Hematological changes including the immune system in patients with visceral Leishmaniasis at Al-Muthanna Governorate

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الخلاصة :

تغيرات كريات الدم والصفائح الدموية مع الجهاز المناعي عند الأطفال المصابين باللشمانية الحشوية في محافظة المثنى

تمت الدراسة على خمسة وثلاثون مريضا تتراوح أعمارهم من سبعة أشهر واثنا عشر سنة , وكان عدد الذكور ثمانية عشر وعدد الإناث سبعة عشر . لوحظ أثناء الدراسة أن أربعة وعشرون مريضا لديهم كريات الدم البيضاء اقل من الطبيعي , وإحدى عشر مريضا كانت كريات الدم البيضاء طبيعية , وكانت نسبة كريات الدم البيضاء المتعددة النواة طبيعية في تسعة وعشرون مريضا ومرتفعة في ستة مرضى . اما الصفائح الدموية كانت منخفضة في كافة المرضى . المسبب الرئيسي لمرض اللشمانية الحشوية هو طفيلي يصيب كريات الدم البيضاء الأحادية النواة (مكروفيج) وكان المرضى يعانون من اللشمانية الحشوية كما هي مشخصة فيهم بسبب خلال في وظائف الجهاز المناعي وظهرت الإعراض في هؤلاء المرضى بسبب خلل في تركيبة الجهاز المناعي الطبيعي والممثل في كريات الدم البيضاء الأحادية النواة والموجودة في الجلد ايضا وكذالك في الجهاز المناعي المتأقلم والممثل في الخلايا المناعية التائية والبائية . ان الهدف الرئيسي من الدراسة كان لايضاح نظرية تأثير الجهاز المناعية على اللشمانية الحشوية

Abstract:

Thirty five patients aged between seven months and twelve years. Eighteen Males and seventeen females were the subject of the study on Leishmaniasis. Twenty four patients had low white blood cell count (WBC) 11 patients were normal. Neutrophils were normal in 29 patients and higher in 6 patients.

Platelets counts were low $36x10^3$-$180x10^3$ in all patients. Lymphocytes counts were 64.5% which is normal compared to the average (30-65.9%).

Introduction:

The causative agent of human visceral leishmaniasis is an obligate intracellular parasite, living in the phagolysosomes of macrophages. Patients suffering from visceral leishmaniasis are characterized by aberrant immune functions (Richard et al.2009). Immunity to leishmaniasis is mediated by both arms of mammalian cellular immune system; innate (by Neutrophils, macrophages, and dendritic cells) and adaptive (T cells) responses, Scott 1989).

Blood was studded to Count Blood Cell (CBC), like Hemoglobin(HB), White Blood Cell (WBC), Lymphocytes, Neutrophils and platelets and compared to normal count fallowed by using the internet web sites and pediatrics text book like (Nelson 2011).

Leishmania donovani is transmitted by the sand fly vector. The parasite will infect macrophage in liver, spleen, bone marrow and some time lymph node resulted in visceral leishmaniasis (Lukasz 2010)

Patients show marked depressed cellular immune response, as demonstrated by the absence of delayed type hypersensitivity reactions to Leishmania antigen (Manson-Bahr,1961 and Rezai, 1978). Also the inability of peripheral blood mononuclear cells (PBMC) to respond to antigen by either lymphoproliferation or cytokine production (Cillari et al. 1988).

Macrophages are the only cells that in vivo allow the growth of the intracellular pathogen Leishmania. They also can present of parasite Ag to CD4+ T lymphocytes known to be involved in protective and counterprotective immune responses, (Prina et al.1996, Behin et al.1979, Handman et al. 1979, and Nacy et al.1983) parasites in macrophage cause production of gamma interferon (IFN--y) by parasite-specific T cells (Sadick,et al.1986).

The Visceral leishmaniasis is acute and resolved if liver is infected , but is chronic during spleen infection, so it is characterized by Spleenomegaly and parasite persistence (Engwerda, et al.2004), Neutrophils, platelets and lymphocytes are all involved in the pathogenesis of Leishmania donovani, (Michael et al.2008).

The infection will be resolved within 6-8 weeks, due to the development of Th1 then granuloma response will occurs, which is caused by production of IFN-gamma (Engwerd et al. 1998).

