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# Immunological detection using immune cytokines for human cutaneous leishmaniasis in Al-Najaf Al-Ashraf governorate

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**Abstract---** The results of the immunofluorescence study with ELISA technique showed ELISA for sera of patients with leishmaniasis L. Major Significant increase in cellular kinetics values, as interleukin reached 6 (IL6)  $4.45 \pm 3.2$  pg | ml, tumor necrosis factor factor -a)  $0.32 \pm 0.15$  pg | The statistical study recorded a significant increase at the  $p < 0.05$  probability level with the control group samples. The laboratory results of hematological covariance assays recorded a significant decrease at  $p < 0.05$  in the hemoglobin level Hb reached  $(12.2 \pm 1.1$  g | deciles compared to the control group  $15.9 \pm 0.66$ ) g | Decylase, white blood cells, neutrophils and lymphocytes were significantly elevated at  $p < 0.05$  when compared with the control group.

**Keywords---** immunological detection, immune cytokines, human.

**Introduction**

Immunological methods have been used to diagnose cutaneous leishmaniasis, such as immune cytokines, which are proteins or carbohydrates secreted by T cells, macrophages and monocytes. Histological examinations lead an important indicator in evaluating the quality of infection with the cutaneous leishmaniasis parasite and for the purpose of differentiating between multiple types of skin infections and other types of skin diseases such as leprosy and deep fungal infection. Baaten BJ, 2012).

## **Cytokines**

The origin of the term cytokines is derived from the Greek word Cyto = cell, meaning cell, and Kinose = movement, meaning movement ( Balk will , 2000 ) . Its low molecular weight (20000-5000) Dalton (Banerjee, 2016) is highly regulated and has hormone-like activity if it affects distant cells in the host body and binds to specialized receptors on the target cell membrane, causing chemical changes as a result of these signals to genes to direct them to do an action A specific biologi (Barazesh ,2018). The effects carried out by cellular kinetics are called according to their location. If they affect the cells that produce them, then they are called autocrine, or on neighboring cells, they are called paracrine, or internally, they are called endocrine. (Barbosa, 2018). Cytokinetics may lead to immunosuppression, producing fibroblasts, white blood cells, and phagocytes. Cyto Caines (Barrera,2013). They are the important basis in the formation of immune system cells that regulate the cellular and humoral immune defenses because they have an important role against inflammation (Ben Salah A, 2013). Cytokines participate in the defense and repair process and in the types of blood cells. Hematopoietic cells are secreted from multiple nucleated cells (Bhattacharya, 2016). So they are pro-inflamaator or anti-inflammatory kinetics as well as they exercise some necessary vital activities in chemical attraction to form unspecialized antigens (Bhattarai NR, 2010). Cellular kinetics also reduce inflammation to prevent tissue damage in the body (Bianchi, 2019) and inhibit target cells due to the presence of surface receptors on them (Biswas, 2017) . Therefore, it is necessary to estimate the level of cellular kinetics, understand the response of the immune defenses in the body, and know the lethal effect of the disease (Cabello-Donayre, 2016). The various cellular kinetics were also classified into several classes, such as:

- Lymphokines, which are produced by lymphocytes.
- Alpha and Beta Interferons (IFNs) Interferon which is produced in cases of virus entry.
- Monokines Monokines produced from Mecrophage monocytes.
- Chemokines, which are produced from mononuclear macrophages, fibroblasts and endothelial cells, which are considered as immune attractions.
- Growth stimulating factor (GFS) that supports cell growth.
- interleukins (ILS) Interleukines secreted from white blood cells (Calderon Guzman, 2019)

## **Interleukins**

It is a type of cytology Kinetics are found inside the white cells in the blood and work to stimulate the immune system by carrying out vital activities that fight disease and expel the pathogen and kill it (Blazkova, 2017). There are more than 25 types of interleukins, each customized according to its role in the body (Campos, 2020).

### **Inter leukin6(IL-6)**

It has a molecular weight of (27-22) kDa and secretes interleukin 6 T lymphocytes

T-lymphocytes, B - lymphocytes, macrophages, eosinophils, polymorphonuclear cells, monocytes, and mast cells osteoblasts, stem cells, chondrocytes, endothelial and skeletal cells, smooth cells, astrocytes, and keratinocytes Keratinocytes Boelaert M,2014). This interleukin works to induce heat in the acute response by raising the body temperature of the host, as it is considered a pro-inflammatory or anti-inflammatory cytokine. Carneiro, 2016). It is also called by scientific nomenclature, including B-cell differentiation factor B-cell differentiation factor , interferon interferonbeta-2 , hybridoma growth factor , B -cell stimulatory factor (BSF-2) , or CTL differentiation factor Browne, 2013 ).

Laboratory experiments conducted on animals showed the significant role of IL-6 in enhancing the immune response to T cells TH2 in cutaneous leishmaniasis CL ,2012) Bualert L). The main and important effects of it are due to its presence in the circulatory system and can be in the center of infection or far from it (Castanys- Munoz E, 2012) and that these effects have a major role in the pathogenesis of the injured and that the use of IL-6 in the treatment of macrophages in vitro works on the inhibition of the production of tumor necrosis factor -inf -a and interferon IFN-Y against L. amazonensis and stimulation of IL-10, IL-17. production (Celes, 2016). Interleukin is IL-6 is a stimulatory and anti-inflammatory cytokine that works at the site of the sandfly sting to stimulate the acute phase response by producing Acute phase proteins. Inflammatory conditions with clinical and biological signs such as heat, redness, swelling and pain (Chakrabarti, 2013)). This is accompanied by an increase in leukocytes, phagocytes and natural killer cells in the area of damage to remove harmful residues of cell debris and prepare the area for repair (Campos, 2020)). Among other effects on B and T cells, there is an increase in immune globin expression and the differentiation of B cells into plasma cells. T doubles and increases its activity, urging the liver to secrete proteins that bind the automata system with microorganisms in addition to molecular adhesion expression and possession of soluble receptors Interleukin-6 receptors (SIL-6Ra) , which have a role in preparing the cellular defense response and activation of inflammatory processes and the emergence of fever through The effect on the neuroendocrine glands, as well as the proliferation and differentiation , and the inhibition of the inflammatory process . Chatterjee, 2015).

