

Study of Some Haematological Parameters for Children Infected With Visceral Leishmaniasis

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Abstract: The present study was carried out on 50 patients infected with suspected visceral leishmaniasis showed that the infection in male's population was more than female's population. Also, the study showed high percentage of disease was in patients with visceral leishmaniasis infection at age group 1 year to 3.11 months, when compared with other age group. And this study showed the rate of prevalence in January and February months compared with other months. Also this study indicated a decrease in hemoglobin ratio and white blood cells counts in patients which infected with visceral leishmaniasis, compared with other groups. The data in this study showed decrease in packed cell volume in patients whose infected with this disease, compared with other groups, while the monocytes should increase within normal range. Finally the present study showed the significant correlation between white blood cells and hemoglobin concentration.

Keywords: Leishmaniasis, visceral leishmaniasis, rate of prevalence, hemoglobin, white blood cells counts, packed cell volume, monocytes.

I. Introduction

Leishmaniasis, a vector-borne disease that is caused by obligate intra-macrophage protozoa, is endemic in large areas of the tropics, subtropics and the Mediterranean basin. This disease is characterized by both diversity and complexity [1]. Leishmaniasis consists of four main clinical syndromes: Cutaneous leishmaniasis; muco-cutaneous leishmaniasis; visceral leishmaniasis (VL) and post-kala-azar dermal leishmaniasis (PKDL) [2]. Visceral leishmaniasis (VL), also known as kala-azar, black fever, and Dum Dum fever [3], is the most severe form of leishmaniasis, this disease is the second-largest parasitic killer in the world (after malaria) responsible for an estimated 500,000 cases each year worldwide [4]. Kala-azar, so called for the darkening of the skin on the extremities and abdomen that is a symptom of the Indian form of the disease. The agent of the disease was also first isolated in India by Scottish doctor William in 1903 and Irish physician Charles Donovan, working independently of each other. As they published their discovery almost simultaneously, the species was named for both of them *Leishmania donovani*. Today, the name kala-azar is used interchangeably with the scientific name visceral leishmaniasis for the most acute form of the disease [5]. In Iraq the infectious agent is *L. donovani*. The yearly incidence of VL was less than 1000 cases before 1990, most of them in the central part of Iraq within a 100 km radius around Baghdad. After the Gulf war 1991, the annual incidence increased to over 3000 cases. In 1989: 491 cases while in 1991: 3,713 cases (incidence rate per 100,000). Many factors could explain the increase of VL in Iraq in recent years: Deterioration of the health status of children below 5 years: malnutrition, immunodeficiency and co-infections are probably the most important factors. And population movements from urban to rural settings due to economic difficulties that followed the imposition of sanctions: this can bring non-immune population into transmission areas: Increased density of sand fly vectors due to increased number of breeding sites generated by the destruction of water and sanitation systems, and shortage of proper insecticides, spraying and fogging machines and other supplies and equipment, also inefficient sewage treatment and disposal system. As well as accumulation of garbage in urban settings. And last but not least increase in canine (dog etc.) population [6]. Therefore the aims of this study were to determine the most of hematological parameters such as the total white blood cell count, the differential count of monocyte, level of blood hemoglobin, and packed cell volume in children infected with visceral leishmaniasis.

II. Materials and Methods

1-Sample collection:

5 ml of Venous Blood had been collected from (50) patients for detection hemoglobin (Hb) & packed cell volume (PCV) & Total WBC count. For (30) of these patients differential count of WBC done for detection the monocyte percentage in addition to the other tests that written above.

2-Materials:

1. Blood sample (5 ml).
2. 0.02 ml pipette (Sahli pipette).
3. Test tube and rack.
4. Diluting fluid (glacial acetic acid).
5. Pasteur pipette or capillary tube.
6. Improved new Bauer chamber.
7. Slides and smooth edges spreader.
8. Leishman stain and staining rack.
9. Distilled water.
10. Microhaematocrit centrifuge.
11. Scale reader.
12. Heparinized capillary tube.
13. Sealing material or soft wax clay.
14. Sterile blood lancet.
15. 70% ethanol.
16. Photometer (colorimeter or spectrophotometer).
17. Standard solution.

3- Methods:

3.1- Total white blood cell count

Method:

1. Measure 0.4 ml of diluting fluid and transfer to test tube.
2. Measure 0.02 ml of blood by micropipette and transfer to test tube which contains diluting fluid.
3. Mix and charge the chamber by capillary tube.
4. Leave to settle for about 2 minutes.
5. Count the cell in the 4 corner squares per cubic mm.

Calculation: Total white blood cell count=number * 50

3.2 Differential count of leukocytes

Method:

1. Write the name of patient on clean and slide.
2. Spread drop of patient's blood on the slide by spreader at 45 angles.
3. Leave to dry in air, and put on staining rack.
4. Put 10 drops of Leishman stain on the film, and leave for 2 minutes.
5. Add double amount of distilled water and mix by blowing.
6. Leave for 10 minutes and wash with distilled water.
7. Clean the back of slide by water and dry it by piece of cloth.
8. Leave the slide dry in air.
9. Examine under oil immersions lens.
10. Count 200 leukocyte and record the percentage of each type.

