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Wasan Abd Al-Hamid Husayn, Ohood Mozahim Shakir and Mostafa Ali Abdulrhamen



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Effect of Leishmania Tropica on Some Haematological Parameters

Wasan Abd Al-Hamid Husayn^{1,a)}, Ohood Mozahim Shakir^{2,b)} and Mostafa Ali Abdulrhamen^{1,c)}

¹College of Education, Department of biology, University of Samarra, Samarra ,Iraq.

²College of Applied Sciences, Department of Pathological Analysis, University of Samarra, Samarra, Iraq.

^{a)} Corresponding author: wsnsamra@gmail.com

^{b)}uhood.m@uosamarr.edu.iq

^{c)}mostafaali20042003@yahoo.com

Abstract. This study was carried out for period November 2020 until February 2021. included (50) patients infected with *Leishmania tropica*, of Samarra General Hospital. Patients aged between 18 - 70 years, the study included effect of parasitic infection on white and red blood cells and blood hemoglobin. The results were recorded a significant increase in the level of (($P < 0.05$) in red blood cells count (RBC) in patients with leishmaniasis, as it reached $(5.5 \pm 0.52) 10^3$ X cells / Mm^3 in males compared with control group by $(5.32 \pm 0.56) 10^3$ X cells / Mm^3 and females with a percentage $(4.8 \pm 0.21) 10^3$ x cells / Mm^3 compared to control group $(4.37 \pm 0.03) 10^3$ x cells Mm^3 and the low hemoglobin (Hb) percentage of the affected patients was significant in males and females at ($P < 0.05$) level. In males infected with cutaneous leishmaniasis, the rate was (14.1 ± 0.94) g / dL compared to the control (14.9 ± 0.58) g / dL, and in females, its rate was (12.6 ± 0.48) g / dL compared to control group, (11.6 ± 0.44) g / dL, The level of PCV decreased significantly ($P < 0.05$) in males (44.8 ± 1.48) g / dL compared to control group, as it reached (45.5 ± 1.7) g / dL, and it increased in females with a rate of (38 ± 1) . G / dL compared to control group, as it reached (37.25 ± 1.22) g / dL. The total number of white blood cells for males increased significantly, as it reached $(8.31 \pm 1.4) 10^3$ x cells / Mm^3 compared to control group, when it reached (6.93 ± 1.40) cells / Mm^3 , while it decreased in females by (7.6 ± 1.2) cells / mm^3 compared to control group, as it reached (10.3 ± 28.3) cells / Mm^3 . As for the number of lymphocytes, it decreased significantly in affected males, reaching $(34 \pm 6.5760) 10^3$ X cells / Mm^3 compared to control $(36 \pm 5.1168) X 10^3$ cells / Mm^3 , While the study indicate a significant increase ($p < 0.05$) in rate of lymphocytes in infected females, as their rate was $(37.127 \pm 4.5254) 10^3$ x cells / Mm^3 compared the control group, as its rate was $(33.22 \pm 11.27) 10^3$ x cells / Mm^3 , As for the monocyte cells, their percentage decreased in males $(9.8 \pm 1) 10^3$ cells / Mm^3 for the infected persons compared with control group, as it reached $(10.2 \pm 0.90) 10^3$ x cells / Mm^3 as in the females, reaching $(8.3 \pm 0.72) 10^3$ X cells / Mm^3 compared to control $(8.73 \pm 0.90) 10^3$ cells / Mm^3 . The total number of neutrophils for infected males and females decreased, as it reached $(55.18 \pm 8.697) 10^3$ X cells / mm^3 in males compared to control, with a rate of (56.007 ± 9.365) . (10^3 x cells / Mm^3), while in the infected females, their rate was $(56.5 \pm 5.658) 10^3$ x cells / Mm^3 compared

INTRODUCTION

Leishmaniasis appears as a result of infection by different types of parasites of the genus Leishmania. The genus Leishmania, it is Protozoa Flagellates Obligate Parasitism [1], where the Leishmania parasite infects the immune system cells of the final host (represented by humans) by stinging the sand fly that represents the insect carrying the parasite, and this parasite spreads in the final host body of the final host according to its type ; Where the infection is either a cutaneous infection that causes skin abnormalities or ulcers, and the infection is called cutaneous leishmaniasis, and this parasite may infect the internal organs (such as the liver and spleen) of the host causing visceral injuries, and the infection is called Visceral leishmaniasis [2].

