Changes of Proportions of Serum Protein and Iron in Children Infected with Kala-Azar

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Abstract

This study were performed to determine two biochemical criteria in the serum of Kala- azar patients include measurement of total serum protein & iron concentration and study the differences between each parameters in patient serum according to age, sex & size of sample study which contain (100) infected children's were (63 male &37 female) those admitted to AL-Batool teaching hospital in Diyala Governorate during the period : (1/10/2012 to 25/3/2013).The result in study were showed a significant increase in the protein & iron In infected as compared to & healthy children's . The concentrations of iron in the infected children is (105 ±7.0g/100ml) while the iron concentration in healthy children is(75.84±2.6 g/100ml).The concentration of protein in serum of infected children is (7.22±0.22 g/*100ml)* while in the healthy children is (7.08±0.12 g/100ml) .

Key words: Kala-azar ,, Serum iron concentration ,, Serum protein Concentration.

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Introduction

Kala-Azar refers to the widespread disease caused by Leishmania spp. sex types are a protozoan species belonging to the level of bilateral kinetoplastida a host Digenetic. The parasite passing two stages in the insect vectors and vertebrate intrudes in phagocytic cells, inside the big cells intracellular amastigotes in human form and the other mammals, the promastigotes developed outside the extracellular cells in the gut of the carrier sand fly [1].

The kala-azar disease is common and important one. that can cause serious health problems in the world, particularly in developing countries, and because of disease caused a rise in the mortality rate in untreated cases [2].

Kala-azar is epidemic diseases which spread in the areas of different environmental conditions ranging from desert to forest areas or rainy rural or civilized areas. The largest statistical Kala-azar epidemics founded in eastern, India and Bangladesh, among immigrants in Sudan, the biggest epidemic that emerged in the urban areas was recorded in the northeast of Brazil. While the observed increase in incidence in the countries of the eastern Mediterranean, which belong to several reasons, including: (flow of the population non-immunized in outposts natural Kala-azar, a change in the environment Alamadaúv tanker and reservoir, and the lack of use of pesticides in the fight against malaria and development in diagnostic methods and recording positive cases [3].

Despite considerable progress in cellular & molecular biology & in the evolutionary Genetics , one is still far from understanding how these organisms act in natural population so they have a complex life cycle & present in very diverse ecological niches and can infect a wide range of host .[4]

Objectives

The aim of our study is to measure the total serum protein and iron in the

Kala –azar children's patients, and to compare the values of these parameters on the

Base of the sex and the age and also with values in serum of healthy children .

Materials and Methods

Samples of blood were collected which are necessary for the study from 100 children admitted to the Al-Batool teaching hospital (male 63 & female 37) with symptoms of the Kala-azar disease (black fever) including swinging pyrexia and pallor. Besides clinical symptoms there is (Hepatospleenomegaly).

(4 ml) of blood was taking from the infected children between the ages of (5 months -- 3 years) and in both sexes, and save it in laboratory tubes (IFAT) .

We select (25) children not infected with Kala-azar disease and they are in good health as a control group & Take (4 ml) of blood from all them for biochemical testing in order to compare with child infected with Kala-azar.

Preparation of Serum Samples

Been placed(4 ml)of blood derived from the infected patients in the tubes free from anti- coagulant to get necessary serum and conduct laboratory analyses of total protein and iron in the serum and then a Centrifuge speed 3000 rpm for 5 minutes to obtained serum required for study .

Solutions Used:

1 - **Biuret solution** consists Detector 100 mmol , sodium hydroxide and 16 mmol / liter sodium - potassium (Na-K-tartrate) and 15 m mol / L -Potassium Iodide and 6 m mol / liter sulfate cupric in the volume of 100 ml, commuted to 400 ml using distilled water which is fixed for 0ne year at 15- 25 degree Celsius.

2 - **standard solution**: - consists of a protein concentration of 10 g / 100 ml.

3 - Blank Solution formed from sodium hydroxide concentration of 100 mmol / L and (Na –K-tartarate) concentration of 16 mmol / l commuted to 400 ml with distilled water, which remains constant for one year at 15-25 degrees Celsius.

Procedure

1 - Test solution: Put 1 ml of **Biuret** solution in a test tube and add 20 Micro letter of infected blood serum and then blending well.

2 - Standard solution: put 1 ml of **Biuret** solution in a second test tube and add 20 Micro letter of standard solution with good mixing.

3 – Blank solution (Zero) Put 1 ml of **Biuret** solution in the third test tube and added 20 Micro letter of distilled water with good mixing, then distribute it in to three tubes in a water bath at 20 ° Selezah for 30 minutes for complete reaction & measure intensity of the color at a wavelength of 540 nm for zero solution.

Calculations of the concentration of total protein in the sample by the following equation:

Protein concentration (g / dL) absorption intensity solution test \ standard absorption solution **x** focus standard solution

Measure the quantity of iron in blood serum:

Materials and solution: used the solutions in special tools manufactured in Spanish company and the kit contains the following solutions**:**

1- **Buffer solution** (R1) contents from clorate condanin 1.5mmo/Liter

2 - solution (R2) Chromogen consisting of Ferrezine by 40 mmol / liter and Sodium acetate 400 mmol / l.

