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Visceral leishmaniasis (Kalazar) and the immune system disorder, in children at Al Muthanna, Governorate.

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Abstract:

Thirty five patients aged between 7 months-17 years were all positive for Leishmania tropica parasite, using Bios Company test kit called "Kalazar Detect Rapid Test" . Blood cell counts for leukocytes were 4 x103, lymphocytes where, neutrophils were and platelets. the cell count were done using Sysmex model KX-21 cell count, Japan (Sysmex Corporation). Using 12 micro liters blood taken from the patient and using glass slide inserted in pocket in the system. Five patients were spleenactomyzed

Introduction:

Leishmania tropica is a tropical parasite, the vector, McHugh, et al.1996 and McHugh, 1993. is sand fly and the intermediate host is the cat probably to play an active role in the disease, in contrast to goats, calves and horses who could act as accidental reservoirs of leishmania, while sheep appears to be not susceptible to experimental infection. In endemic foci for kala-azar in Sudan cows, goats and donkeys had a high prevalence of specific antibodies. Recently in Europe sporadic cases of equine leishmaniasis have been reported: L. infantum was the causative agent Feline leishmaniasis, Mancianti, 2004. Leishmaniasis, an emerging disease found in companion animals in the United States, *Leishmania* spp. are the causative agents of a spectrum of clinical diseases, all termed “leishmaniasis”. These forms vary in clinical presentation from focal cutaneous disease to disseminated visceralizing disease and in severity from non-symptomatic to fatal, Petersen 2009 and Christine, 2009.

Important diseases include tuberculosis, enteric fever and malaria are associated with leishmaniasis, because of their high prevalence in areas where VL is endemic incuriging investigator to link the disease with those infections, Taimur

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Saleem,Umair Khalid, 2010. Leishmaniasis, caused by Leishmania infantum, is an endemic zoonosis in the Mediterranean basin. Dogs are considered the major host for these parasites, as well as the main reservoir for human visceral infection' Maia et al. 2008.

Back ground:

Kala-azar is a chronic multisystemic disease characterized by fever, Hepatospleenomegaly, mild lymphadenopathy, pancytopenia, wasting and weakness, and eventual death due to bleeding or secondary infections**.** The disease is caused by Leishmania parasites, which after inoculation of the skin by sand fly vectors, replicate within macrophages in the liver, spleen, bone marrow, and, sometimes, the lymph nodes, resulting in visceral disease that is usually fatal if it is not treated**.** Pollack et al.1988, reported that, there were a combined anemia, neutropenia and thrombocytopenia in patients with visceral leishmaniasis (kala-azar).

The protozoan parasite *Leishmania donovani* is the causative agent of visceral leishmaniasis (VL), a chronic life threatening disease if untreated. In the experimental model of VL, the two main target organs are the liver and the spleen, Kay et al.1994 . While the spleen stays chronically infected, infection in the liver is self-resolving within 6-8 weeks due to the development of a Th1-dominated granulomatous response, which is characterized by high IFNγ production.

This response is induced by IL-12 secreted by Dendritic cells (DC) Engwerd et al.1998, , Gora, et al.1998 , Scharton-Kersten, et al. 1995 and this interleukin is crucial for parasite control and disease resolution in the liver .Also together with TNFα production and expression of inducible nitric oxide synthase (iNOS) by macrophages, Kaye, et al.2004 can control the infection. One of the key points in the induction of a protective immunity to *Leishmania* parasites is the generation of IFNγ -producing $CD4^+$ T-cells. Although IL-12 production by DC is crucial for the development of Th1 cells, Trinchieri , 2003.

The role of Interferon Regulatory Factor 5 (IRF-5 is responsible for the generation of Th1 responses and in the formation of Th1-type liver

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granulomas in *Leishmania donovani* infected mice. Terminal Long ReapetTLR7)-mediated activation of IRF-5 is essential for the development of Th1 responses to *L. donovani* in the spleen during chronic infection, Khalid et al. 2002.

IRF-5 deficiency leads to the incapacity to control *L. donovani* infection in the liver and to the formation of smaller granulomas. Granulomas in *Irf5*$^{-/-}$ mice are characterized by an increased IL-4 and IL-10 response and concomitant low iNOS expression. IRF-5 as a critical molecular switch for the development of Th1 immune responses following *L. donovani* infections and reveal an indirect role of IRF-5 in the regulation of iNOS expression, Andrea et al 2011..

even though IFNγ production, which is mainly derived from $CD4^+$ T-cells, was severely reduced in Leishmania Donovani infection, Engwerda,1998.

