

Dept. of Biochemistry,
Faculty of Medicine, Assiut University,
Head of Dept. Prof. Dr. S.A. Ghaneimah.

**IMMUNOHAEMATOLOGICAL MANIFESTATIONS OF CUTANEOUS
LEISHMANIASIS IN AL KHARJ AREA, SAUDI ARABIA KINGDOM**
(With 7 Tables & 2 Figs.)

By

**TAHIA H. SALEEM; SOAD M. ABDEL GHANY; S.A. HANTIRAH*
and S.A. AHMED****
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المظاهر الدموية المناعية التي تحدث في
مرضى الليشمانيا الجلدية في منطقة الخرج بالملكة العربية
السعودية

تحية هاشم ، سعاد عبد الغني ، سمير حنقيره ، شرف أحمد

في محاولة للتقصي عن التغيرات التي تطرأ على مكونات الدم وكشف أي اضطراب يمكن أن يحدث في مرضى الليشمانيا الجلدية المزمنة والشديدة ، تم تقدير مستوي هيموجلوبين الدم ، عد دم أبيض كلي ونوعي ، عد للصفائح الدموية ، مستويات البوليما ، البروتين الكلي نسبة الألبومين والجلوبولين في مصل دم ١٠٠ مريض في مستشفى الملك خالد بالملكة العربية السعودية ويعانون من مرض الليشمانيا الجلدية . وقد لوحظ وجود أنيميا غير حادة ، زيادة ذات دلالة احصائية في عدد كرات الدم البيضاء نتيجة الزيادة في الخلايا المتعادلة ، نقص جوهرى في الصفائح الدموية والألبومين مع زيادة جوهرية في الجاما جلوبيولين في هؤلاء المرضى بالمقارنة بالاصحاء ويمكن أن يعزى هذا التغير في خلايا الدم التي تنضم الطحالب التعويضي خاصة في المرضى الذين ليس لديهم مناعة ويعانون مرضا مزمنًا بدون علاج وقد أرجعت الزيادة في كرات الدم البيضاء الى وجود عدوي بكثيرة مصاحبة للأماكن المصابة . وبالإضافة لذلك تم تقدير مستويات أحجام المناعة IgG , IgM , IgA , IgE في ٥٠ مريض (٢٢ مضي ، ٢٨ هندي) من المرضى السابقين وذلك لتقييم حالتهم المناعية ووجد أن مستويات أجسام المناعة الثلاثة IgG , IgM , IgE قد زادت زيادة جوهرية بالمقارنة بالمجموعة الضابطة . وقد فسرت هذه التغيرات بانها استجابة طبيعية للجسم نحو الاصابة بالمرض ولذلك فلا يمكن اعتبار هذه الأجسام المناعية مؤشرا تشخيصيا للتعرف على هؤلاء المرضى . ولكنها بالإضافة الى دلائل الدم الأخرى فانها يمكن أن تفيد في مراقبة استجابة المرضي للعلاج أو التنبؤ بانتكاس الحالات التي تم شفاؤها أو تطورها الي أنماط أخرى .

* Dept. Dermatology & Venereology, King Khalid Hospital, Al Kharj, Kingdom of Saudi Arabia.

** Dept. of Microbiology, Juba University, Sudan.

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SUMMARY

Haemoglobin levels, total and differential white blood cells count, platelets count, blood urea, total plasma protein and percentages of serum albumin and globulins were determined in 100 patients with cutaneous leishmaniasis in an attempt to investigate their haematological status and detect any abnormality that could occur in those patients with chronic severe course of the disease.

Mild anaemia, leukocytosis with significant increase of neutrophils (81%), thrombocytopenia, hypoalbuminaemia together with hypergammaglobulinaemia were detected in these patients. It appears that the compensatory hyperplasia of the spleen in non immune persons with chronic severe untreated cases is the possible factor which might influence the blood cells. Leukocytosis is contributed to the concurrent sepsis of the lesions.

In addition, serum immunoglobulins IgA, IgG, IgM and IgE levels were determined in 22 Egyptians and 28 Indians selected from the patient populations to assess their immune system.

IgG, IgM and IgE levels were found to be significantly increased ($P < 0.001$, for each). These changes could be explained as the body's physiological immune response to chronic infection. So, Immunoglobulins did not have any clinical relevance as diagnostic markers for screening these patients, which is mainly due to their low specificity, but it may provide important information-in addition to blood studies - in monitoring response to therapy and predicting relapse of the cured cases or its development into other forms.

INTRODUCTION

Cutaneous leishmaniasis (C.L.) is a disease of significant morbidity caused by leishmania tropica (Protozoa) in India, Middle East, Mediterranean basin and West Central Africa (SCHMIDT and ROBERTS, 1989). It is transmitted by bites of infected female sandflies (Phlebotomus sp.) (CHEESBROUGH, 1987).

