 36.9% for persons AIDS and acquired opportunistic infection , 12.1% for patients with a CD4 count of less than 200 cells/micron or CD4 percentage of<14 but not clinical AIDS (immunologic AIDS),. 22% of anemia was drug related. The adjusted odds ratios showing excess risk of anemia was associated with clinical AIDS, immunologic AIDS, neutropenia, thrombocytopenia, bacterial septicemia, black race, female sex, prescription of zidovudine, and concurrent diseases, The risk of death for anemic patients with a CD4 count <200 cells/microl was 148 greater, and for persons who did not recover from anemia compared with those who did recover was 170 greater (15).

- *In another study* involving more than 1000 HIV-infected Tanzanian patients, anemia was associated with increased AIDS-related and all-cause mortality, independent of base line CD4count, clinical stage, patient age and body mass index.

  Despite the advent of HAART, HIV-related anemia is still common, and independently associated with decreased survival (18).
- *In a European study* of 6725 HIV-infected patients with a one year follow-up, the incidence of mild (hemoglobin 8 to 12) and severe (hemoglobin <8 g/dl) anemia was 58 and 1.4 percent, respectively. The death rate at one year for this cohort was 3, 16, and 41 percent for those who had no, mild, or severe anemia, respectively (19).

Hemolysis may play a role in HIV-associated anemia. A variety of mechanisms may be involved, including antibody-mediated hemolysis, drug-induced disease in patients with glucose-6- phosphate dehydrogenase (G6PD) deficiency, and microangiopathic hemolytic anemia (20).In one study, a

positive direct antiglobulin test (Coombs' test) was encountered in 18 percent of patients with AIDS, compared with less than 1 percent of non-infected blood donors. Despite this finding, clinically evident hemolysis was not noted in any of the 55 HIV-infected patients (21, 22).

Multiple therapies used in the management of HIV infection and its related conditions can suppress Bone marrow and suppress erythropoiesis.Bone marrow suppression is the most common toxicity observed in patients treated with zidovudine: AZT (23, 24). *In phase I clinical trials* evaluating the efficacy of AZT monotherapy, anemia (hemoglobin <7.5 g/dL) was observed in almost 25 percent of patients treated with 1.5 g/day of AZT; 21 percent of treated patients required transfusions. Subsequent experience has demonstrated a reduced frequency of anemia with lower daily doses of AZT. Patients with advanced immunosuppression are more prone to inhibition of normal erythropoiesis by AZT (24).

Other drugs used for the prophylaxis or therapy of opportunistic processes, including ganciclovir, valganciclovir, hydroxyurea, Amphotericin B, and TMP-SMX can play part in the development of anemia(25,26).

Further evidence of HIV-induced impairment in normal erythropoiesis was provided by a comparative analysis of purified CD34+ progenitor cells from 12 HIV-infected patients and 31 uninfected controls. Investigators identified increased levels of erythroid burst-forming units (BFU-E) in untreated HIV-infected patients compared to controls. BFU-Es decreased significantly, however, in these patients following the initiation of antiretroviral therapy. Antiretroviral therapy was also associated with increases in serum erythropoietin levels and soluble transferrin receptors(27 ,28).

A detailed morphologic review was performed in a blinded fashion on 216 bone marrow specimens from 178 patients with HIV infection. Among the most common bone marrow findings were hypercellularity (53 percent of specimens), myelodysplasia (69 percent), evidence of reticuloendothelial iron

blockade (i.e., anemia of chronic inflammation, 65 percent), megaloblastic hematopoiesis (38 percent), fibrosis (20 percent), plasmacytosis (25 percent), lymphocytic aggregates (36 percent), and granulomas (13 percent). In 7 of 14 patients found to have marrow involvement by malignant cells, the bone marrow represented the initial site of diagnosis (29).

Initiation of HAART reduces both the incidence and degree of anemia in all groups of HIV-infected patients and should be aggressively pursued (22).

In a longitudinal Seattle study including 2493 HIV-infected patients, the incidence of anemia in those treated with HAART was significantly reduced from 23 percent in the pre-HAART period to 4 percent after the introduction of HAART.

The incidence of anemia in this study did not change in patients with a CD4 count <100/micro/L (30).

Another study at San Francisco General Hospital, California, USA**)**, in this study anemia in HIV infection, treatment options, and practice strategies was evaluated to determine the impact of anemia in this patient's population. The working group considers HIV-associated anemia to be an important contributor to the morbidity and mortality of this infection and that recovery from anemia is associated with improved quality of life and survival. Transfusions should be used when rapid recovery is required, and underlying conditions causing anemia should be treated, if possible. Recombinant human erythropoietin (rHuEPO) therapy is appropriate in certain HIV-infected persons and should be considered to maintain hemoglobin concentrations 12 g/l (31).

Another study evaluated medical records of anemic patients with HIV, the impact on hemoglobin (Hb) of initiating zidovudine (AZT)-containing and non-AZT-containing combination antiretroviral therapy was evaluated.

Data was collected from 11 US cities from 1998 to 2004. Baseline anemia was described as mild moderate or severe. They found patients initiating ART, more than half (54%) of patients had improvement of anemia. Most anemic

patients initiating ART (with or without AZT) had increases in hemoglobin level especially those with more severe anemia or immunosuppression (32).

To ascertain the impact of highly active antiretroviral therapy (HAART) on cytopenia and relationship between cytopenia and survival in a large cohort of HIV-infected patients:: A prospective multicenter study of HIV-1 infection in children during their visits every 6 months, The setting was a university-affiliated clinic at 6 sites in the United States. The study included a standardized history, physical examination, and comprehensive laboratory evaluation. Survival analysis was based on overall mortality during the follow-up period: Among HIV-infected pts who were not anemic at baseline, 47% became anemic by 3.5 years of follow-up. The use of HAART was associated with resolution of anemia even when used for only 6 months (P<0.05). A CD4 cell count<200 cells/microL , HIV-1 RNA level>or =50,000 copies/mL and clinical diagnosis of AIDS (P<0.001) were associated with an inability to correct anemia and decreased survival. Similarly, use of HAART for 12 months or more was associated with a protective effect against development of anemia (OR = 0.71; P<0.001). Among HIV-infected patients, cytopenia was independently associated with decreased survival (HR = 2.83; P<0.001). (23)

3.2.2 THROMBOCYTOPENIA

Thrombocytopenia is a common finding in individuals infected with HIV, affecting approximately 40 percent of patients during the course of their illness. HIV-associated thrombocytopenia occurs in patients from all major risk groups including children and adult (33).