Mammalian host responses which prevent progression to clinical VL has been shown to be dependent on promoting T cell IFN-γ production-based immunity and parasiticidal activity within infected macrophages ,Chappuis et al. 2007]. A key immunological feature of T cells from dogs with late stage clinical VL is an inability to proliferate or to produce IFN- γ in response to *Leishmania* antigen, ,de Souza AI et al.2005 and Peterse.

High levels of inflammatory cytokines, including TNF-α have been proposed to stimulate production of regulatory cytokines, specifically IL-10, as a homeostatic response to prevent further inflammation-mediated pathology.

High lesional IL-10 mRNA production is frequently found in human patients with VL Nylen S, Maurya et al. 2007 Nylen S, Sacks et al.2007, and produced by polysymptomatic Foxhounds (Petersen preliminary data). One of the proposed mechanisms of IL-10 promotion of VL is by conditioning macrophages for parasite, de souza, 2005.

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Visceral leishmaniasis (VL) or 'Kalazar' is a serious parasitic infection of the reticuloendothelial system. Unless promptly managed, the disease can result in significant morbidity and mortality especially in the endemic areas, Taimur Saleem ,Umair Khalid, 2010. Jornal of Pakistan Medical Association.

Kasili, 1980 found that of 125 patients with kala azar seen in Nairobi, 90% had a haemolytic anaemia. Half of them had neutropenia and 20% had thrombocytopenia. 74% of the patients showed a low storage iron in the bone marrow and iron deficiency was diagnosed in 84% of the patients. Whereas megaloblastic changes are well known and accepted as occurring in kala azar the finding of iron deficiency was unexpected. A follow-up is proposed to pursue the etiology of the iron deficiency and study the kinetics. *H. Lehmann*. Hematological abnormalities in visceral leishmaniasis. Kasili, 1980

Methods:

Thirty five patients, 17 females and 18 males aging fro five months to 12 years in feminine and children hospital in Al Samawa city in Al Muthanna governorate.

Testing the Kalazar, infection, is using test kit called "Kalazar Detect Rapid Test". The kit was manufacture in The USA, In Bios Company. This kit will detect the anti visceral "leishmaniasis Donovani" antibodies in Human serum.

One ml blood have been taken from patient and added to 5ml plain test tube (No anticoagulant).Then Centrifuged for 3-5 min in FANEM ExcelsaII, Model 206BL, and Applied small amount of specimen to the end of the test strip was dipped in chase buffer.

Waited for ten min at room temperature then the result was recorded. Faint line will appear on the strip telling the result is (+). If the color of the strip stays clear, the result will be (-v).

Result: There was combined anemia, Neutrogena and thrombocytopenia in patients with visceral leishmaniasis (Kaalazar). Using Sysmex model KX-21, Japan (Sysmex Corporation). Treatment with sodium stibogluconate raised the patients'

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platelet, neutrophils and erythrocyte count to make CBC normal due to controlling the parasite.

Due to hyper spleenism, bone marrow suppression was suspected. it is suggested that pancytopenia resulted from rapid destruction of antibody-coated blood cells. Thirty five patients were positive for Kalazar test using the above test kit. Patients were from Al Muthanna governorate in The feminine and children hospital in Al Samawa city. Results showed that WBC were 24 patients were low out of 35 patients, platelets were low in all patients, Lymphocytes one patient were low and the remaining were normal, and Neutrophils, were six high in six patients and the remainder were normal according to table-1. Hepatospleenomegaly were done in all patients. Blood count was done at time of admission.

Table-1: Visceral leishmaniasis test and their leukocytes, platelets, lymphocytes and neutrophils counts in all patients showing Hepatospleenomegaly (HSM) and leishmaniasis positive .

