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Counting of Total and Differential White Blood Cells in Cutaneous Leishmaniasis Patients in Nassiryha city Thi-qar Province

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Alaa Hussein Oleiwi
College of Sciences, University of Thi-Qar
Email: alaah.bio@sci.utq.edu.iq
Fadhial Abbas Munshad, Ali Naeem Salman
Faculty of Education for Pure Sciences,
University of Thi-Qar
Email: Fadhidabass.bio@utq.edu.iq, dr.ali-n@utq.edu.iq

Abstract:

The present study was carried out on 50 cutaneous Leishmaniasis patients , without taken treatments and they were having sore skin infection diagnosed by dermatologist as Cutaneous Leishmaniasis and clinical diagnosis and 25 control groups , attended five main hospitals in Thi-Qar province ,there are Imam Hussein Teaching Hospital , Shattra general hospital, Rifya general hospital , Suq Al-Shayokh general hospital and Al-Chibayish general hospital, during the period from the beginning of January 2017 to end in June in the same year. The statistical analysis were $P \leq 0.05$ considered statistically significant.

There was significant decrease at $p \leq 0.05$ in mean of total WBCs in CL patients(6.36) compared to (controls groups) (7.80), also there was significant differences in mean of basophiles, monocytes , in CL patients(0.75 and 5.65) compared to (controls groups) (1.18 and 6.92) and there was increase and no significant differences were observed in mean of eosinophil's in CL patients compared to (controls groups)(2.31and 2.08) .There was significant were observed increase in mean of neutrophils and lymphocytes in CL patients (65.92 and 32.53)compared to controls groups (58.27 and 25.08).

Keywords: cutaneous leishmaniasis, white blood cells counts, protozoa.

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العدد الكلي والتفريقي لخلايا الدم البيضاء عند المرضى المصابين بداء اللشمانيا الجلدية في مدينة الناصرية محافظة ذي قار

***الاء حسين عليوي ** أ.د. فاضل عباس منشد ** أ.د. علي نعيم سلمان**
***قسم علوم الحياة \ كلية العلوم \جامعة ذي قار**
**** قسم علوم الحياة\ كلية التربية للعلوم الصرفة | جامعة ذي قار**

الخلاصة :

أجريت الدراسة الحالية على ٥٠ مريضًا مصاب بداء الليشمانيا الجلدية ، بدون اخذ علاج مشخص من قبل طبيب الأمراض الجلدية والتشخيص السريري و ٢٥ شخص من الاصحاء كمجموعة السيطرة ، المراجعين لخمسة مستشفيات رئيسية في محافظة ذي قار ، وهي مستشفى الإمام حسين التعليمي ، مستشفى شطرة العام ومستشفى الرفاعي العام ومستشفى سوق الشيخ العام ومستشفى الجبايش العام ، خلال الفترة من بداية كانون الثاني ٢٠١٧ إلى نهاية شهر ايار نفس العام. اكد التحليل الإحصائي على وجود فرق معنوي ($P \leq 0.05$) بين انواع الخلايا كان هناك انخفاض كبير في معدل كرات الدم البيضاء في مرضى اللشمانيا الجلدية (6.36) مقارنة مع مجموعة السيطرة (٧,٨٠) ، وكان هناك أيضا اختلافات كبيرة في معدل الخلايا القاعدية والوحيدة بين المرضى ومجموعة السيطرة (٠,٧٥و ٥،٦٥) (١،١٨ و٦،٩٢) على التوالي , وكان هناك زيادة في معدل الحمضيات ولم يوجد فروق معنوية إحصائية في المرضى CL(٢،٣١) مقارنة مع مجموعات السيطرة(٢،٠٨) لوحظ وجود زيادة كبيرة في متوسط العدلات والخلايا اللمفاوية لدى مرضى CL (٦٥،٩٢ و٣٢،٥٣) مقارنة بمجموعة السيطرة(٥٨،٢٧و٢٥،٠٨).

الكلمات المفتاحية: اللشمانيا الجلدية , خلايا الدم البيضاء , الابتدائيات.

Introduction

Cutaneous leishmaniasis (CL) is a public health problem caused by cell-infecting flagellate protozoa of the genus *Leishmania*. The disease occurs in 98 tropical, subtropical and temperate countries worldwide and it is estimated that 1.2 million new cases of CL occur per year (Avlar *et al*., 2012). Prevalence of leishmaniasis in endemic areas largely depends on the distribution of sand fly vectors and natural *Leishmania* infection in sand fly populations is an important epidemiological parameter for predicting the risk and prevalence of disease, estimation of which depends on the reliable identification of infected sand flies .Estimation of *Leishmania* infection rates in the vector could serve as an indicator of a change in transmission intensity at a given endemic area (Bates, 2007; Kato *et al*., 2010).*Leishmania* species are intra-cellular parasites invading monocytes,

macrophages, and langerhans cell their infection in man induces both humoral and cellular immune responses, but the balance of their expression varies with the type of the disease (De-Souza-Neto *et a*l .,2004). The major elements play role in immune response to parasitic infection were Natural killer cells, T lymphocytes and Macrophages in combination with cytokines. The neutrophils related to parasites during initial stages of infection so that adaptive immunity, which is induced later. In the presence of suboptimal levels of neutrophils, parasite load is not efficiently reduced and adaptive immunity is unable to handle this elevated burden, which ultimately leads to increased mortality (Yisong and Richard ,2009) .The skin is the natural site of entry for infective promastigotes transmitted by sand flies obtaining a blood meal .Therefore , infection with dermal immune system at the initial stage of infection is likely to be important to disease outcome for the reason we evaluated the levels of some immunological markers such ascounting of total and differential white blood cell in CL patients in Thi-Qar province.