3.3 Hematocrit (HCT), packed cell volume (PCV)

3.4 Estimation of Hb by cyanomethemoglobin method

Principle:

It is measuring the ratio of volume occupied by the red cells to the volume of whole blood in a sample of capillary blood after centrifugation, & it is recorded as the (%).

Method:

1. Wipe the tip of the finger with anti-septic.
2. Allow the finger to dry.
3. Stick the tip with sterile lancet.
4. Full 2/3 of heparins capillary tube from the drop of blood by just touching the side of the drop with the tube.
5. Seal one end of the tube with sealing wax.
6. Place the capillary tube in hematocrit centrifuge, the sealed end away from the center.
7. Centrifuge for 5 min.
8. Read, by using the proper, and placing the two ends of the capillary on the zero line & 100 mark line by sliding on the reader.

Principle:

The hemoglobin is converted by the action of ferricyanide to methemoglobin; this is then converted to cyanomethemoglobin by the action of potassium cyanide. The absorption then measured with spectrophotometer at wave length of 540 nm.

Method:

1. Take 5 m of drab kin solution in test tube.
2. Draw 0.02 ml of blood and transfer it to test tube which containing drabkin solution.
3. Mix well.
4. Leave for 5 minute to complete reaction.
5. Read the absorbance at 540 nm by using drabkin solution as a blank.
6. Read the standard solution of test.
7. Refer to calibration curve (standard curve) to find out the concentration of Hb in g/dl. This can simplify by taking the absorbance of the standard equivalent to 15 g/dl and applying the following formula: -Hb

$$\text{Concentration of test} = \frac{\text{Absorbance of the test solution}}{\text{Absorbance of the standard}} \times \text{conc. Of standard.}$$

III. The Results

The following table and figures involved the results for many parameters done to evaluate to evaluate pediatric affected by *L. donovani*. These parameters included following:

1- Prevalence of kala-azar in relation to gender

The table and figure (1) showed that were the males higher infected 54% (52/97) than female 46% (45/97) in patients groups.

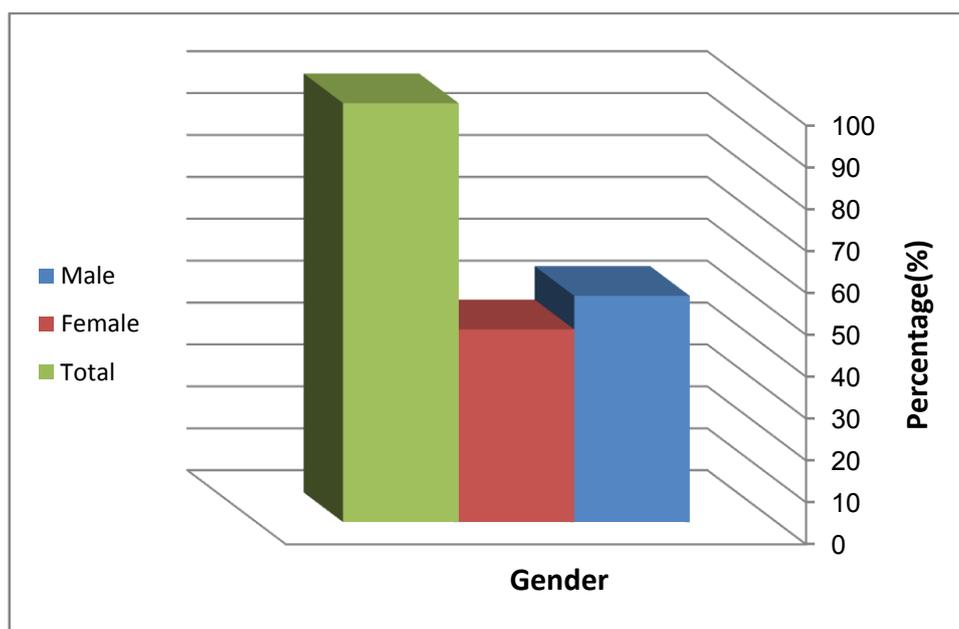


Fig. (1): Prevalence kala-azar depending on gender

2- Distribution of kala-azar according to age group

Results in table and figure (2) showed the highest infection with kala-azar occur in the age group (1 year-3.11 month), which were 61% (59/97) than other age group such as 7 years – 12 years 6% (6/97).