### **Tumor necrosis factor alpha**

It is a protein that participates and emits a cellular signal during the inflammatory process in humans. It belongs to the cytokines. It is of two types: tumor necrosis factor alpha (TNF-a) and tumor necrosis factor (TNF) beta (TNF-B) and tumor necrosis factor alpha (TNF - alpha). And it has a significant impact on activities inside or outside the body that cause regulation of the differentiation of cancer cells, laboratory or internal, such as modifying cell migration or inducing cell death (Chauhan, 2017). The cytokines have a great immune necessity to deal with parasitic and bacterial diseases, yet there is ambiguity in the mechanism of sensitivity to address the disease and achieve the immune response (Chen, 2018).

responsible for the production of TNF-a , including lymphocytes , macrophages , neutrophils, mast cells, B cells , and T cells . There are two TNF-a- binding receptors, TNFR1 and TNFR2 on the surface of the affected cell. The receptors are

distributed in several forms and according to the type of responding cell (das Does Moreira, 2016). TNF -a has an important role in cutaneous leishmaniasis, as treatment with it had a significant role in reducing the skin symptoms of the lesion in terms of size and number of parasite inside the skin ulcers, but when the effect of antibodies to necrosis factor is removed, the complications of the disease will increase (Chikku, 2016)).

The presence of TNF-a as a protein and can pass into macrophages through the membranes, and in a scientific study it was indicated that when injecting anti-TNF into mice infected with leishmaniasis, it leads to the decomposition of the infection-carrying macrophages and a large spread of the parasite L.Major within the macrophages in the body and suppression of the role of immunity. In contrast to the beneficial effect of TNF activating the infected macrophages and fighting L.Major and enhancing the immune capacity to resist the disease, as it has been indicated in many experiments to the ability of TNF to weaken the recurrence of parasitic infection in the body of living organisms (da Silva Vieira, 2019) .

### **Leishmaniasis diagnosis Immunological methods**

It includes multiple diagnostic methods for diagnosing cutaneous leishmaniasis (CL), including:

#### **Skin test**

It is also called the Montenegro test or delayed hypersensitivity, and it is to investigate the injury inside the body (Cogger , 2020). (0.1) ml of the parasite antigen is injected into the dermis area and the positive results are 7 weeks after the injection. Then the hardening and redness of the area reaches a peak after (48-24) hours and the test is positive with an increase in the percentage of hardening of the area at the injection site and this test depends on the body's response leishmaniasis immunoassay (Conceicao, 2016).

#### **Serodiagnosis test**

These tests identify changes in antibodies, serum proteins, or both (Das, 2013).

- Determination of the level of immunoglobulins
- Turban diffusion test. Formal- gel Test
- Complement fixation test (CFT)
- Direct Agglutination Test
- Indirect HaemoagglutinationTest (IHA) \_
- Indirect fluorescent antibodies test (IFAT)
- Enzyme -Linked Immunosorbent Assay (ELISA) (AL-Janabi, 2001; Lainson and Shaw, 1965).

#### **Direct Agglutination Test (DAT)**

Tests of high sensitivity and specificity and that are cheap and simple. Therefore, they are ideal for laboratory and field studies, where their sensitivity in the

absence of tests reaches 100-91 % and the specificity of 100% -72%. 2020).

### **Indirect immune fluorescent antibodies test (IFAT)**

This test identifies antibodies against the antigen present on the surface of the parasite and that there is no difference between the amastigote phase antigen and the promastigote antigen in the same strain of leishmaniasis. (Cunha J, 2012). This test has a high sensitivity in parasitological diagnosis, especially in the early stages of infections, up to 96 %, and the specificity reaches 98 %. Commercial test strips have been manufactured to detect specific antibodies to parasites (Cunha J, 2010).

### **Enzyme linked immune sorbent assay (ELISA)**

This test is of great value in the serological diagnosis of leishmaniasis, as the specificity and sensitivity of the ELISA test are affected by the antigen used. Many antigens were detected in the ELISA test, including Antigen viz for the detection of Gene B protein For L.Major cutaneous and mucocutaneous leishmaniasis, the use of recombinant V12 antigen and major surface recombinant gb63 surface glycoprotein for L.Major. (Cunha J,2011).

### **Immunoblotting**

This test is more commonly used in leishmaniasis Visceral, but it has a low rate with cutaneous leishmaniasis in detecting leishmaniasis antibodies, so it was not widely used (Cunha J, 2013).

## **Materials and Working Methods**

### **Immunological study**

#### **Measure the level of interleuking**

The examination includes a group of people infected with cutaneous leishmaniasis in the current study (318) samples, in addition to the serum of healthy people in the control group (14) samples in order to estimate the level of cellular kinetics (IL-6) through the use of the ELISA test and according to the instructions of the American company that supplied for examination Elabscience

### **Test Principle**

The test method involved adding a serum sample and base solution to measure standard to zero in the plate lined with biotin-conjugate antibodies of interleukin. IL-6 After the reaction between the antibody and antigens inside these pits, the plate is washed to remove the unbound substances, then Horseradish peroxidase (HRP) Avidin-conjugated is added, then the plate is washed again to remove the unbound substances between the antibody and the antigen, and then the base substance (TMB) to the etching and the incubation period of the base material ends, then the reaction is stopped by adding sulfuric acid, where the color changes from blue to yellow. IL-6. cytokinetic assay

amounts	solutions	T	amounts	solutions	T
1 x 50 ml	Sample Diluent	1	1 (96 wells)	Assay plate (12 x 8 coated Microwells )	1
1 x 20 ml	Wash Buffer (25 x concentrate)	2	2	Standard (Freeze dried)	2
1 x 10 ml	TMB Substrate	3	1 x 120 $\mu$ l	Biotin-antibody (100 x concentrate)	3
1 x 10 ml	Stop Solution	4	1 x 120 $\mu$ l	HRP- avidin (100 x concentrate)	4
4	Adhesive Strip (For 96 wells)	5	1 x 15 $\mu$ l	Biotin-antibody Diluent	5
1	Instruction manual	6	1 x 15 $\mu$ l	HRP- avidin Diluent	6

### Method of Work (procedure)

- Dilution: The standard solution was diluted to 1/20 of the dilution solution (N12) to obtain a positive and negative dilution. Sample addition (100  $\mu$ l and as per the pits was covered with tape and incubated for 1 h at 37 °, and the liquid was removed from the pits without being rinsed off. Add Biotin-antibody (100)  $\mu$ l to the etch, place the tape and incubate at 37 °C for one hour.
- Washing: The washing is by filling the pits with a washing solution with distilled water for two or three times, then removing the washing solution and using insulating cleaning paper for 2 minutes each time. Add dilution (100)  $\mu$ l of Horseradish peroxidase to all pits, cover them with new tape, and incubate for 30 minutes at a temperature of 37 °. Repeat the washing process (4-5) times as in the fourth step. Add the base material (TMB) in the amount of (90)  $\mu$ l to the pit cover and incubate it for 15 minutes in the absence of light and at a temperature of 37 °. Adding (50  $\mu$ l) of the stop solution to the pits with gentle pressure to ensure thorough mixing, and the process is called stopping the reaction .
- Assay: The optical density was determined after (5 minutes) for all pits, and then the results were read by a spectrometer with a wavelength of 450 nm.