Cutaneous leishmaniasis is one of health problems caused by one of the common types of leishmaniasis, *Leishmania tropica*. Leishmaniasis affects about 12 million people in 88 countries, and there are between 2 and 3 million new cases diagnosed each year. In addition to the presence of 350 million people in the world annually exposed to infection with cutaneous leishmaniasis [3], and the injuries range from mild or moderate skin injuries to injuries that may lead to death [4]. Iraq is one of the main habitats in which leishmaniasis is common, and therefore the disease is called Baghdad boil is common. In addition to many other names (the oriental blister, the Aleppo boil and the Delhi sore) according to the geographical areas in which the disease is spread [5]. Cutaneous leishmaniasis is caused by a parasitic infection of the skin connective tissue, which leads to ulceration of the skin and inflammation of the tissues, and the severity of the disease depends on; the type of parasite as well as on the immune responses of the host and most cases heal spontaneously in rare cases, as the ulcer becomes chronic. This parasite infected the phagocytes which it Important in the immune response and the first line of defense against infection. These cells represent one of the types of white blood cells that have a major role in the immune system and are the main components of blood that are present in the peripheral blood and defend the body, which are formed inside the bone marrow and then distributed in the blood, so it is an important part of the system Immunity in the body and plays an important role in protecting the body from infections that may arise as a result of the entry of bacteria, viruses, or microbes into the body, as it help fight infections [6].

The white blood cells are often irregular in shape and there are several types of white blood cells and the two most common types are (Lymphocytes and Neutrophils) cells also called multinucleated white blood cells because they contain nuclei inside them with scattered granules and a high percentage of the cytoplasm to the nucleus, unlike cells Red blood [7]. There are five main types of white blood cells, namely, Neutrophils, Lymphocytes, eosinophils, Monocytes, and Basophils [8]. Monocytes are the largest types of white blood cells and they are responsible for attacking and destroying microbes and bacteria that enter the body. Monocytes move from the bloodstream to organs and tissues such as liver, lungs, spleen and bone marrow. The high rate of monocytes is due to the presence of infection or disorders in bone marrow or chronic inflammation, and on the other hand, the lack of white blood cells is a shortage of circulating monocytes and may result from injury to the bone marrow or be a sign of some type of Leukemia [9].

CBC test determines the amounts of blood cells in the blood, including red blood cells, white blood cells, and platelets, also evaluates medical conditions that have a secondary effect on the blood and immune system, which leads to blood changes, and it can provide a census count. Complete blood count (CBC) is a basic look at primary conditions of blood and bone marrow, including disorders such as anemia and leukemia [10].The aim of this research is to study the effect of cutaneous leishmaniasis infection on some hematological parameters.

MATERIALS AND METHODS

Samples Collection

The study included the diagnosis of (50) cases of cutaneous leishmaniasis in Samarra General Hospital, from November 2020 to February 2021. The information for each patient with cutaneous leishmaniasis was recorded according to the questioner information form for each patient. The infection was diagnosed by a dermatologist at Samarra General Hospital.

The Methods

The current study was conducted in Samarra city and its outskirts for patients infected with with *Leishmania tropica* or the so-called Baghdad Boil pill for the Samarra General Hospital; for the period from November to February of 2021. The study included (50) person (25) patients of leishmaniosis, and (25) as a control group, their ages range between 18-70 years and from both sexes (13 males and 12 females) (13 males and 12 males) as a control group.

Diagnosis Methods of the Samples

They were diagnosed clinically (first diagnosis) with cutaneous leishmaniasis by dermatologists of both genders and different age groups and had not previously undergone treatment by taking a swab from the ulcer and watching

the parasite. Then 2 ml of infected and non-infected persons (control group) included in the study were withdrawn and 2 ml of it were placed in a test tube containing an EDTA anticoagulant.

The hematological parameters were measured using a complete blood count, as 2ml of blood was placed in a test tube containing an anticoagulant EDTA , and the tube was shaken slowly as the device drew out a quantity of blood and analyzed it in order to calculate the differential number of white blood cells. The blood was examined by automatic analysis of blood, manufactured in (Sweden) Swelab, where the blood sample is placed by a tape coming out of the machine and the results are shown on the screen.

RESULTS AND DISCUSSION

The current study included the effect of infection with the cutaneous leishmaniasis parasite on some hematological parameters, including the total number of blood cells. The results in Table (1) showed significant differences ($P < 0.05$) in the rate of infection between males and females of cutaneous leishmaniasis patients compared to the control group, the RBC increased in females by $(4.8 \pm 0.21) 10^3 \times 10^3 \text{ cells} / \text{mm}^3$ compared to the negative control group $(4.37 \pm 0.03) 10^3 \times \text{cells} / \text{mm}^3$, as well as its height in males by $(5.5 \pm 0.52) 10^3 \times \text{Cell} / \text{mm}^3$ compared to control with a ratio of $(5.32 \pm 0.56) 10^3 \text{ cells} / \text{mm}^3$. These results are inconsistent with the findings of [11]. The reason for the decrease in the number of red blood cells is the accumulation of red blood cells, which is mostly due to the increase in chances attack it and devour it from macrophages in tissues, and the reason may also be due to the possibility of shortening life of cells as well as the increased fragility of red blood cells with the progression of infection.