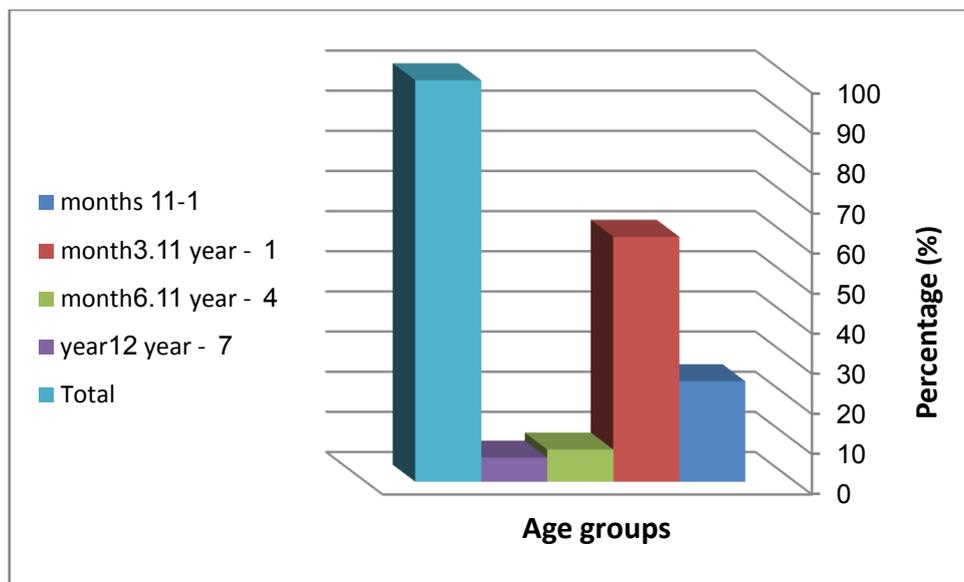


Fig. (2): Distribution of kala-azar patients according to age

3- Monthly distribution of Kala-azar during 2011

The table and figure (3) showed the highest infection rate of Kala-azar were appeared during January and February 2011 which were 16.67% (9/54), while the lowest rate were during May 2011, which were 3.70 % (2/54). But not found any infections were in August and September 2011.

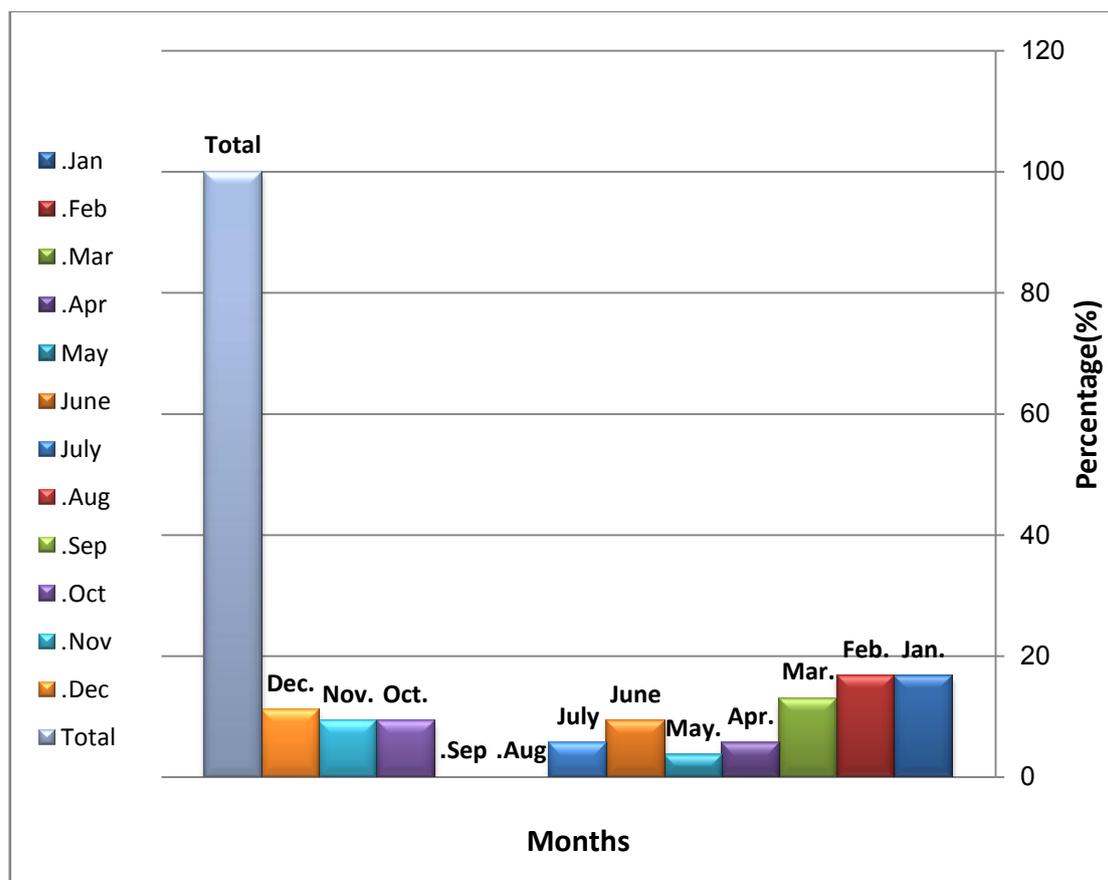


Fig. (3): Monthly distribution of Kala-azar during 2011

4- Monthly distribution of Kala-azar during 2012

Table and figure (4) show the highest infection rates of Kala-azar were appeared during January. 2012, were (10/43), but the lowest rate infection were during August, October and December.2012, which were (2/43), and don't have any infection were in July and November 2012.