Patients may present with thrombocytopenia at any time during the course of HIV infection, from asymptomatic infection to advanced AIDS. It may be the initial manifestation of HIV infection in as many as 10 percent of patients. Therefore, consideration of HIV testing is essential in the assessment of any patient with newly diagnosed thrombocytopenia. The incidence of platelet abnormalities appears to increase with progressive immunosuppression (34).

In one study the incidence of a platelet count below 150,000/microL was found in 8 and 30 percent in HIV-infected patients with CD4 counts of 200 to 500/microL and <200/microL, respectively (35).

In the current era of combination antiretroviral therapy, thrombocytopenia is more commonly encountered among patients with uncontrolled HIV replication and hepatitis C co-infection.
Secondary causes of thrombocytopenia are generally the result of underlying opportunistic infections, malignancy, and co-morbid conditions (36).
Zidovudine (AZT) has been the mainstay of therapy (37).

HAART may also be associated with improvement in thrombocytopenia several retrospective studies support this observation (38).
In one of these studies patients with thrombocytopenia (platelet count <50,000/micro/L) who treated with various combinations of antiretroviral regimens experienced sustained improvement in platelet counts during their therapy (39).

Declines in platelet counts have been reported in patients who discontinue antiretroviral therapy (40).

In another study the exact frequency of HIV-associated thrombocytopenia (TCP), defined as platelet count less than 150 x 10(9)/1, was studied in 435 symptom-free HIV-seropositive individuals. At the baseline control, 23 (5.5%) had TCP. TCP individuals had a significantly lower mean CD4 lymphocyte count than the non-TCP individuals. During a mean follow-up of 30 months, 79 out of the 435 individuals (18%) had TCP at least once. During the study period, only 1% of our patients had a platelet count less than 50 x 10(9)/l. TCP was more frequent in intravenous drug users than in other risk groups. A spontaneous normalization of platelet count was observed in more than 50% of TCP individuals (41).

In another study the Epidemiology of thrombocytopenia in HIV infection in 1992 in Washington D.C. was conducted to determine the frequency of thrombocytopenia and its relationship to HIV infection. They review records of HIV-infected patients attending two outpatient clinics. Fifty-nine percent of the individuals were white, 37% were black and 94% were male. Fifteen percent had AIDS. Thrombocytopenia occurred more frequently in subjects with AIDS (21.2%) than in HIV-infected individuals who did not fit clinical criteria for AIDS (9.2%) ($P < 0.001$). Patients with low CD4-positive cells and an advanced stage of disease were more likely to have low platelet counts: 30% with an absolute CD4 cell count lower than 200/mm3 vs. 8% with CD4 counts between 200 and 500 ($p < 0.00001$), and 18.5% with Stage IV disease compared to 7.6% in Stage II ($p< 0.001$) had platelet counts less than 150,000/mm3.. Of all subjects with platelet counts < 50,000/mm3, 40% reported bleeding and 1 died of an intracranial hemorrhage (36)

3.2.3 LEUKOPENIA AND ABNORMAL LEUKOCYTE FUNCTION

Granulocytopenia is a problem commonly encountered in patients with HIV infection. Although low granulocyte counts usually reflect the toxicity of therapies for HIV infection or associated conditions. The pathogenesis of granulocytopenia in patients with HIV infection is multifactorial. An autoimmune mechanism involving antigranulocyte antibodies and impaired granulopoiesis has been postulated, but not yet proved, to account for granulocytopenia in some patients. Any infiltrative process involving the bone marrow (infection, malignancy) may also produce granulocytopenia. In clinical practice, however, drug toxicity is responsible for most of the granulocytopenia seen in patients with HIV infection (42).

Drug-induced granulocytopenia is common in patients with HIV infection AZT therapy is probably the most common cause of low granulocyte counts in patients with HIV infection. Severe granulocytopenia (< 500 cells/mm3) developed in 16% of AZT-treated patients (43).

Ganciclovir therapy for symptomatic cytomegalovirus infection is another common cause of granulocytopenia in patients with advanced HIV disease drugs may also involved in the development of granulocytopenia such as Trimethoprim-sufamethoxazole and pentamidine and interferon-alpha (24,25).

Leukopenia exists when the total WBC count is less than 4000/mm3. Neutropenia is defined as an ANC of less than 1000/mm3 in infants between 2weeks and 1 year of age and less than 1500/mm3 beyond 1year of age. Severity of neutropenia is graded as: Severe neutropenia: ANC less than 500/mm3 Moderate neutropenia: ANC 500–1000/mm3, Mild neutropenia: ANC 1000–1500/mm3 (44, 45).

Neutropenic patients are usually infected with their own endogenous bacterial flora that resides in the mouth, oropharynx, gastrointestinal tract, and skin. For this reason, the frequency of gram-negative bacterial infections and Staphylococcus aureus (47, 48).

Zon and Groopman noted low granulocyte count in 13% of asymptomatic HIV patients and in 44% of those with frank CDC-defined AIDS (42).

A study on prevalence of neutropenia in HIV infection in 2006. The study was prospective, multicentric study, the result was that neutropenia was common among HIV patients. At base line neutropenia was in 44% with neutrophil count less than 1500/mm3 and 7% less than 1000/ mm3. Followed up to 7 yrs, 79% had neutropenia less than 1500/mm3, and 31% with neutrophil less than 1000 / mm3. They conclude that as the disease worsen there is an increase in neutropenia, and that treatment with HAART without AZT regimen protect against development of neutropenia (48).

3.2.4 HEMOSTATIC ABNORMALITIES

There are several abnormalities of the Coagulation system that may be encountered in patients with HIV infection (34).