Furthermore, this response is induced by interleukin-12 secreted by Dendritic cells(DC) ,(Engwerd et al. 1998, Gorak et al.1998, Scharton-Kersten et al.1995), InterLeukin-2 (IL-2) has resolution in liver together with Tumor necrosis factor(TNF)-& production and expression with the nitric oxide synthase (iNOS) by macrophage which control the infection with *Leishmania donovani* (Kaye et al.2004). Researchers pointed that, Interferon IFNλ production is secreted by Th4 (CD4) T cell and severely reduced in *Leishmania donovani* infection.

High level of inflammatory cytokines including TNF-α will stimulate other cytokines such as IL-10 as homoeostatic response to overcome further inflammatory response and high level of IL-10 mRNA is found in human suffering from visceral leishmaniasis (Nylen, 2007, and Nylen et al.2007).

Objectives:

The objective of the study is to investigate the hypothesis of the immune system influences on visceral leishmaniasis.

Material and methods:

The period of the study was conducted in September 2009 to September 2010. in Al Muthanna Feminine and children Hospitals.The project was designed as follow:

Patients:

Thirty five patients were chosen to be the subject of the study. They were17 females and 18 males aging from seven months to twelve years at Al Muthana Feminine and children Hospital for diagnosing Visceral leishmaniasis (Kala-azar).

All patients come from the surrounding rural area of Al Muthanna Governorate. All families breeding animals like cats, dogs and chicken. All of patients were presented with Hepatospleenomegaly and only five were splenectomized.

Samples for hematology studies:

Blood samples for complete blood count (CBC) like Hemoglobin, White Blood Cells, Platelets, Neutrophils and Lymphocytes were collected in heparinized tube. five ml sterilized tube and the counting done in Sysmex system model KX-21 cell count Japan (Sysmex corporation) using two cubic centimeters of blood, then two micoliters were injected in slide covered with cover slip then the slide was inserted in a pocket located in the system (Langford 2003, Chang 2011.)

Immunology:

Testing Kala-azar, infection or leishmaniasis, by using test kit called "Kala-azar Detect Rapid Test". The kit was manufactured in the USA, Bios Company. It detects the anti-visceral "leishmania Donovani" antibodies in Human serum, (Ryan 2008, Sundar, 2006 and Goswam 2003)

One ml blood have been taken from patient and added to 5ml plain test tube. Then Centrifuged for 3-5 min in FANEM ExcelsaII, Model 206BL, at 12000rpm (Ryan 2008). Half ml (0.5ml)of patients serum were added to the end of the test strip then dipped in chasing buffer , waiting for ten min at room temperature, then result were recorded as a color change. Faint line will appear on the strip telling the result is positive. If the color of the strip stays clear or different color like faint pink, the result will be negative.

Statistical analysis.

Statistical analysis fallowed using t test Quick calculator program by using patients age as a variable.Group-1 included age one year and below, group-2, included more than one year. Using T test value in $P>0.95$ .The data showed that only platelet low in the count contributing to the symptoms of visceral leishmaniasis because there is significant difference between t test and categorized blood cell. The other significance is Hemoglobin concentration among patients.

The two-tailed P value equals 0.0367 for Platelates which tell it is significant

By conventional criteria and the value is ($t = 2.1769$) and for hemoglobin the t test value was 0.3129, but with the rest of patients cells t values were less than 3 which make the significant was rejected.

Results:

Results Showed the lowest count in patients aged between 7 month and 12 years were 16 patients have total WBC less or equal to 3.5x103/cc.The highest count was in one patient which was 10.6x103/cc.Rest of the patients were in between. Platelets count was low in all patients, lymphocytes all of them were normal except one patients was low, and Neutrophils were normal in 29 patients and 6 patients were low in Hemoglobin values the results showed 5 patients were anemic(below 7g/dl)

All patients were positive for Kala-Azar test, using the above test kit. And all of then had hepatospleenomegaly.

Table-1: Patients aged group-1, one year and below explaining HB level and cell count like white blood cells (WBC), Platelet (PLTT), lymphocytes and Neutrophils.