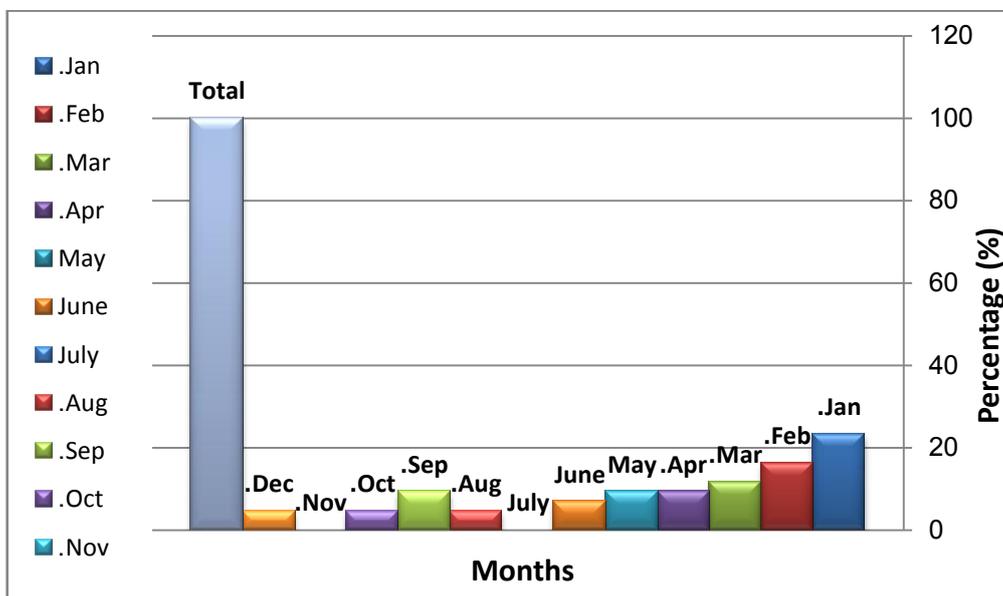


Fig. (4): Monthly distribution of Kala-azar during 2012

5-Prevalence of Kala azar in provinces of Iraq

The higher prevalence of Kala –azar was seen at many Diyala 46.39% (45/97), Baghdad 19.59% (19/97) and Wassit 15.46% (15/97) than in other provinces such as Al-Anbar and Salah Al-Din 1.03% (1/97).

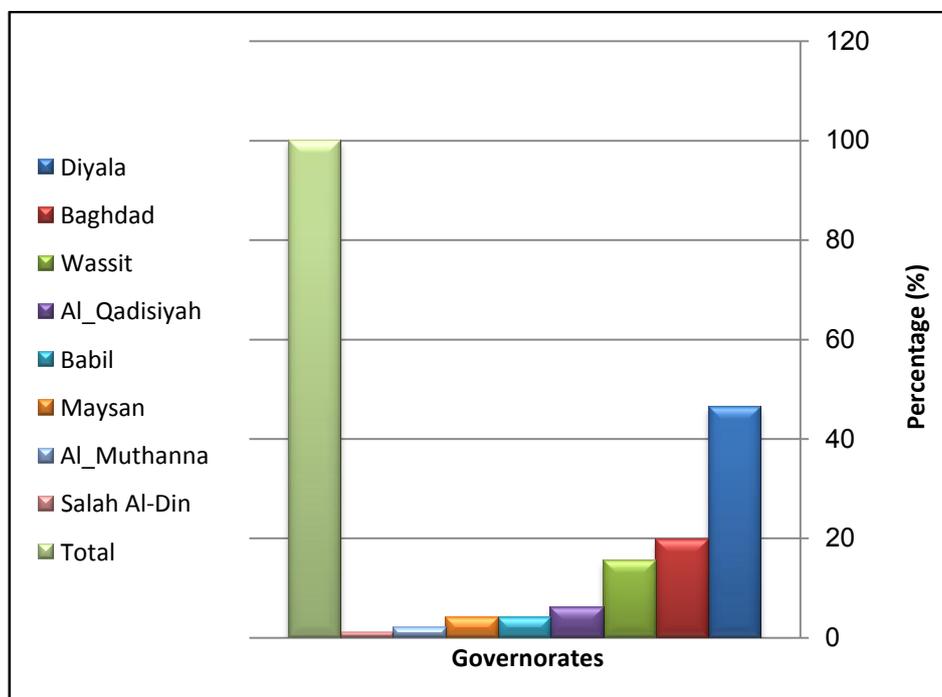


Fig. (5): Prevalence of Kala –azar in provinces of Iraq

6 - WBCs count in patients infected with kala-azar

Table and figure (6) observed that the data showed decrease in number of WBCs counts in visceral leishmaniasis patients especially under normal range, which were 77 % (31/50).