### Calculation of Results

The cellular kinetic (IL-6) concentration values were calculated from the measurement standard curve by using the ALNERA device with wavelength 450 nm and the measured concentration was on the horizontal axis and the chromatic absorbance values on the vertical axis as shown in Figure (3)

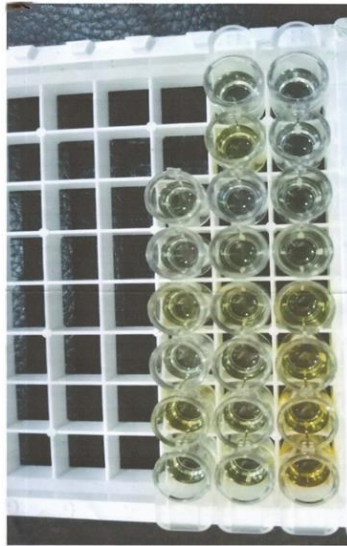
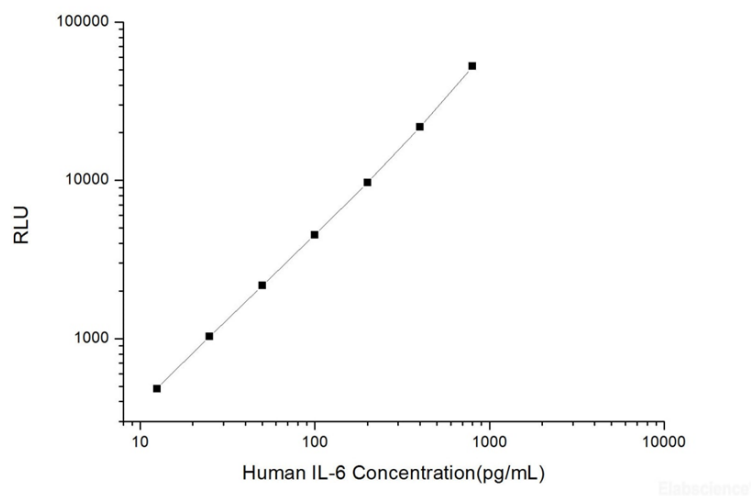


Image showing serum samples in IL-6 . test plate



Standard curve shape for IL-6. Cytokinesis

### Measurement of tumor necrosis factor

The immunoassay ELISA (ELISA) was used, according to the instructions attached to the diagnostic examination kit, which was supplied by the American company Elabscience.

### Test principle

The quantitative sandwich enzyme principle was used, in which antibodies to necrosis factor surrounded TNF-a previously prepared in the microplate, samples were added to the pits in the plate with anti- . antibody TNF-a , the bodies are

bound and then the unbound substances are eliminated after which the biotin-bound body is added to the TNF-a to each hole in the lamina then wash and add avidin -linked peroxidase (HRP) to it. Then washing and removing the irregular reagent avidin - enzyme, then adding the basic solution to etch the plate, then the color changes according to the amount of TNF-a , then its intensity decreases.

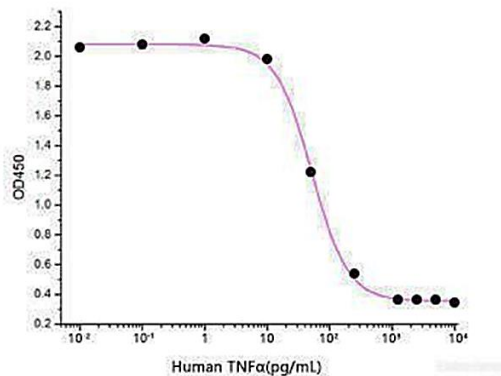
### The method of work

- Dilution : The standard solution was diluted to 1/20 of the dilution solution ( N12) to obtain a positive and negative dilution. Sample addition (100)  $\mu$ l and as per the pits was covered with tape and incubated for 1 h at 37 °, and the liquid was removed from the pits without being rinsed off. Add Biotin-antibody (100)  $\mu$ l to the etch, place the tape and incubate at 37 °C for one hour.
- Washing : The washing is by filling the pits with a washing solution with distilled water for two or three times, then removing the washing solution and using insulating cleaning paper for 2 minutes each time. Add dilution (100)  $\mu$ l of Horseradish peroxidase to all pits, cover them with new tape, and incubate for 30 minutes at a temperature of 37 °. Repeat the washing process (4-5) times as in the fourth step. Add the base material ( TMB ) in the amount of (90)  $\mu$ l to the pit cover and incubate it for 15 minutes in the absence of light and at a temperature of 37 °. Adding (50  $\mu$ l ) of the stop solution to the pits with gentle pressure to ensure thorough mixing, and the process is called stopping the reaction .
- Assay : The optical density was determined after (5 minutes) for all pits, and then the results were read by a spectrometer with a wavelength of 450 nm.

Table 3-11

Components of the kit for the examination of tumor necrosis factor

Materials	Concentrations	T
(12 x 8 coated Microwells )	1 (96 wells)	1
Standard (Freeze dried)	2	2
Biotin -antibody (100 x concentrate)	1 x 120 $\mu$ l	3
HRP- avidin (100 x concentrate)	1 x 120 $\mu$ l	4
Biotin-antibody Diluent	1 x 15ml	5
HRP- avidin Diluent	1 x 15ml	6
Sample Diluent	1 x 50 ml	7
Wash Buffer (25 x concentrate)	1 x 20ml	8
TMB Substrate	1 x 10ml	9
Stop Solution	1 x 10ml	10
Adhesive Strip (For 96 wells)	4	11



Standard curve for TNF-a

## Results

The results of the immunological study : the level of cellular kinetics in infected with the cutaneous leishmaniasis parasite .

### Cytokines (IL-6)

The results of the current study indicated an increase in the level of IL-6 cytokines when comparing patients with cutaneous leishmaniasis  $0.45 \pm 3.2$  pg | ml with healthy subjects in the control group  $0.40 \pm 0.11$  pg | ml, and according to the statistical study and at the level of significance ( $p < 0.05$ ), as shown in Table (11-4).

### Tumor necrosis factor (TNF-a)

The results of the study showed an increase in the level of TNF-a in patients with cutaneous leishmaniasis parasite in comparison with healthy people in the control group, and at a significant level ( $P < 0.05$ ), which amounted to  $0.32 \pm 0.15$  pg | ml and  $0.03 \pm 0.02$  pg | ml and respectively.