#	age/m	Sex	HB, g/dl	WBC	PLTT	Lymph.	Neut.
01-	09	M	10	3	176	38%	50%
02-	12	F	06	3	100	48%	48%
03-	12	F	09	4	120	43%	48%
04-	10	F	08	4	140	39%	50%
05-	12	F	07	4	120	43%	39%
06-	12	F	08	3	90	40%	48%
07-	07	M	08	4	120	40%	42%
08-	12	M	08	4	120	39%	50%
09-	12	M	08	4	120	39%	50%
10-	08	F	07	3	163	46%	22%
11-	07	M	08	5	82	40%	50%
12-	12	M	08	3	50	65%	30%
13-	11	M	09	8	100	40%	55%
14-	07	F	09	3	121	35%	56%
15-	05	M	08	4	122	36%	57%
16-	12	F	06	11	77	40%	50%
17-	11	M	14	05	106	38%	50%

Table-2: Patients age more than one year. It explains the innate immune parameters were white blood cells x 1000cell count, Neutrophils and platelets x 1000 cell count.

#	Age	Sex	HB	WBC	PlT.	Lymph.	Neut
1-	1.3y	M	8	4	150	48%	40%
2-	3y	F	8	3	100	42%	48%
3-	1.4y	F	10	3	180	38%	45%
4-	1.4	F	8	8	150	45%	47%
5-	1.4	F	7	3	100	43%	48%
6-	1.4	F	8	3	90	38%	50%
7-	1.9	M	8	3	70	36%	55%
8-	2	M	8	4	100	34%	50%
9-	1.8y	M	7	6	50	35%	54%
10-	12	M	8	3	60	38%	55%
11-	1.4	M	7	3	90	35%	57%
12-	1.8	M	8	10	100	40%	50%
13-	2	M	8	4	105	36%	53%
14-	1.8	M	6	5	185	58%	55%
15-	1.3	F	9	4	150	49%	53%
16-	5	F	9	4	130	45%	57%
17-	2	F	9	9	55.Re	40%	54%

Tabel-3: Showed the lowest count in patients aged between 7 month and 12 years were 16 patients have total WBC less or equal to 3.5x103/cc.
The highest count was in one patient which was 10.6x103/cc.Rest of the patients were in between. Platelets count was low in all patients, lymphocytes all of them were normal except one patients was low, and Neutrophils were normal in 29 patients and 6 patients were high. In Hemoglobin values the results showed 5 patients were anemic(below 7g/dl).

#	SEX	AGE	HB	WBC	PLT	LYM%	NUT%
1	M	13M	7.5	4X----N	150---L	48%N	40---N
2	F	3YRS	8	3------L	100---L	42---N	48---N
3	F	14M	9.9	3------L	180---L	38---N	45---N
4	M	9M	9.9	3------L	176---L	38---N	50---N
5	F	1YR	5.9	3------L	100---L	48---N	48---N
6	F	14M	8	8------N	150---L	45---N	47---N
7	F	1YR	9	4-----L	120---L	43---N	48---N
8	F	10M	8	4-----L	140---L	39---N	50---N
9	F	7M	7	4----L	120---L	43----N	39---N
10	F	14M	7	3----L	100---L	43---N	48---N
11	F	1YR	7.5	3----L	90-----L	40---N	48---N
12	M	7M	7.5	3.5--L	120--L	40---N	42---N
13	M	12M	8	4----L	120---L	39---N	50--N
14	F	15M	8	3----L	100---L	45---N	40---N
15	F	14M	7.5	3----L	90-----L	38---N	50--N
16	F	8M	6.5	3.2---L	163---L	45.6-N	22.2-N
17	M	7M	7.5	5----L	82----L	40----N	50---N
18	M	12M	8.2	2.4--L	50----L	64.5-N	29.7..N
19	M	5YRS	9.4	4.2---L	36-----L	38---N	55---N
20	M	19M	8	3------L	70----L	35---N	55--H
21	M	2YRS	8	4-----N	100---L	34---N	50--N
22	M	11M	8.7	8----N	101---L	40---N	55---H
23	M	18M	6.7	5.4--N	50----L	35---N	54---N
24	F	7M	8.9	3.2----L	121---L	35----N	56---H
25	M	12YRS	8	3-----L	60---L	38----N	55---N
26	M	14M	7	3-----L	90-----L	35---N	57---H
27	M	18M	7.9	9.9---N	100---L	40---N	50---N
28	M	2YRS	7.5	3.5---L	105---L	38---N	53---N
29	M	5M	8	4----L	122---L	36--L	57---H
30	F	1YR	6.4	10.6--N	77---L	40---N	50---N
31	M	18M	6.1	5.4---N	185--L	38---N	55---H
32	M	11M	14	5----L	106---L	38---N	50---N
33	F	13M	9	4----N	150---L	40---N	53---N
34	F	5YRS	9	5----N	130---L	45---N	57---N
35	F	2YRS	9.4	4.7---N	55---L	40---N	54--N