Old world cutaneous leishmaniasis is also referred to as oriental sore and local names are used as Delhi boil, Aleppo boil or Jericho boil to describe the disease in different parts of the world (CHEESBROUGH, 1987; SCHMIDT and ROBERTS, 1989).

Current reports indicate that in a number of areas, C.L. has become hyperendemic (GRIFFITHS, 1987) and Arabia was considered one of these endemic areas (MANSON, 1898).

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It was found that leishmania tropica infection protects against reinfection with C.L. suggesting permanent immunity (MOSCHELLA, 1988). Consequently, some native people deliberately inoculate their children on a part of their body normally hidden by clothes, which prevents their later developing a disfiguring scar on an exposed part of the body (SCHMIDT and ROBERTS, 1989).

The present study was performed on patients suffering from C.L. from Al Kharj area (80 kilometers south of Riyadh) in order to investigate the immunohaematological changes and quantitate the separate immunoglobulin classes in this disease and check their importance as a diagnostic tool.

MATERIAL and METHODS

One hundred male adult patients with different age groups (20-50 years), diagnosed clinically as having C.L., in King Khalid Hospital, Al Kharj, were included in this study. They were 11 Saudi, 49 Indians, 22 Egyptians and 18 Sudanese.

Males are more often infected than females, this may be due to the more exposure to sandflies because of their profession as farm labourers. This is in accord with that reported by SCHMIDT and ROBERTS (1989).

Evaluation was done for all patients including full history, thorough clinical examination, local examination focused on the total number of lesions, sites affected, severity, duration of illness, consistency of lesions, evidence of secondary infection as oozing, bleeding, lymphadenopathy or necrosis.

The cleaned edges of the lesions were scraped using a sharp sterile blade and the smears were stained by Giemsa's stain. Fine needle aspiration (FNAB) was taken from under the margin of the ulcer using saline aspirates and sterile disposable plastic syringes. The aspirated material was inoculated into N.N.N. (Novy-Nicolle-Mc Neel) medium in addition to its direct examination microscopically after Giemsa staining. Swabs were taken for culture and sensitivity for all lesions with clinical evidence of secondary bacterial infection.

Patients and authorities consent was taken for the study.

Any patient with any associated illness or any concomitant parasite infestation was excluded from the study.

The clinical findings of the patients are shown in table (1).

In addition, 50 normal healthy male subjects were included in the study as the control group. They were comparable with the patients regarding age, nationality, profession and socioeconomic state. They proved to be free from any illness.

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Blood samples were obtained from both the patients and controls, sera were separated and immediately stored deeply frozen.

Both the patients (100) and controls (50) were concurrently subjected for analysis of their haemoglobin levels and total white cell count by Coulter Counter, Bedfordshire, England, differential WBCs count by routine laboratory investigation and platelet count using Thrombo Counter-C, Bedfordshire, England.

Total serum protein were estimated by Miller's modification (1959) of the Lowry method (LOWRY, et al. 1951), serum protein electrophoresis was carried out using Helena electrophoresis machine according to HENRY, et al. (1974) and blood urea was done according to CHANEY and MARBACH (1962).

The serum immunoglobulins IgA, IgG, IgM and IgE were determined in 22 Egyptian and 28 Indian male patients compared to 11 Egyptian and 14 Indian male controls using M-partigen single radial immunodiffusion plates supplied by Behring werke Diagnostics-West Germany.

RESULTS

Table II, shows the frequency of occurrence of C.L. in Al Kharj area. It is evident from the table that the patients often seek advice from October to December and from January to March.

Table III, presents the distribution of patients population, age wise. The susceptible age being from 20-40 y.

Tables (IV) and (V) illustrate the haematological changes obtained in our patients in relation to the controls. The results revealed a significant increase in total leukocytic count ($P = 0.001$) with the higher levels for neutrophils (81%), significant decrease in platelet count and Haemoglobin levels ($P = 0.001$ each). The electrophoretic patterns of serum proteins showed a significant increase in both alpha 2 ($P = 0.001$), beta globulins ($P = 0.01$), gamma globulins ($P = 0.001$) together with a significant decrease in serum albumin ($P = 0.001$) with no alteration of total protein (Data not illustrated).

Tables (VI) and (VII) show the levels of IgA, IgG, IgM and IgE in patients in comparison to the controls. The results showed a significant increase in both IgG, IgM and IgE classes ($P = 0.001$ each) which was observed in Egyptian as well as Indian patients. No alteration in IgA was detected.