Table 11-4

Effect of Leishmania infection on some immune factors in the blood serum of infected persons

TNF-a necrosis factor concentration (pg /ml) M ± SD	Interleukin concentration IL-6 ( pg /ml) M ± SD	standards
		injury
$0.03 \pm 0.02$	$0.40 \pm 0.11$	the healthy
$0.32 \pm 0.15^*$	$4.45 \pm 3.2^*$	the injured
3.4	5.4	Arithmetic v
2.09	2.09	P Tabular $T < 0.05$

### Cellular kinetics ( interleukin- 6 )

The results of the study, as shown in Figure (1-11), showed a significant increase ( $P<0.05$ ) in the level of interleukin cellular kinetics. IL-6 in the blood serum of people with cutaneous leishmaniasis compared with healthy people, where it was  $4.45\pm 3.2$  pg | ml compared to the healthy group,  $(0.40\pm 0.11)$  pg | ml .

### Tumor necrosis factor

The results showed, as shown in Figure (2-11), a significant increase at the probability level ( $P<0.05$ ). leishmaniasis parasite compared with healthy controls, the level of tumor necrosis factor in the group of patients was  $(0.32\pm 0.15)$  pg | ml compared to healthy subjects  $(0.03\pm 0.02)$  pg | ml .

### Blood picture criteria

The current study, as shown in Table (12-4) , showed a standard blood picture of cutaneous leishmaniasis infected and the control group, and indicated the discrepancy between the levels in infected and healthy individuals, including (neutrophil cells , lymphocytes , eosinophils , total white blood cells WBC and hemoglobin) . A significant decrease was recorded at the probability level ( $P<0.05$ ) in the hemoglobin of those infected with the cutaneous leishmaniasis parasite  $(12.2\pm 1.1)$  g | dl compared with the control group  $(15.9\pm 0.66)$  g | dl . Also, both neutrophils and lymphocytes showed a significant increase ( $P<0.05$ ) when compared with the control group  $(63.1\pm 8.61)$  and  $(41.03\pm 2.42)$ , respectively . Also, white blood cells recorded a significant difference when compared to the control group and under the probability level ( $p < 0.05$ ) .

Table 12-4  
Effect of Leishmania parasite infection on some blood elements of infected persons and control group

LYM تركيز concentration M ± SD	Neutrophil % concentration M ± SD	concentration ( $10^9$ /L) M ± SD	Hb concentration (g/dl) M ± SD	standards
				injury
35.1±4.55	50.2±6.21	6.6±0.96	15.9±0.66	healthy
41.03±2.4 2*	63.1±8.61 *	* 9.3±0.96	12.2±1.1 *	afflicted
4.3	7.7	1.9	2.6	Arithmetic v
2.09	2.09	2.09	2.09	P Tabular T < 0.05

### Discussion

#### Immunological study of cutaneous leishmaniasis patients

The results of the current study, after determining the level of (TNF-a, IL6), respectively, in the blood serum of cutaneous leishmaniasis patients showed that there were significant ( $P<0.05$ ) and statistically significant differences as a result

of the immune response, which is a reaction of the patient's body to resistance to parasitic infection, after comparing with other subjects. The healthy controls (control group) where the IL6 level increased and the concentration was  $4.45 \pm 3.2$  pg | In comparison with the control group ,  $0.4 \pm 0.11$  pg | ml also increased the level of TNF-a with a concentration of  $0.32 \pm 0.15$  pg | ml and compared to the control group  $0.03 \pm 0.02$  pg | and this agrees with what was found (De Freitas , 2016) where the IL-6 concentration was high when infected  $1.66 \pm 5.47$  pg | ml and the control group  $1.28 \pm 2.85$  pg | ML, and IL-6 and TNF-a are among the most important physiological indicators associated with the occurrence of infection or some pathological syndromes . Amzar & Iqbal (2017). Macrophages, monocytes , B and T cells are responsible for the production of IL-6 and activation of the body's defenses . And stimulating the liver to form blood (de Moraes, 2018).

The production of IL-6 and TNF-a in cutaneous leishmaniasis is also associated with an increased response in the phase of antibody formation and an increase in body temperature (De Silva, 2016). De Oliveira, (2019) indicated that the immune system plays a key role in cases of infection against pathogens, where macrophages are active against parasitic products, and thus produce cytokines against inflammation and an increase in the level of IL-6 and TNF-a compared to the control group. Thus, these cytokines, after their secretion from macrophages, combine with white and T cells and are attracted at the sites of infection to resist the disease. These results were also in agreement with De Souza, 2012) in his study of 28 cases of cutaneous leishmaniasis, where elevated IL-6 was recorded at a rate of  $9.7 \pm 2.81$  pg | ml compared to the control group  $4.6 \pm 1.61$  pg | This was attributed to the height and immune strategy of the patient's body. The results agreed with (Dennis, 2018) in terms of indicating an increase in IL-6 in the serum of a group of rats infected with Leishmania parasite, where it confirmed the elevation of TNF-a and IL-6 due to inflammation, especially after contact of the parasite with the liver while stimulating the production of these cytos. Kainat.

While it was confirmed by Depledge, (2010), a significant increase occurred as a result of visceral cutaneous leishmaniasis. The current study also agreed with Al-Hassani (2020), where he found a high concentration of IL-6  $0.035 \pm 0.017$  pg | ml compared to control  $0.028 \pm 0.017$  pg | Also, a significant increase of TNF-a was recorded compared to the control group, and respectively  $0.103 \pm 0.201$  pg | ml,  $0.026 \pm 0.031$  pg | This is consistent with what we found in our current study, where TNF-a recorded an increase in concentration compared to the control group. This is due to the body's defenses and the production of hormones like TNF-a and IL-6 , which are produced by macrophages and B-cells for a humoral response. (Desbois N,2014).Recent studies have also demonstrated the ability of the prophase of Leishmania parasite to produce extracellular vesicles that stimulate cells and produce IL-6 and IL-10 and stimulate B-1 cells. to produce TNF-a ( Dostalova A, 2012) . As shown in Table (11-4).