Table-4: Normal blood count, Leukocytes, platelets, Lymphocytes and Neutrophils values according to the age.

(white blood cells/age group)	Thousands of cells per cubic micoliters
To 12 months	7.3 - 16.6
1-2 years	3.6 - 17.0
3-5 years	4.9 - 12.9
6-7 years	4.4 - 10.6
8-16 years	3.9 - 9.9
The average count is	$4.8\text{-}13.7x10^3$
Platelets	Thousands of cells per microliter
To 5 years	217 - 533
6-10 years	181 - 521
11-16 years	154 - 452
Average	$184\text{-}502x10^3$
Neutrophils	Percentage
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
Average is	24.1-58.2%
Lymphocytes	Percentage
To 12 months	38 - 73
To 2 years	34 - 72
To 3 years	29 - 66
To 4 years	25 - 63
4-16 years	25 - 55
Average is	30-65.9

Discussion:

Results claimed that there is no significant in White Blood Cells, Neutrophils and Lymphocytes but the significance in hemoglobin and platelets count. This mean that, platelets suppress d level causing anemia marrow or cause pan cytopenia and hemoglobin decreased also but when hemoglobin increased the reason was due to blood transfusion.

Neutrophil when engraftment during blood transfusion was as expected to suffering from blood transfusion problem make high or low cell count. And suffering from severe pancytopenia. Antibodies against red cells, platelets, lymphocytes and granulocytes were detected in extremely high titers, (Knop et al.2004). Immune-mediated pancytopenia was refractory on multiple immunosuppressive treatment strategies. Proliferation of polyclonal plasma cells of recipient-type that was documented postmortem, was most likely responsible for excessive antibody formation of is due to drug cause immune suppression and the parasite also cause bone marrow supresin (Pancytopenia),their reason of fluctuation to low and high count.

The membrane-associated antiplatelet, antineutrophil and antierythrocytic IgG antibodies seen in visceral leishmaniasis, (Pollack, 1988)

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

Pancytopenia is a reduction in the number of RBCs, WBCs, and platelets in the peripheral blood below the lower limits of the age-adjusted normal range for healthy people. It is therefore the combination of anemia, leucopenia, and thrombocytopenia. It may result from decreased production of blood cells or bone marrow failure, or from their immune-mediated destruction or non-immune-mediated sequestration in the periphery.

In our results lymphocytes were in normal count, and the fact said that lymphocytes has to be in a state of lymphopnia but the result showed the opposite because researcher found that infection with Leishmania will activate T cell then their number will be higher due to the activation due to lipoprotein antigen. , proliferative response of T cells, was mainly due lo activation of Cn2-posilive,T cells (Kemp et al 1991).

Spleen plays an important role in homeostasis of the body by modifying any functional correction especially in spleen concerning disease status. Also acts as a filter through which all blood passes(Kraal et al.,2006). This is why patients when their spleen is splenectomized they will suffer from sever infection due to the loss of active immune system.

Some patients treated by blood transfusion that will make Blood count close to normal , and this is the cause of blood cell count fluctuated between low and high conut.

. As homeostatic mechanism of spleen to control persistent infection induced inflammation. Elevated levels of the regulatory cytokine interleukin (IL)-10 have been reported repeatedly in clinical studies of visceral leishmaniasis. **(**Susanne Nylén,

David Sacks, 2007)**.** Hyper spleenism causes bone marrow suppression. It is suggested that Pancytopenia resulted from rapid destruction of antibody coated blood cells.

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