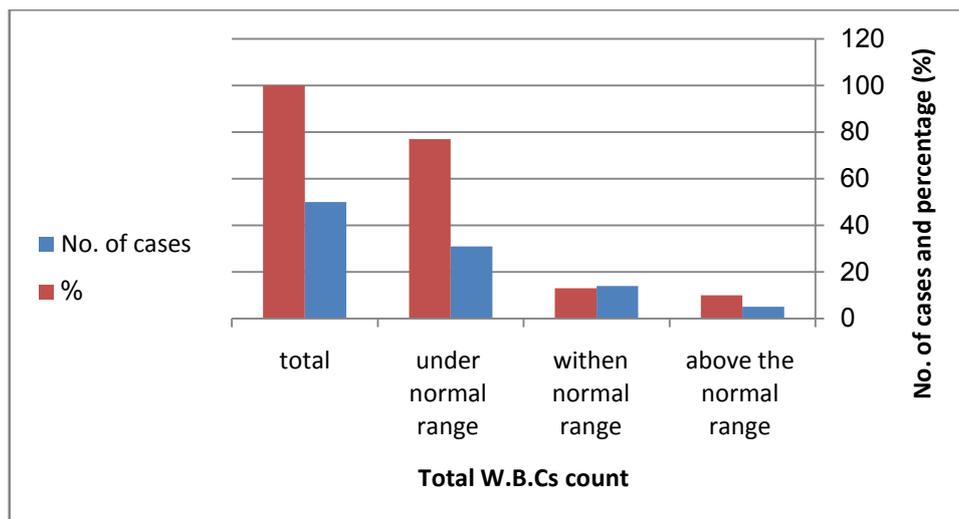


Fig. (6): WBCs count in patients infected with kala-azar

7- Monocytes count in patients infected with kala-azar

The results in tables and figure (7) showed that there were highest percentages of monocyte in within normal range, which were 70 % (21/30) When compared with other ranges.

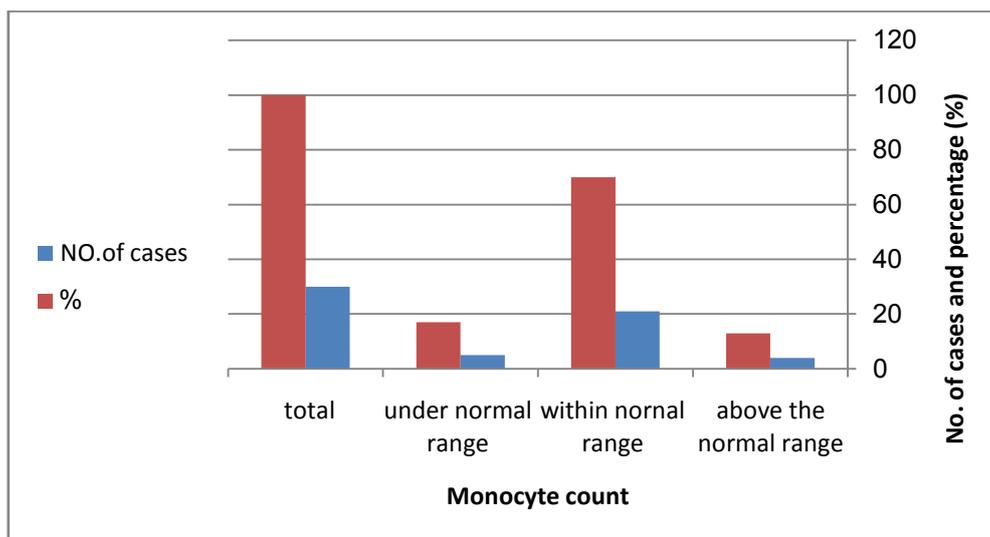


Fig. (7): Monocytes count in patients infected with kala-azar

8 - Packed cells volume (PCV) in patients infected with kala-azar

Results in table and figure (8) showed that there were higher decreases in the concentration of (PCV) in patient infected with kala-azar, which decrease reach to 94% (47/50) in patient who classified as under the normal range comparison with other ranges.