These include the following: An increased propensity for thrombosis, Presence of antiphospholipid antibodies, Abnormalities of several factors involved in the regulation of clotting (49).

Thrombosis — the incidence of venous thrombosis in HIV-infected patients was determined to be 2.6 per 1000 patient. Risk factors significantly associated with the development of thrombosis included age >45 years, and opportunistic infection (50).

Lower CD4 counts , Elevated HIV viral loads , Elevated levels of factor VIII and homocysteine, Elevated lipid levels, Increased tissue factor expression on circulating monocytes , Presence of antiphospholipid antibodies (lupus anticoagulan(la) or anticardiolipin antibodies or autoimmune hemolytic anemia and Acquired deficiencies of proteins S and C and antithrombin(50,51) .

Deficiency of proteins C and S — Acquired deficiencies of proteins S and C have been reported in association with HIV infection which may be related to the presence of acute opportunistic infections. Thrombotic thrombocytopenic purpura hemolytic uremic syndrome has been described among patients with HIV infection, although its incidence in the post-HAART era appears to be declining (52).

Patients who developed thrombotic microangiopathy, compared with those who did not, were more likely to have a lower CD4+ lymphocyte count, and a history of acquired immunodeficiency syndrome (AIDS) and hepatitis C infection (53).

The diagnosis of TTP-HUS should be considered in all HIV-infected patients presenting with thrombocytopenia and anemia (54).

In our study, data on haemostatic abnormalities was not collected, the need to include this in future studies will be recommended.

3.3. OPPORTUNISTIC INFECTIONS

Many people living with human immunodeficiency virus (HIV/AIDS) acquire diseases that also affect otherwise healthy people. In such cases, HIV-infected patients may have a more severe disease course than uninfected people or may develop symptoms that uninfected people do not. However, HIV-infected people are also susceptible to opportunistic infections, which are infections caused by organisms that in a healthy host would not cause significant disease. These are susceptible to opportunistic Infections because of how HIV suppresses the immune system. Many people do not know that they have HIV until the first time that they have opportunistic Infections (55, 56).

3.3.1 HEPATITIS C VIRUS

In HIV–HCV co-infected patients, the hepatitis C (HCV) viral load is higher than in HCV-mono-infected patients in both the plasma and liver tissue. Patients who are HIV positive are commonly co-infected with HCV due to shared routes of transmission percutenous exposure to blood, sexual intercourse, and from a mother to her infant. Infection with HCV can be asymptomatic, self-limiting, or progress to cirrhosis and cancer (57).

The morbidity and mortality caused by HCV has increased since the inception of highly active antiretroviral therapy (HAART) because HIV patients are living longer from potent antiretroviral therapies and prophylaxis of traditional opportunistic infections. The effect of HCV on the natural history of HIV remains inconclusive due to contradictory studies documenting no effect, while others show an increase to an AIDS defining illness or death.

In the United States, approximately 150,000 to 300,000 people are co-infected with HIV and HCV. This represents 15% to 30% of all HIV infected patients and 5% to 10% of all HCV patients. Reduced HCV antibody production, drug interactions, other causes of liver disease, differing epidemiologic characteristics and natural history complicate the management of HCV/HIV patients. Until recently there was little data published regarding treating HIV–

HCV co-infected patients; fortunately recent trials have been published about the safety and efficacy of current treatment options (58).

The primary objective of HCV therapy is permanent eradication of the virus. The secondary potential benefit of eradication is a reduction in the risk of liver failure and liver cancer. Currently, peg -interferon alfa-2a plus ribavirin is the only FDA approved treatment for HIV–HCV co-infected patients. Interferons bind to specific cell surface receptors of virus-infected cells, which induce a complex cascade of protein-protein interactions and a rapid activation of gene transcription. The antiviral effects of interferons are mediated through inhibition of viral penetration or uncoating, inhibiting viral replication or translation of viral proteins, and/or viral assembly and release. The difference between peginterferon and interferon is the addition of a polyethylene glycol (PEG) polymer. The addition of PEG decreases plasma clearance considerably, protects the molecule from proteolytic degradation and reduces its immunogenicity. Ribavirin is a synthetic nucleoside analogue, but its mechanism of action is not clearly established (59).

A study was conducted in Spain 2007 to identify predictors of severe hematological toxicity among HIV-HCV-co infected patients treated with PEG-IFN plus RBV. This retrospective multicentric study included 237 HIV-HCV-co infected patients on PEG-IFN plus RBV. Predictors of severe anaemia, neutropenia, thrombocytopenia and overall hematological toxicity were analyzed. Eighty (34%) individuals showed an episode of severe hematological toxicity. Severe anemia, neutropenia and thrombocytopenia occurred in 32 (13%), 42 (18%) and 26 (11%) patients, respectively. This study concluded that Zidovudine treatment, cirrhosis, baseline low body weight, use of PEG-IFN-alpha2a, and baseline hemoglobin level<11 g/dl are predictors of overall severe hematological toxicity secondary to PEG-IFN plus RBV in HIV-infected individuals. Low pretreatment levels of each hematological series predict a significant decrease of their values during therapy (60).

In another study in Brazil the Hematological abnormality of 701 HIV infected patients in their institute was studied. 61% of the patients were males and 39 females. Anemia was found in 37%, among them 40% were males and 61% with low CD4, 39% had HCV among them 83% with anemia, other patients with no HCV only 40% had anemia (61).

3.3.2 CYTOMEGALOVIRUS

Cytomegalovirus (CMV) is a common viral infection worldwide. Most people with CMV develop few or no symptoms. However, a fetus exposed to CMV can suffer severe consequences, including mental retardation and even death. In patients with HIV/AIDS, the most common complication of CMV is retinitis. CMV can also cause hepatitis, diarrhea, and encephalitis. CMV retinitis is most commonly seen in patients with CD4+ lymphocyte counts of less than 50 cells/μL and can lead to blindness if untreated. One should advise patients to report to the clinic if they notice changes in their vision, including blurry vision or “floaters.” Many patients are asymptomatic. If possible, patients should have regular fundoscopic examinations to check for changes and if necessary should be referred to an eye specialist (62, 63, 64).