TABLE 1.Total number of red blood cells for males and females with cutaneous leishmaniasis.

| RBC count | | |
|-----------|-----------------|------------------|
| Groups | Males M±S. D | Females M±S. D |
| Infected | 5.5 ± 0.52 | * 4.8 ± 0.21 |
| Control | 5.32 ± 0.56 | 4.37 ± 0.03 |

* indicates a significant differences ($p < 0.05$) between groups.

Table (2) shows the percentage of hemoglobin (Hb) for the infected, where the HB level decreased significantly in males with a level of ($P < 0.05$), reaching a rate of $(14.1 \pm 0.94) \text{ g} / \text{dL}$ compared to the control $(14.9 \pm 0.58) \text{ g} / \text{dL}$. In females, it increased, as its rate reached $(12.6 \pm 0.48) \text{ g} / \text{dL}$ compared to the control group, $(11.6 \pm 0.44) \text{ g} / \text{dL}$, and thus our results agree with what was found [12]. This is may be attributed to the process of consuming vitamins such as vitamin C, B6, B12 and folic acid by the parasites during their multiplication and that these vitamins are included in the synthesis of red blood cells.

The results on this study are inconsistent with the findings of studies from Yemen [13], Nepal [14] and Iran [15]. Anemia rate of 96% has been observed in patients with cutaneous leishmaniasis, which may be caused by multifactor, including the isolation and destruction of the red color of hemoglobin (RBC). Immunity, changes in the permeability of the membrane to red blood cells, enlargement of plasma volume, nutritional deficiencies, especially iron, and folic acid and vitamin B12 may also have a major role in this [16,17].

TABLE 2. The percentage of hemoglobin HB for males and females with cutaneous leishmaniasis.

| HB % | | |
|----------|-------------------|-------------------|
| Groups | Males M±S. D | Females M±S. D |
| Infected | * 14.1 ± 0.94 | * 12.6 ± 0.48 |
| Control | 14.9 ± 0.58 | 11.6 ± 0.44 |

* indicates a significant differences (p <0.05) between groups.

Table (3) shows the volume of PCV, as it decreased significantly (P <0.05) in males (44.8 ± 1.48 g / dL) compared to the average in the control group, reaching (45.5 ± 1.7) g / dL, while the results recorded an increase in the PCV in females, but it was not significant, as it reached (38 ± 1) g / dL compared to the control group, as it reached (37.25 ± 1.22) g / dL.

TABLE 3. (PCV) for males and females with cutaneous leishmaniasis .

| Groups | PCV % | |
|----------|-------------------|------------------|
| | Males M±S. D | Females M±S. D |
| Infected | $44.8 \pm 1.48^*$ | 38 ± 1 |
| Control | 45.5 ± 1.7 | 37.25 ± 1.22 |

* indicates a significant differences (p <0.05) between groups.

The reason for the high percentage in females may be attributed to several factors of nutrition, pregnancy, puberty factors, and changing hormones. In males, the parasite may be the reason for the decrease in the percentage because parasitic and viral infection caused the decrease. Table (4) shows the percentage of the total number of white blood cells WBC for infected people , as it increased significantly (P <0.05) in the total number of white blood cells for males, which reached (8.31 ± 1.4) 10^3 x cells / mm^3 compared to the control group, which reached (6.93 ± 1.40). (Cell / mm^3). The results showed a significant decrease total number of WBC for females, reaching (7.6 ± 1.2) cells / mm^3 compared to the control group, as it reached (10.3 ± 28.3) cells mm^3 ,this results is in agreement with [18], who concluded that parasitic infections activates platelets, in addition to its role in inflammation and tissue repair, the reason for this increase in the WBC rate of white blood cell count may be due to the activation of the cellular defense immune system due to the entry of parasite antigens into the body leading to the production of a number of cells, especially the macrophage cell, which is an important defense criterion in intracellular parasitic infections as well as lymphocytes, whose importance lies in the specific immune response and the increase in the total number of white blood cells may be due to the parasite's possession of antigenic determinants such as (GP 63) Glyco protein 63, which can stimulate the host's immune system [19].

TABLE 4. Total number of WBC count for males and females with cutaneous leishmaniasis

| Groups | WBC count X 10^3 | |
|----------|--------------------|-----------------|
| | Males M±S. D | Females M±S. D |
| Infected | * 8.31 ± 1.4 | * 7.6 ± 1.2 |
| Control | 6.93 ± 1.40 | 10.3 ± 28.3 |

* indicates a significant differences (p <0.05) between groups.