#	SEX	AGE	HB*		PLT***	T+	LYM%	NUT%	H+
1	M	13M	7.5		150---L		48%N	40---N	
2	F	3YRS	8		100---L		42---N	48---N	
3	F	14M	9.9		180---L		38---N	45---N	
4	M	9M	9.9		176---L		38---N	50---N	
5	F	1YR	5.9		100---L		48---N	48---N	
6	F	14M	8		150---L		45---N	47---N	
7	F	1YR	9		120---L		43---N	48---N	
8	F	10M	8		140---L		39---N	50---N	
9	F	7M	7		120---L		43----N	39---N	
10	F	14M	7		100---L		43---N	48---N	
11	F	1YR	7.5		90-----L		40---N	48---N	
12	M	7M	7.5		120--L		40---N	42---N	
13	M	12M	8		120---L		39---N	50--N	
14	F	15M	8		100---L		45---N	40---N	
15	F	14M	7.5		90-----L		38---N	50--N	
16	F	8M	6.5		163---L		45.6-N	22.2-N	
17	M	7M	7.5		82----L		40----N	50---N	
18	M	12M	8.2		50----L		64.5-N	29.7..N	
19	M	5YRS	9.4		36-----L		38---N	55---N	
20	M	19M	8		70----L		35---N	55--H	
21	M	2YRS	8		100---L		34---N	50--N	
22	M	11M	8.7		101---L		40---N	55---H	
23	M	18M	6.7		50----L		35---N	54---N	
24	F	7M	8.9		121---L		35----N	56---H	

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25	M	12YRS	8		60---L		38----N	55---N	
26	M	14M	7		90-----L		35---N	57---H	
27	M	18M	7.9		100---L		40---N	50---N	
28	M	2YRS	7.5		105---L		38---N	53---N	
29	M	5M	8		122---L		36--L	57---H	
30	F	1YR	6.4		77---L		40---N	50---N	
31	M	18M	6.1		185--L		38---N	55---H	
32	M	11M	14		106---L		38---N	50---N	
33	F	13M	9		150---L		40---N	53---N	
34	F	5YRS	9		130---L		45---N	57---N	
35	F	2YRS	9.4		55---L		40---N	54--N	

*g/dl, **WBC: x10^{3}cell/µl, L: low, H: high and N: normal***platelets x10^{3}cells/µl,HSM:Hepato SpleenoMegaly, #:Count per age: see tabl-2

The lowest count in patients aged between 7months and 12 years were16 patients with total WBC were ≤ 3.5x10^3/cc. The highest count was in one patient which was 10.6x10^3/cc. The rest of the patients were in between.

T*: Test, H*: Hepatospleenomegaly

The lowest count in patients aged between 7months and 12 years were16 patients with total WBC were ≤ 3.5x10^3/cc. The highest count was in one patient which was, 10.6x10^3/cc. The rest of the patients were in between.

Table-2: Normal blood count, Leukocytes, platelets, Lymphocytes and Neutrophils values according to the age.

Leukocytes (white blood cells)	Thousands of cells per microliter
To 8 days	9.0 - 18.4
To 12 months	7.3 - 16.6
1-2 years	3.6 - 17.0
3-5 years	4.9 - 12.9

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6-7 years	4.4 - 10.6
8-16 years	3.9 - 9.9
Over 16 years	
Platelets	Thousands of cells per microliter
To 5 years	217 - 533
6-10 years	181 - 521
11-16 years	154 - 452
Over 16 years	150 - 440
Neutrophils	Percentage
To 8 days	24 - 51
To 12 months	16 - 50
To 2 years	18 - 54
To 3 years	21 - 60
To 4 years	24 - 65
4-9 years	32 - 64
10-14 years	35 - 65
15-16 years	37 - 65
Over 17 years	30 - 70
Lymphocytes	Percentage
To 8 days	32 - 62
To 12 months	38 - 73

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To 2 years	34 - 72
To 3 years	29 - 66
To 4 years	25 - 63
4-16 years	25 - 55
Over 17 years	25 - 40

Discussion:

From table-1 above all patients experience low in platelet count (thrombocytopenia) and one patient showed low lymphocyte count. Neutrophils high in six patients and low White Blood cell Count (WBC) in 24 patients.

Those results which explain any insult of the cell due to leishmania infection may be cytopenia of immuno-related pancytopenia IRP and some patients caused by the qualitative abnormality of the hematopoietic stem cells by the destruction or suppression of hematopoietic stem cells from certain extrinsic insults such as low hemoglobin, or anti cell antibodies.

The imbalance of the lymphocytes subtypes and over function of Th2 lymphocytes played important roles in the pathogenesis mechanism of IRP leading to increased and over functional B lymphocytes, which produced autoantibody destructing or suppressing hematopoietic in IRP, Zhonghua, et al.2004.

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