### **Blood parameters**

The results of the current study showed a significant decrease in the hemoglobin level of people with cutaneous leishmaniasis, which amounted to  $12.2 \pm 1.1$  compared to the control group, which amounted to  $15.9 \pm 0.66$  , according to the statistical study and at a significant level ( $P < 0.05$ ). These results are consistent

with Al-Hassani study (2020). ) in Qadisiyah, where the result was close and reached in the injured  $15.82 \pm 0.83$  compared to the healthy group  $13.37 \pm 1.77$  . This decrease is attributed to cases of anemia resulting from malnutrition or lack of iron absorption in the intestines or as a result of broken red blood cells and this conclusion is consistent with what the researcher Felek (2019) also pointed out the role of leishmaniasis in its effect on the liver and spleen, and the occurrence of defects and the decrease in hemoglobin in the blood, with loss of appetite in the sufferers. Because of malnutrition and a lack of folic acid, iron and vitamin B12 , as well as the possibility of a deficiency of red blood cells or their destruction as a result of an enlarged spleen or a defect in some body functions (El-Beshbishy HA, 2013) .

Our current study showed in terms of the total number of white blood cells by recording a significant increase and difference at the level of  $p < 0.05$  , and the WBC in the injured was  $9.3 \pm 0.96$ , and compared with the control group, it amounted to  $7.65 \pm 0.96$  and it did not agree with what was reached by Al-Hassani (2020) where no significant differences were recorded. It was within the normal rates and this was attributed to several factors, including the time of taking the sample, the duration of infection, the number of parasites ingested in mononuclear cells, and the slight decrease due to their presence in the liver, spleen and bone marrow, and their absence in the bloodstream. (El-Khadragy, 2018) Our study agreed with Al-Mansoori (2007), who pointed to a decrease in white blood cells in the infected to the accumulation of blood cells as a result of the large parasite numbers and their accumulation in the liver and spleen, and their dysfunction and lack of numbers in the bloodstream. She also agreed with Moker (2006) . Which indicated an increase in the number of white blood cells.

As for the neutrophils and lymphocytes, their concentration in the blood increased, reaching higher in the infected than the healthy group. The neutrophilic cells in our current study reached  $63.1 \pm 8.6$  , a significant difference according to the statistical study and at a probability level of  $p < 0.05$  compared with the control group, which amounted to  $50.2 \pm 6.21$  The results of our study agreed with the findings of Al-Hassani (2020). Where it recorded an increase in neutrophil cells  $52.99 \pm 7.29$  compared to the control group  $62.98 \pm 9.07$ , as well as an increase in the concentration of lymphocytes, and it was  $36.24 \pm 5.51$  in the infected group and in the control group  $40.31 \pm 3.61$  Also our study agreed with the findings of Al-Mousawi (2015) in Dhi Qar from An increase in the concentration of neutrophils and lymphocytes. These results were explained by the role of these cells in defending the body against parasitic protozoa, as they digest and phagocytize them, so their number increases in the case of infections caused by parasites and bacteria, and in chronic cases, and as a result of these cells containing lysosomes , their numbers decrease, including infection with the Leishmania parasite Erejuwa , 2013 ) ) Our results also agreed with the findings of Esmaeeli , (2019), who indicated an increase in neutrophils in patients compared to the control group.

As for the concentration of lymphocytes, our study recorded a significant increase in it, which amounted to  $35.1 \pm 4.55$ , compared with the control group, which amounted to  $(41.03 \pm 2.42)$ , where there were significant significant differences. The patients in his study were  $36.24 \pm 5.51$  and the control group  $40.31 \pm 3.61$ .

This is due to the activity of the cytotoxic T -cell cells , which are characterized by fighting the cells that form inside and stimulating them to programmed death (European, 2010) and it agreed with the results of Evans KJ, (2017) .

### **Conclusions**

- I used the ELISA technique ELISA and gave results of high sensitivity in the immunological test for the diagnosis of cutaneous leishmaniasis and at a lower cost.
- The high level of cellular kinetics (TNF-a) and (IL6) in the serum of people with cutaneous leishmaniasis compared to the serum of healthy people (control group) is an important indicator of the immune response in the body.
- The parasite has an effect on some hematological variables such as low levels of Hb , pcv and an increase in other levels such as white blood cells, lymphocytes and neutrophils where several of these indicators are important in the immune response against leishmaniasis in humans.

### **Recommendations**

- Emphasis on the use and development of vaccines against the parasite and the identification of surface antigens for the parasite such as Gpb3 to stimulate immunity against the disease and strengthen the immune system to address it and reduce infection.
- Emphasizing the dissemination of health education and awareness among citizens through introductory seminars on parasite and disease and how to prevent and treat, in addition to combating the host vector and storage by spraying chemical pesticides, controlling rodents and loose dogs, urging cleanliness and disposal of waste and organic materials that are an environment conducive to the growth of the vector (Sand fly).
- Conducting scientific studies and extensive research on biological indicators related to cutaneous leishmaniasis, such as Cyto Kinetics, antioxidants and many trace elements and their effectiveness in the body's immune response.
- 4 - Emphasis on the development of effective treatments to reduce the severity of skin infection, and the search for plant extracts with positive efficacy to cure the disease.

### **References**

- Abu Dawanij , Hassan Zaati Abadi (2014 ) A study of some epidemiological aspects of leishmaniasis . Leishmaniasis and Parasite Diagnosis Using Nested - Kinetoplast Polymerase Chain Reaction Technique Minicircle DNA-PCR In Maysan Governorate, Master Thesis, College of Education for Pure Sciences, University of Basra - Iraq.
- Al Shukr, Ban Shaker Abdul Amir (2012) . Diagnosis of cutaneous leishmaniasis Al-Abdullah, Shtiwi (2012) . Physiology, first edition. Dar Al-Masira for Publishing and Distribution, Amman, Jordan.
- Al-Hassani, Mohamed Kamel Karim Taher (2020) Study of the levels of some trace elements in blood serum and their relationship with some antioxidant enzymes and immune factors in patients with cutaneous leishmaniasis and white rats .