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DISCUSSION

In this study, we demonstrated marked thrombocytopenia ($P < 0.001$), also mild normochromic anaemia in our patients which is reflected mainly in a significant decrease in the haemoglobin levels ($P < 0.001$). No abnormal RBCs were seen. These changes could be explained by the chronicity of the disease (about 76% of cases had 2-6 months and 24% between 6-24 months duration) and splenic hyperplasia, due to the compensatory production of macrophages and other phagocytes which are so important in defending the parasite. Also, the liver and bone marrow may be affected rendering the blood cell production and sequestration greatly depressed.

In non immune persons (when cell mediated immunity is defective) with secondary infection, the parasites spread in cutaneous tissues by way of blood or lymphatics (direct extension) (CHEESBROUGH, 1987) and there they multiply in the reticuloendothelial system and lymphoid cells of the skin (SCHMIDT and ROBERTS, 1989).

GOLDSMITH (1983) reported that the distinctions between visceral, cutaneous and mucocutaneous are not rigid, because in the course of illness, one type may develop into another and leishmaniasis that normally are visceral may become dermal (SCHMIDT and ROBERTS, 1989). So, there may be a metastatic extension for the disease.

The marked leukocytosis that observed in our patients is not the case for other reports where leukopenia is found to be marked (REES and KABER, 1987). Leukocytosis may be attributed to the concurrent sepsis due to secondary bacterial infection which occur in more than 90% of our patients & proved by the presence of high percentage of neutrophils (81%). Staph aureus was the predominant organism that was isolated from the patients lesions.

Blood urea is not affected and non of the patients had impaired renal function (Data not illustrated).

The electrophoretic profile of serum protein revealed a significant increase in both alpha 2 ($P < 0.001$), beta globulin ($P < 0.01$), gamma globulin ($P < 0.001$) together with a significant decrease in albumin ($P < 0.01$). No alteration of the total serum protein was observed.

Hypergammaglobulinaemia is due to non specific immune response and this was supported by the finding that serum values of IgG, IgM and IgE was significantly increased in these patients when compared to controls ($P < 0.001$ for all) whereas IgA value was not different from that of controls.

FARAH (1979) indicated that *L.tropica* infestation in mice and guinea pigs elicited antibodies against the infesting parasites whose levels or class did not correlate with the stage or extent of the disease.

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ADLER (1965) reported that the infection and lesions in the human, persist despite the immune response which is not able to eradicate the existing infection immediately but it does prevent reinfection in the majority of cases. This was contributed to the immune response induced by these agents which was characterized by little antibody but strong cell mediated immunity. According to FARAH, et al. (1975) and CHEESBROUGH (1987) macrophages of the skin appears to play a central role in leishmanial infections. It is a natural habitat of the parasite (amastigote) in which it grows and multiplies, perhaps protected by its intra cellular status from the effects of the immune response. Not only so, but the macrophages also possess soluble antigens from the parasite and by presenting these antigens on its surface membranes, it may aid in the stimulation of B and T cells involved in the development of the immune response.

IgG immunoglobulins are formed particularly in response to soluble antigens as toxins, bacterial or protozoal products (WHITBY, et al. 1988). Following an antigenic stimulus often in tropical diseases IgM formation usually precedes IgG formation, thus, IgM is considered to be an early defence mechanism against the intravascular spread of infecting organisms (WHITBY, et al. 1988), whereas, IgA may form part of the defence mechanism against local viral and bacterial infections.

On the other hand, IgE includes the reagins which bind to cells as the mast cells. In the presence of antigen, one result of the antigen-antibody reaction is the release of histamine and other amines and polypeptides from the cell giving rise to a local hypersensitivity reaction (WHITBY, et al. 1988).

So, the increase in IgG, IgM and IgE of our patients is a part of the body's physiological response to infection especially in chronic infection as the case of our patients who were suffering from L.tropica for a long period.

The patients, immune response to the infection determines chiefly the form taken by the clinical disease. If the patient mounts an adequate but not excessive cell mediated immune response to the parasite i.e. the ulcer penetrated by macrophages, plasma cells and lymphocytes, usually resulting in a reducing of the parasite numbers, healing of the ulcerative lesions and specific protection result (HEYNEMAN and MACKERROM, 1987).

The overdeveloped cell mediated immune response produces L.recidivans caused by L.tropica in which no ulcerated lymphoid nodules form at the edge of the primary lesion. These lesions persist indefinitely although the parasites are not easily demonstrated. Thus a spectrum of host responses to C.L. exists ranging from multiple disseminated parasite filled ulcers with diminished reactivity to the host, (anergic response), to single spontaneously cured immunizing sores, to L.recidivans hyperactive host responses with few or no parasites (allergic response).

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ZUCKERMAN (1975) stated that the parasite may be responsible for depressing cell mediated immunity locally and generally in the presence of heavy parasite loads as in diffuse C.L. and Kala-azar. The suppression of lymphocyte activity seems to be species specific following the same pattern of relationship as between the parasite and the macrophage.

