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TO DETERMINE THE FREQUENCY OF VISCERAL LEISHMANIASIS IN PERIPHERAL CYTOPENIC PATIENT IN PATHOLOGY DEPARTMENT HAYATABAD MEDICAL COMPLEX.

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ABSTRACT

BACKGROUND: Visceral Leishmaniasis is a chronic disease and was first described in 1903, by LIESHMAN and DONOVAN. The disease is common in tropical and sub tropical areas of the world with various hematological manifestations. It is characterized by fever, visceromegaly, weight loss, pancytopenia and hypergammaglobulenemia. The disease is silent killer, invariably killing almost all untreated patients, but curable with hematological improvement within 4-6 weeks of treatment.

OBJECTIVE: To determine the frequency of Visceral Leishmaniasis in patints with cytopenias .

MATERIAL AND METHODS: A descriptive study conducted in Pathology department, Hayatabad Medical Complex, Hayatabad from September 1, 2012 to August 31, 2013. This study comprises of 126 patients, subjected to complete blood counts. Diagnosis were confirmed by finding Amastigote(L/D body) from bonemarrow aspirate. All the patients who were referred to pathology Department of the hospital for bone marrow examination, with the results of peripheral blood using automated Haematology analyzer, Sysmex KX 21 showing cytopenia were included in the study. Consent was taken from the patient for bone-marrow aspiration procedure. After consent detailed history, physical examination was done.

Laboratory investigations i.e. full blood count, which includes hemoglobin estimation, white blood cell, red blood, and platelet count.

Bone marrow cytology (Giemsa stain) was recorded on the designed profroma.

Posterior superior iliac spine (PSIS) was used as the site for aspiration in adults and children over 2 years of age

RESULT: Descriptive case series study of 126 patients of peripheral cytopenia. In which 77 (61.1%) patients were males and 49 (38.9%) were female with male to female ratio of 1.57: 1 It was also found in this study that visceral leishmaniasis was present in 29 (23%) of cases and the male: female were 1.6: 1. Result of the automated hematology analyzer of peripheral cytopenic patients in visceral leishmaniasis show that all of the patients were having total leukocyte count less than 4000/cmm (100%). The hemoglobin level wass less than 10gm/dl in 26 cases (87.7%) and more than 10gm/dl in three cases (10.3%). In case of platelets count, 27 cases (93.1%) were having platelets count less than 150000/cmm.

CONCLUSION: Incidence of visceral leishmaniasis is highier in children age group 1-10 years, also males are more prone than females. Leukopenia is recorded in all (100%) of the cases, followed by thrombocytopenia (93.1%) and anemia (Hb <10gm %) 87.7% cases.

KEY WORD: Visceral Leishmaniasis, Kala Azar, Amastigote (L/D body)

INTRODUCTION

Leishmaniasis is a chronic protozoal infestation disease associated with three main pattern i.e. visceral, cutaneous and mucocutaneous.Visceral leishmaniasis (VL) is also known as Kalazar is transmitted to human being by the bite of infected female sandfly (phlebotomus).[1] It is a systemic infection of the reticuloendothelial system. The parasite has two forms i.e. aflagellate or amastigote and flagellate or

promastigote forms. The amastigote form proliferates in mononuclear phagocyte system(MPS), especially spleen, liver and bone marrow with resultant hyperplasia of the organs leading to hematological manifestations.Visceral leishmaniasis is characterized clinically by a chronic febrile course with associated weight loss and hepatosplenomegaly. The diagnosis is usually established by identification of parasite is aspirate in from bone marrow, spleen or lymph nodes.Visceral Leishmaniasis is one of the major health problem,the World Health Organization (WHO) estimates the annual global rate for visceral leishmaniasis prevalence and incidence at 2.5 and 0.5 million cases respectively. Visceral Leishmaniasis causes 60-70 thousands deaths every year.[2]

Visceral leishmaniasis is usually associated with heamatological manifestations. The detection and diagnosis of cytopenia namely anemia, leucopenia and thrombocytopenia alone or in combination (pancytopenia) is frequently the focus of attention in care of the patient, because accurate quantification is difficult to achieve and rational analysis of problem is not possible. The cytopenia alone is not a diagnosis in itself but is objective sign of disease. Visceral Leishmaniasis is one cause of haematologi · cytopenia.[3,4]