- PhD thesis. Faculty of Education. Al-Qadisiyah University.
- Al-Moussawi, Azhar Musa Jaafar (2015) . Molecular and Immunological Study of Cutaneous Leishmania in Central and Southern Governorates . PhD thesis . College of Education for Pure Sciences. Karbala University.
- Al-Musawi, Nabil Abdul-Jabbar Abdul-Kadhim ( 2015 ) Investigation of cutaneous leishmaniasis and knowledge of the role of heat shock protein HSP70 in the immune response in Dhi Qar Governorate, Master's thesis, College of Education for Pure Sciences, University of Dhi Qar, Iraq.
- Azpurua J, De La Cruz D, Valderama A, Windsor D. *Lutzomyia* sandfly diversity and rates of infection by *Wolbachia* and an exotic *Leishmania* species on Barro Colorado Island, Panama. *PLOS Negl Trop Dis*. 2010; 4: e627, doi : to. 1371 PMID: 20231892 .
- Baaten BJ, Tinoco R, Chen AT, Bradley LM: Regulation of Antigen-Experienced T Cells: Lessons from the Quintessential Memory Marker CD44. *Frontiers in immunology* 2012, 3:23 .
- Babiker , MAA Determination of Some Micronutrients In Traditional Sudanese Food. Sudan University of Science and Technology.
- Banerjee, S., Bose, D., Chatterjee , N., Das, S., Chakraborty , S., Das, T., & Saha , KD (2016). Attenuated *Leishmania* induce proinflammatory mediators and influence leishmanicidal activity by p38 MAPK dependent phagosome maturation in *Leishmania donovani* co-infected macrophages. *Scientific Reports*, 6(1), 1-14.
- Barazesh , A., Motazedian , MH, Sattarahmady , N., Morowvat , MH, & Rashidi , S. (2018). Preparation of megalumine antimonate loaded albumin nanoparticles and evaluation of its anti-leishmanial activity: an in vitro assay. *Journal of Parasitic Diseases*, 42(3), 416-422.
- Barbosa, FMC, Dupin , TV, Toledo, MDS, Reis, NFDC, Ribeiro , K., Cronemberger -Andrade, A., et al. (2018). Extracellular vesicles released by *Leishmania (Leishmania ) amazonensis* promote disease progression and induce the production of different cytokines in macrophages and B1 cells. *Front. Microbiol* . 9, 1—14. doi : 10.3389/ fmicb . 2018. 03056
- Barrera, P.; Sülsen , VP; Lozano, E.; Rivera, M.; Beer, M.F.; Tonn , C.; Martino, VS; Sosa, MA Natural Sesquiterpene Lactones Induce Oxidative Stress in *Leishmania Mexicana* . *Evid . Based Complement. Alternate . Med*. 2013, 2013, 163404. [ CrossRef ] [PubMed]
- Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A, Ben Alaya N, et al (2013) Topical paromomycin with or without gentamicin for cutaneous leishmaniasis . *N Engl J Med* 368: 524-532.
- Ben Salah A, Ben Messaoud N, Guedri E, Zaatour A. Ben Alaya N. et al. Topical paromomycin with or without gentamicin for cutaneous leishmaniasis . *N Engl J Med* 368: 524-532 .
- Bhattacharya, PT, Misra SR, & Hussain , M. (2016). Nutritional aspects of essential trace elements in oral health and disease: an extensive review. *Scientifica* , 2016.
- Bhattarai NR, Dujardin JC, Rijal S, De Doncker S, Boelaert M, Van der Auwera G: Development and evaluation of different PCR-based typing methods for discrimination of *Leishmania donovani* isolates from Nepal. *Parasitology* 2010 , 137(6): 947-957.
- Bianchi, C., Kostygov , AY, Kraeva , N., Záhonová , K., Horáková , E., Sobotka , R., Yurchenko , V. (2019). An enigmatic catalase of *Blastocritidia* . *Molecular*

- and *Biochemical Parasitology*, 232, 111-119.
- Bianchi, S., Bernardi, S., Continenza, MA, Vincenti, E., Antonouli, S., Torge, D., & Macchiarelli, G. (2020). Scanning electron microscopy approach for evaluation of hair dyed with *Lawsonia inermis* powder: in vitro study. *Int. j. morphol.*, 38(1), 96-100.
- Biswas, A., French, T., Düsedau, HP, Mueller, N., Riek-Burchardt, M. Dudeck, A., Dunay, IR (2017). Behavior of neutrophil granulocytes during *Toxoplasma gondii* infection in the central nervous system. *Frontiers in cellular and infection microbiology*, 7, 259
- Blazkova, J., Gupta, S., Liu, Y., Gaudilliere, B., Ganio, EA, Bolen, CR Hasni, S. (2017). Multicenter systems analysis of human blood reveals immature neutrophils in males and during pregnancy. *The Journal of Immunology*, 198(6), 2479-2488.
- Boelaert M, Sundar S. (2014). Leishmaniasis . In: Farrer J, Hotez P, Junghanss T, Kang G, Lalloo D, White NJ, editors. *Manson's Tropical Infectious Diseases*. 23rd ed. Philadelphia: Elsevier Saunders. p. 631—51.
- Browne, N., Heelan, M., & Kavanagh, K. (2013). An analysis of the structural and functional similarities of insect hemocytes and mammalian phagocytes. *Virulence*, 4(7), 597-603.
- Bualert L, Charungkiattikul W, Thongsuksai P, Mungthin M, Siripattanapipong S, Khositnithikul R, et al. Autochthonous disseminated dermal and visceral leishmaniasis in an AIDS patient, southern Thailand, caused by *Leishmania siamensis* . *Am J Trop Med Hyg* . 2012; 86: 821—824. doi : 10.4269/ajtmh.2012.11-0707 PMID: 22556080.
- Cabello- Donayre , M., Malagarie-Cazenave , S., Campos-Salinas, J., Gálvez , FJ, Rodriguez-Martinez, A., Pineda-Molina, E., Estévez , AM (2016). Trypanosomatid parasites rescue heme from endocytosed hemoglobin through lysosomal HRG transporters. *Molecular Microbiology*, 101(6), 895-908.
- Calderón Guzmán , D., Juárez Olguin , H., Osnaya Brizuela , N., Hernández Garcia, E., & Lindoro Silva, M. (2019). The use of trace and essential elements in common clinical disorders: roles in assessment of health and oxidative stress status. *Nutrition and cancer*, 71 (1), 1320.
- Campos, TM, Novais , FO, Saldanha , M., Costa, Re, Lordelo , M., Celestino , D., Machado, P. (2020). Granzyme B produced by natural killer cells enhances inflammatory response and contributes to the immunopathology of cutaneous leishmaniasis . *The Journal of Infectious Diseases*, 221 (6), 973-982.
- Carneiro , PP, Conceição , J., Macedo , M., Magalhães , V., Carvalho , E. Me, & Bacellar , O. (2016). The role of nitric oxide and reactive oxygen species in the killing of *Leishmania braziliensis* by monocytes from patients with cutaneous leishmaniasis . *Plos one*, 11 e0148084.
- Castanys -Munoz E, Brown E, Coombs GH, Mottram JC: *Leishmania mexicana* metacaspase is a negative regulator of amastigote proliferation in mammalian cells. *Cell death & disease* 2012, 3:e385 .
- Celes , FS, Trovatti , E., Khouri , R., Van Weyenbergh , J., Ribeiro , SJ, Borges, VM, De Oliveira, C. 1. (2016). DETC-based bacterial cellulose bio-curatives for topical treatment of cutaneous leishmaniasis . *Scientific Reports*, 6(1), 1-11.
- Chakrabarti , S.; Sarkar , S.; Goswami , BK (2013): Clinico-haematological Profile of visceral leishmaniasis in immunocompetent patients. *Southeast Asian J Trop Med Public Health*. 44: 143-149.
- Chatterjee , N., Das, S., Bose, D., Banerjee, S., Jha , T., & Saha , KD (2015).