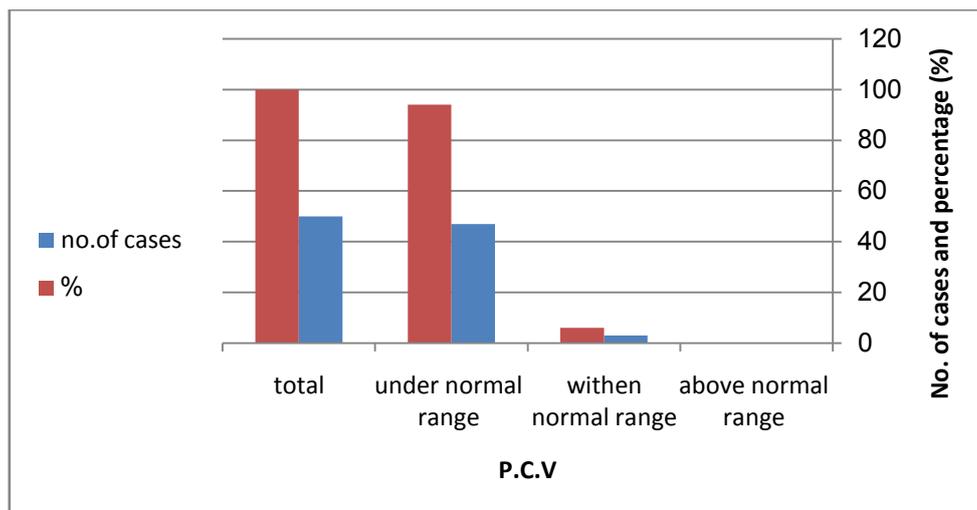


Fig. (8):Packed cells volume in patients infected with kala-azar

9- Concentration of Hb in patients infected with kala-azar

Results in table and figure (9) showed that there was decrease in the concentration of hemoglobin in patient infected with VL, Which were 97% (47/50) when compared with other mean concentration of hemoglobin in normal range and above normal range.

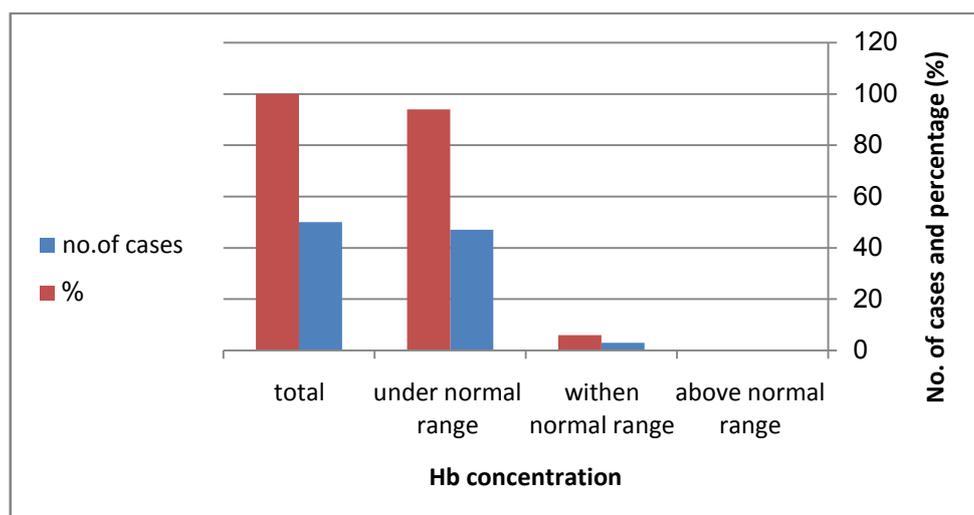


Fig. (9)Concentration of Hb in patients infected with kala-azar

Tab. (10): Mean and Standard Deviation for Total W.B.Cs and Hemoglobin

Descriptive Statistics			
	Mean	Std. Deviation	N
Total wbc	7.1776	4.1659	50
Hemoglobin	8.3286	2.3294	50

Tab. (11):The correlation between Total W.B.Cs and Hemoglobin

		Total wbc	Hemoglobin
Total wbc	Pearson Correlation	1.000	.171
	Sig. (2-tailed)	.	.235
	N	50	50
Hemoglobin	Pearson Correlation	.171	1.000
	Sig. (2-tailed)	.235	.
	N	50	50