In conclusion, this study provides some informations about the immunohaematological abnormalities that could occur in patients with chronic severe C.L. The study provides also an evidence that immuno-globulins quantitation is not a reliable parameter for screening these patients due to its low specificity but it might with the haematological parameters, be of special interest in monitoring response to therapy and predicting relapses (CHEESBROUGH, 1987) of the cured cases by other *Leishmania* species or sub species, or its development into other forms.

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Table (1): Clinical Data of Patients suffering from Cutaneous Leishmaniasis.

	Percentage of patients
1- Nationality	11% Saudi, 89% Indians, Egyptians & Sudanese.
2- Profession	87% farm labourers.
3- Residence	92% from agricultural rural areas
4- Severity of lesion	92% severe & 8% moderate
5- Site of lesion	100% extremities.
6- Duration of illness	76% between 2-6 months & 24% between 6-24 months.
7- Culture of aspirate in N.N.N.	100% (50 positive patients selected from 100 patients studied).
8- FNAB direct exam.	88% positive.
9- Direct exam. of skin scraping	73% positive.
10- Organisms isolated	Staph. aureus 42%, Staph. epidermatis 22%, mixed growth 29% & the remaining are sterile.

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Table (II): Frequency of occurrence of C.L. in KKH, Al-Kharj.

Period from	Total Patients Seen		Patients with C.L. Seen	
	M	F	M	F
October-December 1988	782(62.31%)	473(37.69%)	133(79.16%)	35(20.84%)
January-March 1989	674(53.58%)	584(46.42%)	141(78.33%)	39(21.67%)
April -June 1989	238(64.85%)	129(35.15%)	38(79.17%)	10(20.83%)
July -September 1989	575(61.04%)	367(38.96%)	73(79.35%)	19(20.65%)
October-December 1989	1082(63.09%)	633(36.91%)	290(79.67%)	74(20.33%)
Total	3351	2186	675	177

Table (III): Age Distribution.

Age	12-20 Y	21-30 Y	31-40 Y	41-50 Y	51-60 Y	↑60 Y	Total
No. of patients	9	38	42	9	-	2	100
Percentage	9%	38%	42%	9%	-	2%	100%

Table (V): Percentages (mean \pm SD) of serum albumin, alpha 1, alpha 2 globulins, beta and gamma globulins as obtained from patients with cutaneous leishmaniasis in comparison to controls.

	Albumin %	Alpha 1 %	Alpha 2 %	Beta %	Gammaglobulins %
Patients (100)	49.03 \pm 3.6	3.33 \pm 0.9	11.65 \pm 2.6	12.7 \pm 2.1	22.98 \pm 5.2
Controls (100)	57.9 \pm 3.9	3.1 \pm 1.1	10.1 \pm 1.9	11.9 \pm 2.1	14.8 \pm 1.9
P	<0.001	N.S.	<0.001	<0.01	<0.001

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Table (IV): Mean \pm SD Haemoglobin levels, total white blood cell counts, and platelets count as obtained from patients with cutaneous leishmaniasis in comparison to controls.

	Haemoglobin g/dL	Total WBCs $\times 10^3$ /ml	Platelets $\times 10^4$ /ml
Patients (100)	15.2 \pm 3.1	8.8 \pm 2.9	173.37 \pm 48.9
Controls (100)	16.8 \pm 2.2	6.7 \pm 3.1	281.110 \pm 36.7
P	<0.001	<0.001	<0.001

Table (VI): Mean \pm SE of serum IgA, IgG, IgM and IgE levels in 22 Egyptians with cutaneous leishmaniasis in comparison to controls.

	IgA (mg/dL)	IgG (mg/dL)	IgM (mg/dL)	IgE (IU/dL)
Cutaneous Leishmaniasis No. (22)	199 \pm 33.4	2100 \pm 88.7	283 \pm 28.5	812 \pm 98.6
Control group No. (11)	193 \pm 22.8	1113 \pm 112.9	103 \pm 19.6	119 \pm 29.6
P	N.S	<0.001	<0.001	<0.001

Table (VII): Mean \pm SE of serum IgA, IgG, IgM and IgE levels in 28 Indians with cutaneous leishmaniasis in comparison to controls.

	IgA (mg/dL)	IgG (mg/dL)	IgM (mg/dL)	IgE (IU/dL)
Cutaneous Leishmaniasis No. (28)	207 \pm 29.7	1922 \pm 103.6	302 \pm 33.1	806 \pm 79.3
Control group No. (14)	188 \pm 19.1	1037 \pm 97.6	123 \pm 16.7	127 \pm 30.3
P	N.S.	<0.001	<0.001	<0.001)

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