- Leishmanial lipid affords protection against oxidative stress induced hepatic injury by regulating inflammatory mediators and confining apoptosis progress. *Toxicology letters*, 232(2), 499-512.
- Chauhan , P., Shukla , D., Chattopadhyay , D., & Saha , B. (2017). Redundant and regulatow roles for TLRs in Leishmania infection. *Clin Exp Immunol .*, 10. 1111.
- Chen, J., Yu, B., Chen, D., Huang, Z., Mao, X., Zheng , P., He, J. (2018). Chlorogenic acid improves intestinal barrier functions by suppressing mucosa inflammation and improving antioxidant capacity in weaned pigs. *The Journal of nutritional biochemistry*, 59, 84-92.
- Chiku , VM, Silva, KLO, de Almeida, BF Ms , Venturin , GL, Leal, AAC, de Martini, CC, de Lima, VMF (2016). PID-I function in apoptosis of T lymphocytes in canine visceral leishmaniasis . *Immunobiology* , 221(8), 879-888.
- Chu N, Thomas BN, Patel SR, Buxbaum LU: IgG1 is pathogenic in *Leishmania mexicana* infection .
- Cogger , VC, Hunt, NJ, & Le Couteur , DG (2020). Fenestrations in the liver sinusoidal endothelial cell. *The Liver: Biology and Pathobiology*, 435-443.
- Conceicao , J., Davis, R., Carneiro , PP, Giudice , A., Muniz, AC, Wilson, Bacellar , O. (2016). Characterization of neutrophil function in human cutaneous leishmaniasis caused by *Leishmania braziliensis* . *PLoS neglected tropical diseases*, 10 (5) .
- Covre , LP, Devine, O., Garcia de Moura , R., Vukmanovic-Stejic , M., Dietze , R., Rodrigues, RR, Akbar, AN (2020). Companmentalized cytotoxic immune response leads to distinct pathogenic roles of natural killer and senescent CD8+ T cells in human cutaneous leishmaniasis . *Immunology*.
- Cunha J (presenting author), Carrillo E, Sánchez C, Tavares J, Moreno J and Cordeiro -da-Silva A: Characterization of the virulence of *Leishmania infantum* isolates from human patients. *Immunology 2012*, 137, Issue Supplement s1 (Special Issue: Abstracts of the European Congress of Immunology, 5-8 September 2012, Glasgow ,
- Cunha J (presenting author), Carrillo E, Sánchez C, Tavares, J, Moreno J and Cordeiro -da-Silva A: Characterization of the virulence of *Leishmania infantum* isolates from human patients. 3rd European Congress of Immunology, September 5-8, 2012, Glasgow, Scotland, Poster 1168
- Cunha J (presenting author), Moreno J and Cordeiro -da-Silva A: Comparative study between naturally attenuated and virulent strains of *Leishmania infantum* : culture conditions and infectiveness. *Acta Parasitológica Portuguesa 2010*, 17(2; Congresso Português de Parasitologia , 8-10 September 2010, Porto, Portugal: 97 .
- Cunha J (presenting author), Moreno J, Cordeiro -da-Silva A: Comparative study between naturally attenuated and virulent strains of *Leishmania infantum* : culture conditions and infectiveness. XIV Congress Português de Parasitologia 2010, Porto, Portugal, Poster P-31
- Cunha J (presenting author), Moreno J, Cordeiro -da-Silva A: In vitro comparative study between naturally attenuated and virulent strains of *Leishmania infantum* . 2nd 13S Scientific Retreat, May 5-6, 2011, P6voa de Varzim , Portugal, Poster 12
- Cunha J, Carrillo E, Sánchez C, Cruz I, Moreno J and Cordeiro -da-Silva A: Characterization of the biology and infectivity of *Leishmania infantum* Viscerotropic and dermatropic strains isolated from HIV+ and HIV- patients in

- the murine model of visceral leishmaniasis . *Parasites and Vectors* 2013, 6: 122
- Cunningham, AF, Flores- Langarica , A., Bobat , S., Dominguez Medina, CC, cook, CN, Ross, EA, Henderson, I. R. (2014). B cells recognize protective antigens after natural infection and vaccination, *Frontiers in immunology*, 5, 535.
- Cutaneous Leishmaniasis by PCR technique with studying the effect of plant extracts on parasite numbers and viability *ex vivo*. Master's Thesis. College of Education for Girls. University of Kufa
- da Silva Vieira, T., Arango Duque, G., Ory , K., Gontijo , CM, Soares , RP, & Descoteaux , A. (2019). *Leishmania braziliensis* : strain-specific modulation of phagosome maturation. *Frontiers in cellular and infection microbiology*, 9, 319.
- Dafi'i , Rana Saleh Sahib Mahlol ( 2013 ) , The prevalence of cutaneous leishmaniasis in Al-Qadisiyah Governorate with evaluation of the response to treatment with Bent and TTM by RT-PCR , Master's thesis, College of Education, University of Al-Qadisiyah .
- Daham, Amal Kamel and Al- Alusi , Tawfiq Ibrahim (2011) . Epidemiological survey of cutaneous leishmaniasis in Salah El-Din Governorate. Diyala Locality for Agricultural Sciences.
- das Does Moreira, N., Vitoriano -Souza, J., Roatt BM, de Abreu Vieira, PM, Coura -Vital , W., de Oliveira Cardoso, JM Carneiro , CM (2016). Clinical, hematological and biochemical alterations in hamster ( *Mesocricetus auratus* ) experimentally infected with *Leishmania infantum* through different routes of inoculation. *Parasites & vectors*, 9(1), 181.
- Das, M., & Das, R. (2013). Effect of Modern Instructional Aids for Developing Awareness Towards Zinc Deficiency Among Rural Females of Sagar District, Madhya Pradesh, India.
- De Freitas , EO; Leoratti , FM; Freire -de-Lima, CG; Morrot , A.; Feijó, DF The Contribution of Immune Evasive Mechanisms to Parasite Persistence in Visceral Leishmaniasis . *Front. Immunol* . 2016, 7, 153. [ CrossRef ] [PubMed]
- de Moraes , ARDP, Tavares, GD, Rocha, FJS, de Paula, E., & Giorgio, S. (2018). Effects of nanoemulsions prepared with essential oils of copaiba-and andiroba against *Leishmania infantum* and *Leishmania amazonensis* infections. *Experimental parasitology*, 187, 12-21.
- De Oliveira, C I., Brandão , LO, celes , FS, & Paiva , CN (2019). The paradoxical leishmanicidal effects of Superoxide Dismutase (SOD)mimetic Tempol in *Leishmania braziliensis* infection *in vitro*. *Frontiers in cellular and infection microbiology*, 9, 237.
- De Oliveira, CI, Brandão , LO, celes , FS, & Paiva , CN (2019). The paradoxical leishmanicidal effects of Superoxide Dismutase (SOD)mimetic Tempol in *Leishmania braziliensis* infection *in vitro*. *Frontiers in cellular and infection microbiology*, 9, 237.
- De Silva, L., Somaratne , V., Senaratne , S., Vipuladasa , M., Kahatapitiya , S., De Silva, D., Wickremasinghe , R. (2016). Analysis of clinical features with microscopy of cutaneous leishmaniasis suspected patients attending the dermatology clinic at general hospital Hambantota .
- De Souza, C. d. SF, Calabrese, K. d. S., Abreu-Silva, AL, Carvalho , LOP, Cardoso, F. d. O., Dorval, M., Pacheco, R. d. S. 2018). *Leishmania amazonensis* isolated from human visceral leishmaniasis : histopathological analysis and