The first case of Visceral Leishmaniasis in Pakistan was reported in year 1970 from Himalya region and since then is frequently seen in Northern areas and Kyber Pukhtunkhawa, and believed to be endemic in those areas.[2,5]

Transmission has also been reported through blood transfusion in Northern Europe. The disease can present unexpectedly in H.I.V and renal transplant patients.[5,6]

The most important haematological changes are neutropenia, anemia, thrombocytopenia, and pancytopenia.[7]

The clinical presentation varies from acute to chronic form of disease. The incubation period varies from weeks to months, and occasionally to several years.

The first sign of infection is high grade fever associated with chills and rigors. There is loss of appetite, weakness chest infection and diarrhea.[8]

Massive splenomegaly, hepatomegally and lymphadenopathy are also seen in majority of patients.[9]

Pancytopenia, and secondary infections like tuberculosis, pneumonia, serious ameobic or bacillary dysentery, gastroenteritis, herpes zoster, chicken pox, and skin infection are common.[10]

The disease carries high morbidity and mortility if left untreated .The Visceral Leishmaniasis can be diagnosed by various specific and non specific tests. The gold standard for diagnosis is visualisation of amatigote (L.D body) in splenic, bone marrow or lymph node aspiration .This is a technically challenging procedure.[11,12]

Buffy coat prepared from venous blood are sometimes helpful, culture of leishmania is possible on Schneider's Dorsophilia, RPMI medium1640 and NNN medium. Also Montenegro (Leishmanin) skin test, Antibody detection by ELISA and Immunoflourescent techniques is also available for diagnosis.[13] PCR can be performed on lesion aspirate, bone marrow aspirate and blood and biopsy material, is also an efficient method diagnosis.[14]

Keeping in view the importance of these changes in the peripheral cytopenic patients suffering from visceral leishmaniasis infection, this study has been designed to see occurrence of these changes in patients suffering from this disease in a tertiary care hospital at Peshawar. This will help in diagnosis, early treatment of complications as well as will be helpful in reducing morbidity and mortality from these complications of Visceral Leishmaniasis.

The epidemiology of visceral leishmaniasis is changing due to the increasing rate of co-

infection with HIV. According to the WHO, over 70% of HIV cases in southern Europe are also co-infected with visceral leishmaniasis.

Infection with visceral leishmaniasis and HIV is particularly harmful due to the parasites' ability to suppress immunity and stimulate the replication of the HIV virus. Co-infection with visceral leishmaniasis and HIV is usually spread between individuals who share needles, usually intravenous drug users.

Currently, the WHO is working to control the epidemic by setting up surveillance systems in 28 institutions worldwide. By providing common guidelines for diagnosis and computerized case report forms, the WHO can continue to track the trajectory of the disease.

The objective of this study is To determine the frequency of Visceral Leishmaniasis in patints with cytopenias as cytopenias caused by leishmaniasis is curable if properly diagnosed and treated in time and can save many lives especially of childern.

STUDY DESIGN: It was a one year descriptive study.

MATERIAL AND METHODS

This cross sectional descriptive study was carried out in the Department of Pathology, Postgraduate Medical Institute (Hayatabad Medical Complex) Peshawar. The duration of this study was one year from Sept 1, 2012 to Aug 31, 2013. The sample size was 126 patients, using 20% prevelance, 95% Confidence level and 5% margin of error under WHO software for sample size. Sample technique was purposive (non-probability) sampling.

Inclusion criteria: All age patients of both sexes with hematologic cytopenias were included.

Exclusion criteria: Patients with other causes of hematologic cytopenia like bleeding disorders, various deficiencies anemias, and drugs induced cytopenias were excluded from the study.

DATA COLLECTION PROCEDURE:

All the peripheral cytopenic patients who were referred to Pathology department of hospital for Visceral Leishmaniasis were studied. Those cases meeting diagnostic criteria like prolonged fever > 02 weeks, splenomegally and hematologic cytopenia were included. Detailed history ,physical examination.and laboratory investigation like peripheral smear were performed amongst them, those patients with peripheral cytopenia were subjected to bone marrow cytology.