Materials & Methods

Collected samples:

Blood samples were collected from 50 cutaneous Leishmaniasis patients , without taken treatment and they were having sore skin infection diagnosed by dermatologist as Cutaneous Leishmaniasis and clinical diagnosis was confirmed by laboratory demonstration of the parasite in the lesions by direct smears. Lesions were cleaned with ethanol, and punctured at the margins with a sterile lancet. Exudate materials was smeared, dried in air and fixed by methanol. The smears were stained with Giemsa's stain and examined by light microscope. Microscopic diagnosis was made when amastigotes were identified in the smears and 25 samplesfrom healthy person as control , attended five main hospitals in Thi-Qar province ,there are Imam Hussein Teaching Hospital , Shattra general hospital, Rifya general hospital , SuqAl-Shayokh general hospital and Al-Chibayish general hospital, during the period from the beginning of January 2017 to end in June in the same year.

One ml of blood were collected from patients by using disposable syringes. We the blood in EDTA tube and shake it quietly, to determine of WBC count, basophils, lymphocytes, monocytes, eosinophil's and neutrophils, analyzed then the data were processed by the computer obtained of results.

Statistical analysis :

Data were entered into SPSS version 20 and P value equals or less 0.05 considered statistically significant Differences between groups were tested with the Student's t-test.The values of $P \leq 0.05$ were considered significant.

Results & Discussion

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The present study evaluates the percentage of some blood parameters, in CL patients and non-infected (controls groups), there was significant differences at $p \leq 0.05$ between patients and controls .

There was significant decrease at $p \leq 0.05$ in mean of total WBCs in CL patients compared to (controls groups), also there was significant differences in mean of basophiles, monocytes , and there was increase and no significant differences were observed in mean of eosinophil's in CL patients compared to (controls groups).There was significant were observed increase in mean of neutrophils and lymphocytes in CL patients compared to (controls groups).

Total a count of leukocytes (WBCs), neutrophils, monocytes and lymphocytes in both groups, in table (1) explain the details.

White Blood cells play a role in the immune response to pathogens as a result of these infections, there is a change an increase or a decrease or remain within the normal range (4000-11000 cells / mm3) in parasitic infections(Ajoka *et al*.,2002).

Total count of leukocytes (WBCs) and basophils increase numbers in CL patients compared to (controls groups) the results of the current study was agreed with Al- Obaidi (2011) and Al-Mosawi (2015) which found there is an increase in the mean of white blood cells in CL patients .

Monocytes was increased in the peripheral blood of patients with *L. braziliensis*, compared with uninfected controls ,that intermediate monocytes are increased in frequency soon after infection, express CCR2,

Table(1) : Percentage of Total and Differential White Blood Cell in CL patients and not infected (Controls groups).

Blood parameter	CL patients	No.	Mean× 10^3	SD	t	p	α
WBC	patients	50	6.36	2.52	-2.673	0.01	0.05
	Control	25	7.80	1.76			
Basophils	patients	50	0.75	0.75	-3.268	0.002	0.05
	Control	25	1.18	0.34			
Monocytes	patients	50	5.65	2.76	-2.471	0.016	0.05
	Control	25	6.92	1.43			
Neutrophils	patients	50	65.92	13.37	-2.27	0.005	0.05
	Control	25	58.27	8.74			
Eosinophil's	patients	50	2.31	2.30	0.496	0.622	0.05
	Control	25	2. 08	1.44			
Lymphocytes	patients	50	32.53	10.48	-2.981	0.03	0.05
	Control	25	25. 08	8.10			

Which would promote their migration into the lesions and, owing to their production of TNF, can enhance the inflammatory response (Passos *et al*., 2015). The results of the current study disagree with results Saha *et al*.,(1999) which observed Monocytes was increased in the blood of CL patients is one of the distinctive sign associated with cutaneous leishmaniasis.

While studies of early lesions illuminate the fact that cells other than macrophages serve as hosts for parasites at different times during infection. Indeed, a model of natural infection transmitted by sand fly bites demonstrated that during the initial 2 h of infection, neutrophils

accumulating at the site of *L. major* inoculation readily take up parasites .Taken together, these studies that (1) "tissue injury" caused by either natural infection or experimental inoculation enhances the local early inflammatory infiltrate and (2) that the formation of skin lesions correlates with the development of adaptive immunity (Belkaid *et al*.,2000; Peters *et al* .,2008)

The results of the current study was agreed with previous studies by Blanchard and Rothenberg (2009) and AL-Ghezy (2012) which observed an increase mean of eosinophils in parasitic infections. Eosinophils have been associated with immune responses to helminthic parasites usually, eosinophils are not implicated in the immune response against protozoa because their levels are not elevated during these infections. However, several lines of evidence support the involvement of eosinophils *L. amazonensis* promastigotes, and in vivo studies have demonstrated eosinophil recruitment to the infection site of *L. amazonenesis* and *L. major* . During the initial hours of *L. mexicana* infection, eosinophils have been observed close to de granulating mast cells at the site of parasite inoculation, suggesting a role in parasite clearance, it has been demonstrated that eosinophils have anti-microbial activity against *L. mexicana* (Rodríguez and Wilson,2014)

This results of the current study was agreed other previous studies by Moker (2006) in Basra province and Al-Mosawi (2015) in Thi-qar province, which found increase the percentage of lymphocytes and in CL patients compared to control group.

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