3 - Iron standard consisting of Ferric ion concentration of 100 mg / dL is prepared Working Solution blends four volumes of buffer solution (R1) with one volume of the (R2) Chromogen) and this solution shoulled

be established for a period of six months in temperature(2 -8 c).

The natural distributions of iron in the body:

We have estimated a quantity iron which is distributed as follows:

• 2500 mg in hemoglobin
• 200 mg Hemoglobin
• 200 mg in enzymes that contain iron (cytochrome 45P Monoaoxeginaz catalase … **(5)**
• 1000-1500 mg savers in savings Centers (bone marrow, spleen, liver**) (6) .**

Results

The effect of Kala-azar on the level of total protein, showed the results of measuring the level of total protein. The average level of total protein for the infected children (7.22± 0.22) gram / 100 ml) compared of rate in non infected(7.08± 0.12 g/100m) as clear in the table (1)

Table (1): The Impact of Kala-Azar about some of Blood Criteria.

Patients	Total protein concentration g / 100 ml	Serum iron concentration in g /100 ml
infected	7.22±0.22	105± 7.0
Healthy	7.08±0.12	75.84±2.6

Serum iron concentration:

From the results the concentration of iron in healthy is 75.84±2.6 while the iron concentration of serum in infected children is 105 ±7.o g / 100 ml versus 75.84±2.6 g / 100 ml not no infected as in table (1) .

Normal value :
Iron--- (5—34 g /L).
Protein—(6.5—8.5 g /L).

In Table (2) there is no significant difference in the rate of total protein concentration for infected male children Kala-azar (7.23±0.19 g / 100 ml) against the concentration of infected female children with this disease (7.21± 0.19 g / 100 ml).

Table (2): Biochemical Changes According to Sex in Kala-azar Cases.

sex	Total protein concentration g / 100 ml	Serum iron concentration in g / 100 ml
Males (63)	7.23±0.19	105.01±3.6
Females (37)	7.21±0.19	105.13±4.9

The present study shown as in Table (2) there is no significant difference in the rate of iron concentration for Infected males with Kala-azar**.** the concentration reached 105.01 ±3.6 g / 100 ml, while the average concentration in infected female with same disease 105.13±4.9. g / 100 ml.

Normal value :
Iron--- (5—34 g /L).
Protein—(6.5—8.5 g /L).

The results, as shown in the table (3) there is no significant differences in the rate of concentration of total protein for three age groups of Kala-azar the range of concentration was 7.43±0.28 g / 100 ml and 7.22± 0.17 g / 100 ml respectively.

Table (3): The Effect of Age on some Kala-Azar Cases.

Age groups	Total protein concentration in g / 100 ml	Serum iron concentration in g / 100 ml
Under one year	7.43±0.28	106 . 11±5.9
From (1-2) years	7.22 ± 0.17	105 .04±2.1
From (2-3) years	7.22±1.35	104 .02±2.2

Iron concentration in the serum of children infected with Kala-Azar.

By the results of the study and statistical analysis as shown in Table (3) there is no significant difference in the average of iron concentration in the serum of the affected child with Kala-azar in the first age group 106.11 ±5.9 g / 100 ml, compared with concentration rate in the second age group 105.04 ±2.1 g / 100 ml and for third age group 104.02 ±2.2 g / 100 ml .

Normal value:

Iron--- (5—34 g /L).

Protein—(6.5—8.5 g /L).

Discussion

Through the results of the current study can see the standards biochemical changes in blood especially the level of serum protein, as well as the proportion of iron.

1 – The effect of Kala-azar on some standards biochemical agents:

Total serum protein concentration:

Although mentioned some of the sources that Kala-azar cause a decrease concentration of serum protein specially the albumin [7]. But the current study did not indicate a significant difference in serum protein concentration in Kala-azar disease, and could explain this decline in albumin concentration paralleled by a significant increase in immunoglobulins, especially IgG in the case infected by Kala-azar [8].

The serum iron concentration:

The results of this study and having high rate in concentration of serum iron when the children infected with Kala-azar, but in other studies they confirmed a high level of ferritin (iron protein saver) and hemosideren when injured by Kala-azar [9]. Because increase the level of red blood cells (RBC) due to parasitic infestation, which is led to increase in the proportion of iron in serum, or iron ferritin plays an important role in the control of iron absorption, that prevents the absorption by the intestines or prevent the free iron which absorb in blood flow. So this will be prevent the absorption process when the stored iron level is reduce in the body, thereby causing low level of ferritin in the mucosal cells lining the gut Otherwise, the presence of a lot of iron reducing the absorption process [11].