Ganciclovir intravenously or valganciclovir orally are the antiviral medications recommended for treating CMV (63, 65).

3.3.3 TOXOPLASMA GONDII

Toxoplasma gondii is transmitted via raw or undercooked meat, particularly pork, lamb, and venison, and immunosuppressed individuals should avoid contact with stray cats and cat feces. Good hand washing can prevent infection(66).

Toxoplasmosis in the immunocompromised host usually causes central nervous system disease, specifically brain abscesses. Toxoplasmosis commonly reactivates, causing repeated infections. Patients have focal neurologic deficits,

including seizures, hemiparesis, hemiplegia, cerebellar tremor, cranial nerve palsies (e.g., facial nerve palsy), hemisensory loss, visual problems or blindness, personality changes, and cognitive disorders. Severe localized headache that does not respond to analgesics may be present. Toxoplasma gondii infection is classically seen as multiple-ring enhancing lesions on a computed tomography scan (66, 67). Antibodies often can be detected in the blood or other body fluids (cerebrospinal fluid). This disease is much more common in adults than in children. However, infants infected in utero are at high risk for toxoplasmosis encephalitis (67).

Treat toxoplasmosis with pyrimethamine and sulfadiazine. Treatment for toxoplasmosis should continue for at least 4 weeks after complete resolution of disease. Primary prophylaxis is recommended with TMP-SMX daily for severely immunocompromised patients (68).

(4) AIMS OF THE STUDY

Most of HIV infected children live in limited resources countries. Little information about the hematological abnormalities among these children. Some studies were reported from USA, parts of Africa Nigeria and Ethiopia, other studies from Jamaica, Brazil and India.

In Libya few studies have been conducted about HIV in general and no single study about hematological abnormalities in HIV infected children. So the aim of our study is:-

1-To review hematological abnormalities including various cytopenia of all major blood cell lines (anemia, neutropenia, lymphopenia, and thrombocytopenia)

2-To evaluate the influence of contributing factors as:

(a) Demographic characteristics

(b) Co infection with HCV, CMVand Toxoplasmosis,

(c) The stage of the disease according to (CDC classification) in HIV infected children at Infectious Disease Center in Benghazi- Libya.

(5) PATIENTS AND METHODS

A total of 426 patients with HIV are registered and are followed up at Infectious Disease Center of Children in Benghazi. These children are the victims of the largest documented incident of nosocomial infection of HIV in history. The crisis first came to light in November 1998. The Libyan public was enraged and many foreign medical workers were arrested. It was eventually revealed that over 400 children had been infected. Libya requested and received an emergency WHO team which was sent in December and stayed through to January 1999 for investigations. This epidemic happened during the period 1997-1998 but the exact time, the details of the epidemic, the cause of this tragedy still a mystery and still under investigations.

From this total, the first 100 patients' charts as they organized in the register were included. Exclusion criteria: Patients over 16 years of age and those who had malignancy and hypersplenism.

This study was a descriptive case series study. Data was collected retrospectively from the 100 patient's charts using a designed Performa which already present in the data base in the infectious center. The following data was collected; patients' Demographics: gender, date of birth, nationality, residence and investigations (complete blood count: hemoglobin, leukocyte, neutrophils, lymphocytes, platelet count. The following definitions of cytopenia was used in this study: leukopenia as total leukocyte count less than $4.5x10^9$/L. Neutropenia as an absolute neutrophil count $\leq 1.5x10^9$/L ,lymphopenia as absolute lymphocyte count $\leq 1.5x10^9$/L , anemia as hemoglobin$\leq$ 11g/L mild (hemoglobin 8 to 11) and severe (hemoglobin <8 g/L) and thrombocytopenia as platelet count < $150x\ 10^9$/L. All cytopeniawas adjusted according to age (44).

The serological tests for (HCV, CMV, Toxoplasmosis) as well as the stage of the disease (CDC classification) which were performed at time of their diagnosis as

well as during their follow up from the time they were diagnosed until February 2012.

- **DATA ANALYSIS:** Data was analyzed. Each variable was analyzed for number of episodes, severity and its correlation with co infection. Data was interpreted in term of percentage, and test as chi squire, and p value was used to determine statistical significance of association between categorical variable.

(6) R E S U L T S

A total of 100 patients are included in this study:

- 87% from Benghazi.
- 13% from outside Benghazi
- 98% were Libyan
- 2% were non Libyan

6.1. DEMOGRAPHIC CHARACTERISTC (n=100)

Age:

Their age at time of the study had a mean age of 14.1 $\pm$ 1 years and at time of diagnosis of HIV infection had a mean age of 17 $\pm$ 4 months

Gender:

- 55% were male
- 45% were female

Figure (1) below illustrates the gender of patients included in the study

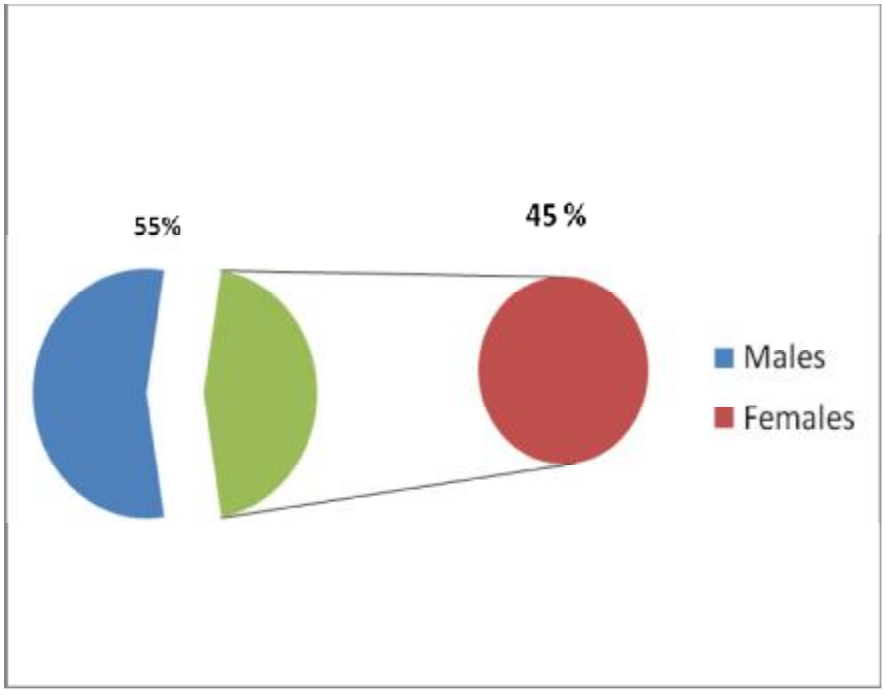

Figure -1: Distribution of the patients (n=100) according to gender

6-2. HEMATOLOGICAL ABNORMALITY AT TIME OF DIAGNOSIS

53% of patients had hematological abnormality at their time of diagnosis of HIV infection of these: anemia was the most frequently observed 33(62%), followed by lymphopenia 10(19%). Thrombocytopenia 4(7.5%), leukopenia4(7.5%), (Note: leukopenic patients were not followed up subsequently) and neutropenia 2(4%). Figure **(2)** below illustrates the prevalence of the various types of cytopenia.

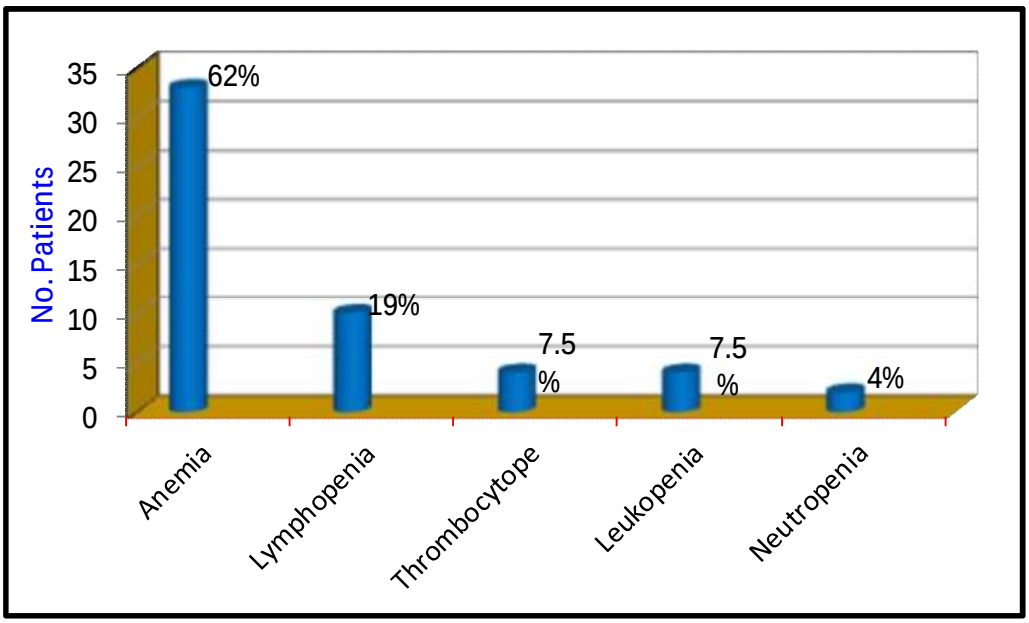

Figure -2: Distribution of patients according to Hematological Abnormality at Time of Diagnosis

6.3. TIME OF CYTOPENIA

From total cases who had hematological abnormality either at time of diagnosis or develop it during course of their disease 59 of them had anemia:- (33at time of diagnosis and 26 developed subsequently(33+26)), 49 :- neutropenia(2+46), 40:-lymphopenia(10+30), 38:-(4+34)thrombocytopenia, most of patients had more than one type of cytopenia.

Figure (3) illustrates the time of hematological abnormalities either at time of diagnosis or develop it latter during their follow up and number of patients for each abnormality.

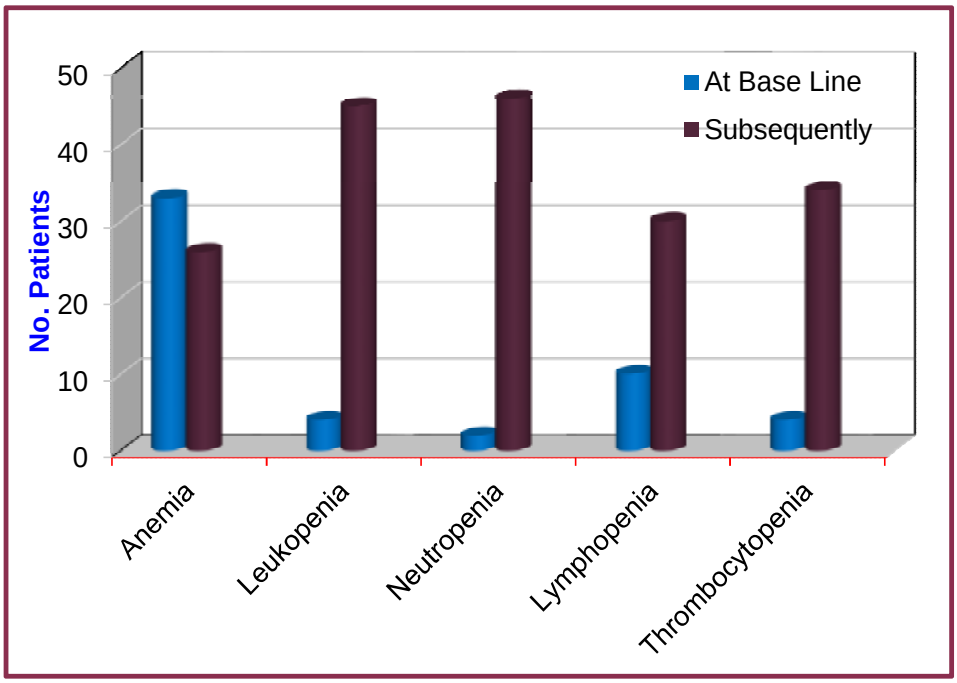

Figure -3: Distribution of Patients According to Time of Cytopenia

6.4. STAGES (CDC CLASSIFICATION) OF HIV ASSOCIATED DISEASE AND IT IS CORRELATION WITH CYTOPENIA.

All patients were classified into class N, A, B and C according to clinical symptoms and immune category (According to CD4 count)into 1, 2, 3.The cytopenia is more in advanced disease, but there is no significant association between cytopenia and stage of the disease with p=0.88 (p value is significant if it less than 0.05). **Table (2)** below illustrates the CDC classification of HIV associated disease and its correlation with cytopenia.