The results in Table (5) showed a significant decrease (p <0. 05) in the number of lymphocytes in infected males, reaching (34 ± 6.5760) 10^3 cells / mm^3 compared to the control (36 ± 5.1168) 10^3 x cells / mm. Our results are consistent with the findings of (12) which proved that infection with cutaneous leishmaniasis leads to an increase in the number of lymphocytes. While the study recorded a significant increase (p <0.005) in the rate of lymphocytes in the infected females, as their rate was (37.127 ± 4.5254) 10^3 x cells / mm^3 compared to that of the control group, as its rate was (33.22 ± 11.27) x 10^3 cells / mm^3 The results of our study are consistent with the findings off[20] and do not agree with the results of [12].T-lymphocytes play a major role in generating a specialized response and building memory in intracellular parasite infections [21]. These cells are stimulated by the glycoprotein of the cutaneous leishmaniasis parasite and thus produce the important INF γ in inducing INOS and producing nitric oxide. Several researches indicates the important role of lymphocytes in defending and defining leishmaniasis infections and that the protective effect is by producing pro-inflammatory or anti-inflammatory or equilibrating cellular kinematics.

That is, lymphocytes can differentiate into Th1 or Th2 and this differentiation depends mainly on the promoter type of promoter initiating during cellular locomotor differentiation IL-4, it was seen to stimulate Th2 leading to disease progression, while cytokine IL-12 stimulates Th1 differentiation which they both play major role on intracellular pathogenesis [22].

TABLE 5. Number of lymphocytic leukocytes for males and females with cutaneous leishmaniasis.

| lymphocytes count X 10 ³ | | |
|-------------------------------------|--------------|-----------------|
| Groups | Males M±S. D | Females M±S. D |
| Infected | * 34 ±6.5760 | *37.127 ±4.5254 |
| Control | 36 ± 5.1168 | 33.22 ± 11.27 |

* indicates a significant differences (p <0.05) between groups.

As for monocytes, Table (6) showed no significant differences (P <0.05), as the results showed a decrease in the rate of their percentage in males (9.8 ± 1) X 10³ cells / mm³ for the infected compared with the control, as it reached (10.2) X 10³. The results of the study showed a decrease in females, reaching (8.3 ± 0.72) X 10³ cells / mm³, compared to the control (8.73 ± 0.90) X 10³ cells / mm³.

TABLE 6. Monocytes for males and females with cutaneous leishmaniasis.

| monocytes count X 10 ³ | | |
|-----------------------------------|--------------|----------------|
| Groups | Males M±S. D | Females M±S. D |
| Infected | 9.8 ± 1 | 8.3 ± 0.72 |
| Control | 10.2 ± 0.90 | 8.73 ± 0.90 |

* indicates a significant differences (p <0.05) between groups

The study did not agree with [12], as monocytes increased, and some studies indicated that the high percentage of mononuclear blood cells is one of the distinguishing signs associated with cutaneous leishmaniasis, and it must be noted that tissue injury with pathogens leads to mononuclear cells migrating from the peripheral blood to the affected body tissues to settle in that tissue and it is called the macrophage, which have a high digestive capacity for the pathogens that invade the body [22]. Also studies have shown that the Promastegote phase of the Leishmaniasis parasite can stimulate the migration of neutrophils by releasing for Leishmania chemotactic factor (LCF), which has the strong effect of attracting only the clue cells and no other immune cells such as mononuclear cells and natural killer cells [23]. The results in Table (7) showed the total number of neutrophil cells, as it decreased significantly (p <0.05) for infected males and females, reaching (55.18 ± 8.697) 10³ X cells / mm³ compared to the control, as its rate was (56.007 ± 9.365) 10³ X cells. / mm³, while the study recorded an increase in infected females, with a rate of (56.5 ± 5.658) 10³ cells/mm³ compared to control, as their rate reached (61.37 ± 9.2655) 10³ x cells / mm³

TABLE 7. Number of neutrophils for males and females with cutaneous leishmaniasis.

| neutrophils count X 10 ³ | | |
|-------------------------------------|-----------------|----------------|
| Groups | Males M±S. D | Females M±S. D |
| Infected | * 55.18 ± 8.697 | 56.5 ± 5.658 |
| Control | 56.007 ± 9.365 | 61.37 ± 9.2655 |

* indicates a significant differences (p <0.05) between groups

Our study agrees with the results of [23-25]. The cause of neutropenia may be the result of the destruction of early white blood cells (especially neutrophils) by the parasite [17], and we noticed that its results are inconsistent with the results of [26], as it was found that an increase in the number of Phagocytic neutrophilic cells of Leishmania parasite by secreting some attracting cellular motility, such as IL-8, which are important in attracting other neutrophils to the site of injury to perform their function. IL-8It is rapidly produced in the skin after infection with the L-Major type Leishmaniasis [26].

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