The peripheral smears were prepared by taking three ml of venous blood by aseptic measure using disposable syringes. The blood was mixed with anticoagulant EDTA. Complete blood count was done on hematology analyzer, Sysmex KX 21 after carefull caliberation and were crosschecked manually during peripheral smears examination.

The blood smears both thick and thin were prepared and fixed with methanol for 03 minutes and stained with Giemsa's stain. Also hemoglobin was estimated by Sahli's hemoglobinometer and TLC, total RBC count and platelets count was performed by Neubar's chamber.

Both the manual methods and analyzer findings were compared and in case of any discrepancy were confirmed by both means and finalized. For differential counts 100 white blood cells were counted with the help of a differential counter. For reticulocyte count Brelliant Cressyl Blue was used as vital stain and their percentage noted.

Bone Marrow aspiration was done after a written consent from the patients, while adapting a standered aseptic techciques. About 0.5 - 1 ml of the marrow was sucked in 50 ml disposal syringe. Smears were made from the aspirate, without delay.The wound was then closed with sterilized dressing. Well spread smears with enough fragments were selected for Giemsa's staining. Two slides were stained from each case. The giemsa's stain used in peripheral smears was

also used in bone marrow aspirate as well, only the staining timings were doubled. After preparation of stained slides, the slides were examined for L.D. bodies, cellularity and any abnormal cells like storage cell or extramedullary cells etc.

The data of all the studied variables including demographic feature were analyzed for descriptive statistics like frequencies and percentages. For age, hemoglobin level, total leukocyte count and platelet count mean ± standard deviation was calculated. For gender distribution, male to female ratio was calculated. All the data was processed by computer Statistical Program for Social Sciences (SPSS) version 14.

RESULTS

It was a hospital based descriptive case series study of 126 patients of peripheral cytopenia. This study represented cases from all districts of KPK as well as from Afghanistan. In this study out of 126 peripheral cytopenic patients, 77 (61.1%) patients were males and 49 (38.9%) were female with male to female ratio of 1.57: 1. Visceral leishmaniasis was present in 29 (23%) cases and the male: female ratio in visceral leishmaniasis was 1.6:1. Age wise distribution of peripheral cytopenic patient show that majority of them belong to age group of 1-20 years followed by, 21-40 years etc (Table 1).

Result of the automated hematology analyzer of peripheral cytopenic patients in visceral leishmaniasis show that all of the patients were having total leukocyte count (TLC) less than 4000/cmm (100%). The hemoglobin level was less than 10gm/dl in 26 cases (87.7%) and more than 10gm/dl in three cases (10.3%). In case of platelets count, 27 cases (93.1%) were having platelets count less than 150000/cmm, whereas in 2 cases(6.9%) it is more than 150000/cmm.(Table.2).

Table no 01
Bone Marrow Findings In Different Age Groups Patients (n=126)

S.No	Age group in year	L.D. +ve Bone Marrow9(n=29)	L.D. -ve Bone Marrow9(n=99)
1	1-20	28(28.3%)	71(71.7%)
2	21-40	01(5.9%)	16(94.1%)
3	41-60	--	08(100%)
4	>60	--	02(100%)

Table No 02
Hematological parameters in visceral leishmaniasis patients (n=29)

S. No	Hematoliogical Parameters	No of Cases	Number of Abnormal Cases/%age	Number of Normal Cases/%age
1	Hemoglobin	29	26(89.7%)	3(10.3%)
2	Total Leukocyte count	29	29(100%)	00
3	Platelets Count	29	27(93.1%)	2(6.9%)

DISCUSSION

Visceral Leishmaniasis is a major health problem in the World and affects predominantly infants and children .According to World Health Organization (WHO) Leishmaniasis affects around two million people annually, 500,000 cases of which are visceral form. It is estimated that 350million people are exposed to the risk of infection.[14]

Unacceptable high levels in the region. Pakistan is a tropical and agricultural country with urbanized population of 35%, where as rural population is. 65% of its total population.[15]

The incidence of visceral leishmaniasis has decreased from 15.57% in 1960 to 0.45% in 1979, because of implementation of malaria eradication programme. According to World Health Organization (WHO) report following large scale anti malarial insecticide (Dichlorodiphenyltrichloroethane) (DDT) compaign implemented in 1950s, VL almost completly disappeared from the Subcontinent, but unfortunately the disease re- emeged when DDT compaign was discontinued.[16]