Table-2: Distribution of patients according to CDC classification and cytopenia.

Cytopenia	CDC Classification				
	N*	A*	B*	C*	**Total**
Anemia	15 25%	7 12%	26 44%	11 19%	59 100%
Lymphopenia	8 20%	6 15%	18 45%	8 20%	40 100%
Neutropenia	8 17%	7 15%	27 55%	6 13%	48 100%
Thrombocytopenia	10 26%	3 8%	18 47%	7 19%	38 100%
Total	41	23	89	32	185
Chi –square test	X^2= 4.3 df= 9 P= 0.88				

Table -2 Notes:

1-N*Asymptomatic stage: latent period between HIV infection and clinical signs and symptoms, CD4 more than500.
2-A*Mild clinical stage: minor symptoms start to appear, generalized lymphoadenopathy,candidiasis, CD4 rang between350-499.CD4% more than 28%
3-B*Advanced Stage: HIV patients with weakened immune system can develop life-threatening infection, fungal infection, tuberculosis CD4 200-399.CD4% 14-28%.
4-C* severe stage: clinical AIDS.

6-5. SEVERITY OF CYTOPENIA

Anemia at time of diagnosis was mild in most of cases 73% .**Table (3)** below illustrates the level of Hemoglobin at the time of the study.

Degree of cytopenia ranged from mild to severe. **Table (4)** below show the number of patients in each type of cytopenia and the median of each one.

Table-3: Distribution of patients (n= 33) according to the severity of anemia at time of diagnosis.

Hb Level	No. of Patients	%
>9-11g/dL	24	73
8-9 g/dL	8	24
< 8g /dL	1	3
Total	33	100

Table-4: Distribution of patients according to the severity of Cytopenia.

Severity	Type of Cytopenia				
	Anemia	Leukopenia	Neutropenia	Lymphopenia	Thrombocytopenia
Minimum	6.3g/L	$1.7x10^9$/L	$0.3 x10^9$/L	$0.3 x10^9$/L	$11 x10^9$/L
Maximum	11.3g/L	4.410^9/L	$1.4 x10^9$/L	$1.4 x10^9$/L	$150 x10^9$/L
Median	8.8 g /L	$3.1x10^9$/L	$0.8 x10^9$/L	$0.8 x10^9$/L	$80 x10^9$/L

6.6. FREQUENCY OF CYTOPENIA

Most of anemic patients 22(37%) had up to 5 episodes, persistence cytopenia were develop in 31%, 21%, 20%, 10%, 7% of anemic, thrombocytopenic , leukopenic, neutropenic and lymphopenic patients respectively. **Table (5)** below illustrates the episodes of various cytopenia.

Table-5: Distribution of the patients according to number of episodes of Cytopenia.

No. of Episode	Type of Cytopenia									
	Anemia		Leukopenia		Neutropenia		Lymphopenia		Thrombocytopenia	
	No	%	No	%	No	%	No	%	No	%
≤ 5	22	37%	21	43%	35	73%	28	70%	26	68%
>5 - 10	19	32%	18	37%	8	17%	9	23%	4	11%
Persistent*	18	31%	10	20%	5	10%	3	7%	8	21%
Total	59	100%	49	100%	48	100%	40	100%	38	100%

*More than 6 months.

6-7. EFFECT OF GENDER IN CYTOPENIA

Anemia is more in females 33/59 in contrast to other cytopenia which more in males but there is no significance association between gender and development of cytopenia with P = 0.57. (Significant p value less than 0.05). **Table (6)** below illustrates the type of cytopenia and its correlation with patients gender.

Table- 6: Distribution of the patients according to Gender and Cytopenia

Type of cytopenia	M.	F	Total	Test chi square
Anemia	26	33	59	χ^2=1.97 df=3 p=0.57
Nuetropenia	27	21	48	
Lymphopenia	21	17	38	
Thrombocytopenia	21	19	40	
Total	58	90	148#	

NB:# there is an overlap between various cytopenia

6-8. EFFECT OF CO INFECTION ON CYTOPENIA

CMV was the most common co infection affecting more than 85% of HIV infected children as general. It was present in 95%, 89%, 88%, 85% of neutropenic, anemic, lymphopenic and thrombocytopenic patients respectively. Approximately 50% of patients were HCV positive. The infection was present in 56%, 52%, 47%, and 43% of neutropenic, anemic, thrombocytopenic and lymphopenic patients respectively. With approximately 10% with toxoplasmosis, But there is no significance association between co infection with CMV, HCV, Toxoplasmosis and development of cytopenia with P > 0.05 in all of them.We also noted that coinfection is more in neutrpenic pts

. Tables (7, 8, 9) illustrates the above findings.

Table -7: Distribution of the patients (100) according to CMV and Cytopenia.

Cytopenia		CMV Infection		Not Done	Total	Test chi sequare
		+ve	-ve			
		No of Cases	No of Cases			
Anemia	Yes	50	6	3	59	χ^2=0.26 df=1 p=0.61
	No	30	5	6	41	
Neutropenia	Yes	42	2	4	48	χ^2=2.01 df=1 p=0.15
	No	40	6	6	52	
Lymphopenia	Yes	30	4	6	40	χ^2=0.02 df=1 p=0.81
	No	50	6	4	60	
Thrombocytopenia	Yes	29	5	4	38	χ^2=0.31 df=1 p=0.57
	No	50	6	6	62	

NB: significant p value less than 0.05

Table -8: Distribution of the patients (100) according to HCV and Cytopenia.