- parasitological burden in different inbred mice. *Histol Histopathol* , 33(7), 705-716.
- Deak E, Jayakumar A, Cho KW, Goldsmith- Pestana K, Dondji B, Lambris JD, McMahon-Pratt D: Murine visceral leishmaniasis : IgM and polyclonal B-cell activation lead to disease exacerbation .
- Deak E, Jayakumar A, Cho KW, Goldsmith- Pestana K, Dondji B, Lambris JD, McMahon-Pratt D: Murine visceral leishmaniasis : IgM and polyclonal B-cell activation lead to disease exacerbation .
- Dennis, S.G. (2018). *Engineering Soft Tissue Replacements and the Development of Novel Technologies to Heal These Wounds*. Clemson University.
- Depledge DP, MacLean LM, Hodgkinson MR, Smith BA, Jackson AP, Ma S, Uliana SR, Smith DF: Leishmania -specific surface antigens show sub-genus sequence variation and immune recognition. *PLOS neglected tropical diseases* 2010, 4(9):e829 .
- Desbois N, Pratlong F, Quist D, Dedet J. *Leishmania ( Leishmania ) mattiniquensis n. sp. ( Kinetoplastida : Trypanosomatidae )*, description of the parasite responsible for cutaneous leishmaniasis in Martinique Island (French West Indies). *Parasite*. 2014; 21: 12. doi : 10.1051/parasite/2014011 PMID: 24626346 .
- Dostálová A, Volf P. *Leishmania* development in sandflies: parasite-vector interactions overview. *Parasite Vector*. 2012; 5: 276-288 .
- Dostalova A, Volf P: *Leishmania* development in sand flies: parasite-vector interactions overview. *Parasites & vectors* 2012, 5:276 .
- El - khadragy , M., Aiolayan , EM, Metwally , DM, El-Din, MFS, Alobud , SS, Alsultan , NI, Abdel Moneim , AE (2018). Clinical efficacy associated with enhanced antioxidant enzyme activities of silver nanoparticles biosynthesized using moringa oleifera leaf extract, against cutaneous leishmaniasis in a murine model of leishmania major. *International journal of environmental research and public health*, 15(5), 1037.
- El- Beshbishy HA, Al-Ali KH, El- Badry AA (2013) Molecular characterization of cutaneous leishmaniasis in Al- Madinah Al- Munawarah province, western Saudi Arabia. *Int J Infect Dis* 17: e334-c338 .
- El- Beshbishy HA, Al-Ali KH, El- Badry AA (2013) Molecular characterization of cutaneous leishmaniasis in Al- Madinah Al- Munawarah province, western Saudi Arabia. *Int J Infect Dis* 17: e334-e338.
- El Hajj, R. (2018). Efficacy of the Imiquimod analog EAPB0503 against Cutaneous Leishmaniasis : A promising new treatment paradigm.
- Erejuwa , OO, Sulaiman , SA, & Ab Wahab , MS (2013). Evidence in support of potential applications of lipid peroxidation products in cancer treatment. *Oxidative Medicine and Cellular Longevity*, 2013.
- Esmaeeli , S., Hoseinirad , SM, Rajabian , M., Taheri , AR, Berenji , F., & Hashemy , SI (2019). Evaluation of the oxidant-antioxidant balance, isoprostane and quantitative CRP in patients with cutaneous leishmaniasis . *Microbial pathogenesis*, 137, 103738.
- European journal of immunology* 2010, 40(5): 1355-1368 .
- Evans KJ, Kedzierski L: Development of Vaccines against Visceral Leishmaniasis . *Journal of tropical medicine* 2012, 2012:892817.
- Kashkol, Abbas Hayawi ( 2009 ). Some Environmental and Biological Aspects of Harms Phlebotominae ( Diptera : Phlebotomidae ) and the epidemic of cutaneous leishmaniasis in Al- Diyaniyah governorate . Master.College Sciences . Al-

- Qadisiyah University: 99 p.
- Mayali , Hadi Madlool Hamza (2014) . Evaluation and use of some immunological tests in the epidemiological study of leishmaniasis in Al-Qadisiyah Governorate. PhD thesis . College of Education for Girls, University of Al-Qadisiyah.
- Moker, Hanadi Mohsen Mahdi (2006) . Cutaneous leishmaniasis in Basra Governorate, an epidemiological, immunological and therapeutic study, a master's thesis. College of Science, University of Basra.
- Parasite, Rasha Amer Nouri (2003) The epidemiology of leishmaniasis and its relationship to the vector insect Harms. stinger Psychodidae : Diptera in Najaf Governorate. Master Thesis . College of Science, University of Kufa.
- Salman, Reem Ihsan Youssef (2017) . Study of some immunological aspects and histological effects of patients with cutaneous leishmaniasis in Najaf Governorate. Master Thesis . College of Education for Girls, University of Kufa.
- Shaban, Maha and Nahas, Samar (2004) . Epidemiology of cutaneous leishmaniasis for auditors of the Dermatology and Venereology Hospital and Comprehensive Clinics in Damascus. Damascus University Journal of Basic Sciences Volume 19 First Issue: 124-113
- Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550–561. <https://doi.org/10.1002/cae.22202>
- Yasiri , Saleh Mahdi (2012) . Study of the relationship of sand fly species with leishmaniasis in Maysan Governorate. *Maysan Research Journal*, Volume Eight, Issue Sixteen.