All cases of visceral leishmaniasis are potentially severe and life threating, especially when managed inappropriately. A major reason for progression from mild to severe disease is either missed or delayed diagnosis. Once diagnosed, the treatment is parenteral administration of anti Leishmanial drugs, in an intensive care unit.[17]

In this study of 126 cases of Peripheral Cytopenic patients it was found that 29 cases (23%) were positive for visceral leishmaniasis. A study conducted by Rahim[18] on bone marrow examination of cytopenic patients revealed 25% cases. Another observation made by Altaf et al was 42% cases.

In our study no association with HIV was noted. There are some epidemiological studies conducted in Africa and Europe having association with HIV, because of depressed immunity in VL where about 80% coinfection with Human Immunodeficiency Virus (HIV) is stated especially in adults.[18,19]

Males are more susceptible to many protozoan infections than females, field and laboratory studies link this increased male susceptibility to circulating steroid hormones. In our study males were more affected 55.2% than females 44.8% with the ratio of 1.3: 1. Male predominance is also observed in other studies conducted locally in Abbotabad,[19] Muzafarabad,[20] and Dir[18] and conducted abroad in Iraq,[21] and Iran[22] also show male predominance. While studies conducted in Yamen[23] and Brazil[24] show female dominance.

Average age of the patients who suffered from visceral leishmaniasis in this study was 4.8 years. In study conducted locally it was noted that mean ages were 2.57 and 1.7 years[19,20]. It was 2.6 years and 2.8 years respectively in studies conducted abroad.[22,23] The reason for this high incidence in under five (pre school) age may be due to developing immune system of the body.

Visceral leishmaniasis is an annual killer of over 50,000 people globally and its essential co-morbidity is cytopenias. Cytopenias due to visceral leishmaniasis is a major health problem in endemic areas, particularly for young children and pregnant women. These cytopenias are caused by excess removal on non-parasitized erythrocytes in addition to immune destruction of parasitized red cells, white blood cells and platelets, also an impaired compensation for this loss due to dysfunctioning bone marrow. Though hyperspleenism is the predominant cause of cytopenias and its complication.[23]

Leukopenia is found with variable frequencies among visceral leishmaniasis patients.In our study Leukopenia was found in 100 % cases, where as study conducted by Hamid[23] leucopenia occurred in 67% of the cases, Dhingra[27] found leucopenia in 72.22% of cases. Thus our study in term of leukocyte count is slightly different from these studies.

Thrombocytopenia was another common finding present in 65% of patients in a study conducted by Rahim et al in KPK. In our study thrombocytopenia was found in 93.1% of cases, which is also different from our study. Few other studies carried out by Zardad et al, Altaf et al and Hassan et al found thrombocytopenia in 64.06%, 83.6% and 89.9% of cases respectively. Hamid et al[23] and Haider et al[26] have reported thrombocytopenia in 56%and 64.06% of cases respectively. It is postulated that thrombocytopenia observed in peripheral blood may be due to hyperspleenism and partly due to poor platelet formation.

In our study anemia was recorded in 87.7% of the cases. In a comparative study conducted by Altaf[2] and Zardad et al[25] anemia was present in (98%) and (95.71%) patients respectively, the anemia was hypochomic and microcytic in almost all cases.

Pancytopenia is the most frequent hematological abnormality in V/L patients. Mathur et al found pancytopenia in 88% cases. The reason for the higher frequency of panctopenia is probably the long duration of symptoms and splenomegaly before presentation to the hospital and increased

peripheral destruction rather than bone marrow failure of production.Anemia, leucopenia, and thrombocytopenia were found with variable frequency among V/L patients.[28]

CONCLUSION

Visceral leishmaniasis is one of the curable disease causing significant morbidity and mortality in developing countries especially in children in the age range of one to ten years (under developed immune system) with common hematological manifestation. It is concluded that children with hematologic manifestations my be investigated for visceral leishmaniasis considering geographic and relevant clinical features. Also it is concluded that patients with cytopenia and high or normal reticulocyte count provides a useful clue towards visceral leishmaniasis in a febrile patient with hepatosplenomegaly.

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