Type of Cytopenia		HCV infection		Not done	Total	Test chi square
		+ve	-ve			
		No of cases	No of cases			
Anemia	Yes	30	27	2	59	χ^2=0.76 df=1
	No	17	22	2	41	p=0.38
Neutropenia	Yes	26	20	2	48	χ^2=1.5 df=1
	No	22	28	2	52	p=0.22
Lymphopenia	Yes	16	21	3	40	χ^2=1.65 df=1
	No	34	26	-	60	p=0.19
Thrombocytopenia	Yes	17	19	2	38	χ^2=0.53 df=1
	No	34	28	-	62	p=0.46

NB: significant p value less than 0.05

Table-9: Distribution of the patients (100) according to Toxoplasmosis infection and Cytopenia

Cytopenia		Toxoplasmosis Infection		Note Done	Total	Test chi sequare
		+ve	-ve			
		No of cases	No of cases			
Anemia	Yes	7	48	4	59	χ^2=0.41 df=1
	No	6	28	7	41	p=0.52
Neutropenia	Yes	8	36	4	48	χ^2=2.72 df=1
	No	3	42	7	52	p=0.09
Lymphopenia	Yes	4	31	5	40	χ^2=0.05 df=1
	No	7	47	6	60	p=0.8
Thrombocytopenia	Yes	5	28	5	38	χ^2=0.38 df=1
	No	6	50	6	62	p=0.53

NB: significant p value less than 0.05

6.9. CAUSS OF DEATHS

Five patients (5%) died from different causes all of them had pancytopenia and persistence anemia at time of their deaths. **Table (10)** below illustrates the patients causes of death and age at death

Table-10: Distribution of the patients according to causes of deaths.

Case No.	Age at Death	Causes of Death
1	16 yrs.	Pancytopenia*
2	14 yrs.	Sepsis Pancytopenia , persistaence anemia and thrombocytopenia
3	12yrs.	Cardiomyopathy, HIV wasting syndrome, anemia Persistence pancytopenia
4	10 yrs.	Cerebral palsy, growth failure pneumonia persistence pancytopenia
5	7yrs.	HIV encephalopathy, wasting syndrome, persistence anemia and thrombocytopenia.

NB: * direct cause not known pt. died outside the hospital

(7) DISCUSSION

In Libya there is lack of base line data on HIV or HIV treatment related hematological abnormalities, the results of this study try to fill part of the gap. But small number of patients in addition to large number of variables in the study limits the generalization of the results.

Most of literature available on the Hematological changes in HIV infected patients were studies performed on adolescent and adult patients and this is a pitfall in all the studies conducted on children as comparing with was not feasible and we acknowledge this fact in our study.

The number of children infected with HIV has increased dramatically in developing countries (1). There are 2.5 million new infections. Among the new infections, 420,000 were children younger than 15 years (2). Cytopenia of all major blood cell lines were increasingly recognized among HIV infected patients. And appears to increase with progressive immunosuppression as a result, HIV infection should be considered in the assessment of patients presenting with any type of cytopenia (3).

In the Infectious Disease Centre in Benghazi were this study was conducted a total of 429 are registered .Of these 237 child were less than 16 years at the time of the study.

The 100 patients who were included in this study were diagnosed between June-1998 to May-2000 and their age at time of data collection was 13.5 to 16 years with mean age of 14.1+-1. With 55% were males as seen in study done in Washington DC (36) as well as in other places (7, 61).

In one series of patients with AIDS, anemia was noted in approximately 70 percent, lymphopenia in 70 percent, neutropenia in 50 percent, and thrombocytopenia in 40 percent(1).In other HIV infected children various

hematological manifestation were studied , anemia was found in 70% , 28% leukocytosis, 24% lymphopenia , 10% thrombocytopenia , 2% leucopenia with neutropenia (10). In Ethiopia: Anemia was found in 21.9%, thrombocytopenia 7.8%, neutropenia 4.7% severe anemia in 14.3% (11).

In our study 53(53%) patients had hematological abnormality initially at time of diagnosis 33(62%) of them had anemia, 10(19%) had lymphopenia, 2(4%) neutropenia, 4(7.5%) leukopenia, and another 4(7.5%) had thrombocytopenia.
The hematological abnormality which was present at the time of diagnosis or developed subsequently during the course of their disease was anaemia in 59 patients, (33initialy+26subsequently) leukopenia in 49(4+45), neutropenia in 48 (2+46),lymphopenia in 40(10+30) , thrombocytopenia in 38(4+34).Anemia was the commonest hematological manifestation in Libyan HIV infected children. These results are comparable with that reported from Africa, India and Jamaica in the literature (9, 11, 12, 13).

These figures show that cytopenia were low initially at time of diagnosis and most of these patients develop cytopenia later on during their course of the illness with progression of the disease. Except in anemia where more than half of cases were at the time of diagnosis can the cause of can be suggested by that at time of diagnosis many of them at age of physiological anemia in addition of iron deficiency anemia in those less than2years.

Table (11) below shows our study results in comparison to the other world results.

Table-11: Our study results and the other world results.

STUDY	HEMATOLOGICAL ABNORMALITIES				
	Anemia	**Leukopenia**	**Neutropenia**	**Lymphopenia**	**Thrombo-cytopenia**
Allauria M., Burns E.R 2000. Newyork 100 patients	74%	47%	----------	----------	33%
Adetifa IM, Temiye E.O, 2006 Nigeria 68 patients	77.9%	----------	17.5%	6%	2.5%
PieereR.B, Lewis K.P. 2007 274 Patients	80.7%	19%	----------	----------	11.9%
Muluneh A.B Fessahaye A.L 2009 46 patients	21.9%	----------	4.7%	----------	7.9%
Cynthia F., Laura S. 2010 Africa,America, South America 1571 patients	12%	----------	14%	----------	7%
Shah I., Katira B. 2011 50 paients	70%	----------	2%	24%	10%
Our study 2012 Benghazi – Libya 100 patients	59%	49%	48%	46%	38%

The above table showed that our children are equally affected by different cytopenia in spite that anemia was the most common; this is similar to study done by Cynthia I. and Laura S in which the patients were in early stages of the disease.