IV. Discussion

Visceral leishmaniasis has become an arising public health problem in various regions and countries, with the highest prevalence of the disease. In Iraq it has been prevalent for more than eight decades causing a serious public health problem [7]. These results showed that the number and percentage of male patients were higher than the number and percentage of female patients, a result similar to other studies such as Mahdy [8] who found that the number and percentage of male patients (40, 56.3%) were higher than female patients (31, 43.7%). Also the study of Kumar *et al.* [9] who found that infection of V.L. had higher prevalence in males than females in India. The prevalence of V.L. in patients with age groups ranging from 1 year – 3.11 months were more prevalent than other age group ranges, this means that the disease affects infants and young children and decreases with increasing age. These findings might be due to immunological mechanisms including humoral and cell mediated immune response that will be considered in an infant is not well developed at an early stage of life [10]. Also the children with age 1-3 years were more susceptible to infection, because they started to develop their own immune system [11]. But older children in age 3-7 years were less liable to infection probably, because they developed their own immune system depended on an acquired immunity [12]. Similar results were obtained in study of Mahdy [8] who found that the patients in age groups (1-2.9 and 3-4.9) years were respectively more prevalent than other age group ranges. But this study disagreed with Mohammed [13] who found that the patients with age groups (<1, 1-2.9) years were higher than other age groups. In this study, a comparison between Diyala, Baghdad and Wassit and with other provinces in Iraq, which showed a highly percentage difference. Therefore, the proportion of kala-azar infection in Diyala, Baghdad and Wassit are higher than other provinces in Iraq. This may be due to many reasons, such as the living standard, way of living, the presence of reservoir or domestic animals, and above all the viability of infected sand fly due to suitable environmental conditions [14]. Additionally, all risk factors favorable for an outbreak of V.L. such as inefficient sewage treatment and disposal system and accumulation of garbage in urban setting, increase density number or breeding sites generated by the destruction of water and sanitation system [15]. This study disagreed with other study such as study of Al-Gunima [16] who found that the infection with Kala-azar in Wassit higher than in Baghdad. But this result agreed with Nuseif [17] who found that the increased of V.L. cases in middle and southern governorates of Iraq. While monthly distribution of cases registered during winter season mainly January and February months, due to growth and propagation of vector and declined in July and other months. These results are disagreed with Korzeniewski [18] who found the peak incidence of cases was during December. Distribution of kala-azar during January month. The present data showed in WBCs counts in V.L. patients. The decreased in WBCs counts considered to be the results of pooling and probable reduction of neutrophils in spleen and possibly the liver due to the presence of infection with *L. donovani* [19]. Our result obtained disagreement with Grechet *al.* [20] who stated that decrease in the number of WBCs counts in patients with VL. Comparison with normal individuals, but this decrease was within abnormal value of WBCs counts. But these results agreed with Herwaldt [1] who stated that decreased of WBCs counts was one of signs and symptoms of V.L. patients. This study showed that there was higher decrease in the mean concentration of hemoglobin in patients, who's infected with kala-azar. The result of this study agreed with other studies such as Abd [21]. When found that the anemia was almost a variable finding in kala-azar patients. Anemia was probably due to reduction of life span of erythrocyte or hemolysis occurring in both the massively enlarged spleen and liver due to hyperplasia of Kupffer cells which are packed with amastigote form of the parasite. The anemia that happened in VL patients which also can be explained by the effect on the red cell precursors in the bone marrow by the infected with *L. donovani* [22]. The percentage of the monocyte in this study was within normal range, therefore this result disagreed with Pearson *et al.* [23]. When found increase the monocytes and lymphocytes, but occurred decreases in thrombocytopenia. This difference in the results may be due to the number of monocytes in the samples were less, or may be dependent on the number of parasites which ingested by the monocytes. Then refers to the results of examination was apparent after many days in the laboratory [24]. But the correlation between the counts of white blood cells and hemoglobin was significantly relationship. This result agreed with Lafuse *et al.* [25]. Who's found that the hamsters infected with leishmaniasis were anemic with significantly decreased hemoglobin levels and hematocrit. Leucopenia was present with numbers of red and white blood cells in the blood. But our result disagreed with Carneiro de Freitas *et al.* [26]. When found the groups of animals that infected with leishmaniasis, there were no changes observed in the parameters of the white blood cells. But there was a reduction in the mean values of erythrocyte, hematocrit and hemoglobin. The results in this study refers to the white blood cells which consider as a first line for immunity system which effected when infection with visceral leishmaniasis, then the patients suffer from decreases in all number of white blood cells, generally accompanied increased in percentage in monocytes and anemia due to short in life cycle of erythrocytosis in blood [23].

V. Conclusions

1. According to this study, an increased male: female ratio was found in patients with visceral leishmaniasis.
2. A higher prevalence of patients at age range groups of (1 year 3.11 month) among other age range groups.
3. The Kala-azar infection was higher in Diyala and Baghdad province than other provinces in Iraq.
4. The highest infection rate of Kala-azar was appeared during January and February in 2011-2012 than other months.
5. The laboratory results showed decreased in WBCs counts in V.L. patients compared with normal and above the normal range.
6. The blood test showed high significance in percentage of monocyte especially in the normal range comparison with other ranges.
7. This study recorded highest increased in concentration of PCV especially in under the normal range comparison with other ranges.
8. The laboratory results showed higher decrease in mean concentration of hemoglobin of patients infected with V.L. when compared with other ranges.