CDC classifications an attempt to classify HIV disease in a progressive sequence from least to most severe. each higher stage having poorer prognosis.CDC system combine 3categories of CD4 count,1,2,3 with 3clinical symptoms categories A ,B and C. A-mild ,B- advanced or middle stage ,C-severe (6).

In one study 8% of asymptomatic HIV-seropositive patients, 20% of those with symptomatic middle-stage HIV disease, and 71% of those with AIDS were anemic (16).In another Investigators from a cohort longitudinal study of HIV disease found anemia in 18% of asymptomatic HIV-seropositive patients, 50% of those with symptomatic middle-stage HIV disease, and 75% of those with CDC-defined as AIDS and increase with progression of disease.Neutropeia is also common and increase with the progression of disease(47,48).

In agreement with previous reports our study revealed that anemia in asymptomatic seropositive patients was only 12% was present but present in 63% in those with advanced disease (middle and severe stage). Lymphopenia in early stage present in15% while in advanced disease in 65% as well as neutropenia present in 15% in early stage and in advanced 69%, also thrombocytopenia present in 8% in early stage and 65% in advanced stage. But there is no significance between the stage of the disease and the development of cytopenia with $P = 0.88$.

Anemia at time of diagnosis was mild in most of cases 24/33,(73%) and only 1/33(3%) had severe anemia .and it is degree ranged from 6.3 g/dl to 11 g/dl with median of anemia ,leucopenia, neutropenia, lymphopenia and thrombocytopenia were (8.8 g /L, $3.1x10^3$/ L ,$0.8x10^3$/ L,$0.8x10^3$/ L,and $80x10^3$/ L) respectively.

The number of patients developed anemia subsequently was less than those with other cytopenia but the severity of anemia clearly increased with the progression

of the disease. In other cytopenias the number of the patients and the severity of cytopenias increase markedly with the progression of the disease.

Most of anemic patients 22(37%) had up to 5 episodes, with18 patients (31 %) had persistence anemia, approximately 20% of leukopenic as well as patients with thrombocytopenia became chronic(persistent more than 6 months) .

We found in our study anemia affected more females 33/59 in contrast to other cytopenia which more in males but there is no significance association between gender and development of cytopenia with P =0.57. This is similar with other researcher where they found anemia is frequent complication of HIV infection and it is incidence is associated with female gender in study done in Brazil (61), and with progression of disease, neutropenia, thrombocytopenia, black race and prescription of certain chemotherapy (15).

Other study they also show factors associated with excess risk of anemia include demographic factors (female sex) and concurrent disease, in contrast to thrombocytopenia which more frequent in white males and older patients were they found 95% of cases with thrombocytopenia were male (33).

CMV is worldwide infection causing no symptoms in immune competent persons but it causes severe even fatal infection in immsuppressed HIV patients. In USA 60%of HIV adult patients are serologically positive (63). In Libya no information about the prevalence of CMV in HIV or normal population.

CMV were the most common co infection affected our patients it present in95%, 89%,88%,85% in neutropenic, anemic, lymphopenic and thrombocytopenic patients respectively.

Hepatitis C confection is estimated to occur in ~30% of HIV-infected individuals in the United States (58).In a retrospective case-control study of HIV-infected patients, thrombocytopenic patients (i.e., platelet counts

<100,000/micro/L for more than three months) were significantly more likely to have HCV co-infection (58).

Haematological adverse events related to pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy could affect the patients' quality of life; however, the risk factors for severe haematological toxicity associated with this therapy in patients co infected with hepatitis C virus (HCV) and HIV are unclear (59,60).

Cytopenia is more commonly encountered among patients with uncontrolled HIV replication and hepatitis C co-infection (35, 61)
In our patients HCV infection affect 56%, 52%, 47% and 43% of neutropenic,anemic, lymphopenic and thrombocytopenia patients respectively. Toxoplasmosis co-infection present in only 10% of HIV infected Libyan children.

As we find CMV was the most common co infection followed by HCV co infections and the least was toxoplasmosis, but there is no significance association between co infection with CMV, HCV and Toxoplasmosis, and development of cytopenia with $P > 0.05$ in all of them. We find also that the neutropenic patients were more susceptible to co-infection.

Cytopenias that does not resolve is associated with shorter survival of HIV infected patients , the risk of death was 170% greater for patients who did not recover from anemia, neutropenia, thrombocytopenia and with CD4 count < 200cells/ microl as well as in patients with clinical AIDS and Opportunistic infection(15).

HIV-associated cytopenia is an important contributor to the morbidity and mortality of this infection. And that recovery from them is associated with improved quality of life and survival (31).

We have 5 patients died from Sepsis. Cardiomyopathy and HIV wasting syndrome. Cerebral palsy, growth failure and pneumonia. HIV encephalopathy, wasting syndrome and all of them had pancytopenia at their time of death.

(8) CONCLUSION

- Our study showed that hematological abnormalities were common among HIV infected Libyan children and it increases with the progression of the disease. Anemia was the commonest hematological abnormality followed by neutropenia.
- Females are more affected in anemia; males are more in other cytopenia.
- Regarding co-infection CMV infection was the commonest, followed by HCV.
- All children with HIV in our study who died had pancytopenia.
- This study was the first study in Libya conducted on HIV infected children and is intended to provide a baseline study which looked at a number of parameters relating to hematological manifestation.
- This study was restricted by a small sample size and by the large number of parameters studied. More studies will be required to obtain more meaningful result.

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(9) R E C O M M E N D A T I O N S

- Regular updating of the data base is recommended
- Establishment of a more advanced laboratory investigations for proper diagnosis of co-infections.
- Further study should include all the HIV children registered in the centre
- Further studies for each type of cytopenia individually is required to investigate the causes, the disease burden and therapy received.
- Establishment and maintaining a national disease registry for conducting further studies in the whole country is paramount.

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