References

- [1]. Herwaldt, B. L. (1999). Leishmaniasis. *Lancet*, 354:1191-1199.
- [2]. Arevalo, J. (2007). Influence of leishmania (viannia) species on the response to antimonial treatment in patients with American tegumentary leishmaniasis. *J. Infect. Dis.*, 195, 1846-1851.
- [3]. James, William D.; Berger, Timothy G. (2006). *Andrews' Diseases of the Skin: clinical Dermatology*. Saunders Elsevier.
- [4]. Desjeux P. (2001). "The increase of risk factors for leishmaniasis worldwide". *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 95 (3): 239-43.
- [5]. Jean, Francois. (1995). "Sudan: Speak no Evil, Do no Good" *Life, Death and Aid: the Médecins Sans Frontières Report on World Crisis Intervention*.
- [6]. World health organization. Communicable disease profile for Iraq – (2003).
- [7]. Taj- Eldin, S.; Nouri, L.; Jawad, J. and Kalaki, N. (1969). Kala-azar in Iraq. *Analysis of new series. J.Fac. Med. Baghdad*, 11:7-15.
- [8]. Mahdy, Aseel Saeed (2001). *study of some immunological parameters for children infected with visceral leishmaniasis*. B, Sc thesis, college of health and medical technology. Baghdad: 113 pp.
- [9]. Kumar, R.; Kumar, P.; Chowdhary, R.; Pia, K.; Mshra, K.; Kumar, K.; Pandey, H.; Singh, V. and Sunder, S. (1999). Kala azar epidemic in Varanasi district, India. *Bull. WHO*, 77(5):371-373.
- [10]. Busuttill, A. (1974). Kala azar in Maltese islands. *Trans. Roy. Soc. Trop. Med. Hyg.* 236-240.
- [11]. Bucheton, B.; Kheir, M.; El-Sofi, S.H.; Hammad, A.; Mergani, A.; Mary, L. and Dessein, A. (2002). The interplay between environmental and host factors during an outbreak of visceral leishmaniasis in Sudan. *Microb. Infect.* 4(14):1449-1457.
- [12]. Enerink, M. (2000). Infectious disease. Has leishmaniasis become endemic in the United States. *Science*, 290:1881-1883.
- [13]. Mohammed, Dina Moussa (2007). *immunological study in patients infected with visceral leishmaniasis*. Higher diploma, college of health and medical technology. Baghdad: 62 pp.
- [14]. Yassir, M. (2004). *Leishmaniasis*. PhD. thesis, college of medicine. Baghdad University, p: 1-7.
- [15]. Jassim, A.K.; Maktoof, R.; Budosan, B. and Combell, K. (2000). *visceral leishmaniasis control in Thi-Qar governorates, Iraq, 2003*. *East. Mediterr. Health. J.*, vol.12 (supplement 2):230-237.
- [16]. Al-Gunima, Abdulsadah Abdul Abbas (2008). *A comparison between molecular and serological methods in the diagnosis of kala azar*. PhD Thesis. College of science, Al-Nahrin University: 141 pp.
- [17]. Nusief, A.W. 1997. *National program for control of leishmaniasis in Iraq WHO Leishmania control follow up for East Mediterranean countries Amman 20-22 Oct.*
- [18]. Korzeniewski, K. (2004). *Iraq Warszawa. Wydawnictwo Akademickie DIALOG.*, 73(2):54-56.
- [19]. Zijlstra, E.E. and El-Hassan, A.M. (2001). *leishmaniasis in Sudan: visceral leishmaniasis*. *Trans. Roy. Soc. Trop. Med. Hyg.* 95(1):27-58.
- [20]. Grech, V.; Mizzi, J.; Mangoin, M. and Vella, C. (2000). *Visceral leishmaniasis in Malta an 18 years pediatric, population based study*. *Aroh. Dis. child.* 82:381-385.
- [21]. Abd, Inas S. (2012). *A comparison between ELISA and Diagnostic Methods in Iraqi Hospitals for Diagnosis of Kala-azar*. M.Sc. thesis, college of health and medical technology. Baghdad: 72 pp.
- [22]. Al-Marzoki, J.M. (2002). *Clinical and laboratory study of kala azar in Itallia*. *Iraq. J. Babylon university. Med. Sci.* 4(7):715-731.
- [23]. Pearson, R.D.; Sousa, A.Q. and Jeronimo, S.M.B. (2000). *Leishmaniasis species: visceral (kala-azar), cutaneous leishmaniasis in: principles and practice of infectious diseases*. By: Mmandell, G. 1.5th edition. Churchill Livingstone, Philadelphia, p: 2831-2834.
- [24]. Pass well, J.; Shor, R.; Keren, G. and el-on, J. (1985). *Human monocytes infected with Leishmania amastigotes enhance lymphocyte proliferation*. *Clin. Exp. Immunol.*, 60(3):565-571.
- [25]. Lafuse, W. P.; Story, R.; Mahylis, J.; Gupta, G.; Varikuti, S.; Steinkamp, H.; Oghumu, Steve and Satoskar, A. R. (2013). *Leishmania donovani infection induces anemia in Hamsters by differentially altering erythropoiesis in bone marrow and spleen*. *Journal. Pone. pLoS ONE*, 8(3): diol. 10-137.
- [26]. Carneiro de Freitas, J. C.; Nunes-pinheiro, D. S.; Neto, B. E. L.; Santos, G. J. L.; Amaral de Abreu, C. R.; Braga, R. R.; Campos, R. and Fernandes de Oliveira, I. (2012). *Clinical and laboratory alterations in dogs naturally infected by Leishmania chagasi*. *Rev. Soc. Bras. Med. Trop.*, 45(1): diol. 0037-8682.