The chronic Kala-azar disease Lead to decrease in the production of (RBC) then gets a reduction in the quantity of hemoglobin way as a case of anemia caused by iron deficiency and for this reason produces red cells wich contain a low percentage of hemoglobin [12] or the iron surplus be linked to some of the proteins that do not allow the transfer of iron to plasma [12].

2 – The effect of sex on some standards infective children:

Total protein concentration

The results showed there is no significant difference in the rate of concentration of total protein among infected children (male and female) with Kala-azar. The reason for similar of public proteins function for both sexes in the maintenance of viscosity and amount of blood, and maintain the osmotic slimy pressure which important to pull fluids from tissues to the blood and in another side

as an anti-bodies proteins ,its impact in the clotting process also [13].

The serum iron concentration

The result of study indicated that there is no significant difference in rate of the serum iron concentration for male children infected with Kala-azar compared with female children in same disease, the reason for increase in the amount of iron due to heading the decline in the transfer of iron from the places of storage to the plasma [14].

The reduced in response of bone marrow accord to changes in the level of hormone Erthropoietin which is produced in the kidney that activate the use of iron in the production of mature RBC [15].

3 – The effect of age on some biochemical agents:

Total serum protein concentration

The study show no significant difference in the rate of serum protein concentration for children infected with Kala-azar in the age groups. may be due to the similarity in the categories and function in maintaining the blood viscosity and its amount, and maintain osmotic pressure which pull the interstitial fluids to blood, as well as act as an anti bodies and their role in the coagulation process [16].

Iron concentration in the serum

The results indicate there is no significant difference in the rate of concentration in serum iron in children infected with Kala-azar between the age groups studied. The reason for this may return to breakdown of red blood cell at the same level in all age groups, or because of a weak resistant of immunity system [17] or perhaps belonging to an increase in the level of the amount of iron and to a decline happening in the transfer of iron to plasma [18]., or an increase in the concentration of storage iron protein will Lead to increase transfer of iron from RBC and cause anemia. [4]

References

[1] WHO, (2002). Special program for research and training in tropical disease (TDR) Geneva.3 – 1

[2] Zuckerman, A. and Lainson, (1977). Leishmania In: Parasitic protozoa. Vol. Julius. R. AndKreier, Eeds. Academic press.New Yerk, pp75-133.

[3] Markell, E. K.; Vage, M. and John, DT (1990) Leishmaniasis In: Medical Parasitology 6thed pp 121-130. saundres Company philadephia.

[4] Zijlestra, E. and EL-Hassan, A.M.(2000).Kala-Azar in Sudan with special reference to Kala-azar. Trans. Roy.Soc.Trop. Med. Hyg ., 95,1,S1/27-S1/58.

[5] Dacie, J. V. and Lewis, S.M. (1995). Practical Hemat0l0gy 6th, ed prentice Hall Intrnat. INC.

[6] Keisu, M. and Ost A. (1990). Dignosis in patient with sever pancytopenia suspected of having a plastic anemia. Eur. J.Haematol. 45:11 -14.

[7] Hossain, M. A. Akond, A. K. and Chwdhary M. K. (1992). Pancytopenia, Astudy of cases, Bangladesh J.Pthol: 9 -12.

[8] Vermal, N. and Dash, S (1992). Reappraisal of ubderlying pathology in adult patients presentiy with pancytopenia Top. Mfd .44:322 - 327.

[9] Gill, D, and Obrien, N. (1998) pediatric Clinical examination. First edition, churill living stone comp.; 5.

[10] Behaman, R. E.; Kliegman, R. M. and Jensen, H, B. (2000) Nelson textbook of pediatrics, 16th ed WB saunders company.

[11] Firkin, F.; Chester man, C.; penington, D.and Rush, B. (1989). Clinical Hematology in Medical practice. 5 th, edn, Blackwell, Sci. publ, Oxford

[12] Thabet, F. ; Tabark, B.; Fehen, R.; Yacoub; Selma, H. and Essonssi, A. S. (1999). Syndromene of inappriat macrophage activation associated with visceral infantile.

[13] Tarish, H. R. (2006). Some serological and biological test for the visceral in Med-euphrate Leishmaniasis in pediatric patients ph. D. Thesis education, university of AL-Qadisiya. p: 130.
[14] Worood, M. (1997). The laboratory assessment of iron status Anupdatye.Clin. Chem. Acta; 259: 3 - 23.
[15] AL-Muhammad, O. A.; Gafil, H.A.and Mude, H.N. (2004). Heamatological changes in children surffering form visceral Leishmaniasis (kalazar), Medical Journal of Babylon vol. (1) :3-4.
[16] Dennis, V.A.; Champman, W.L.; Hasson, W.L. and Lujan, R. (1995) Leishmania donovani; Clinical, heamatologic and epatic changes in squirrel monkeys (Samiri sciureus), Journal of parasitology, 71 (5).
[17] Cotterell, S.E.; Engwerda, C.R.; Kaye, P.M. (2000) Enhanced hematopoitic activity accompanies parasit expansion in thespleen and bone marrow of mice infected with visceral leishmaniasis Infec. Immun.; 68 (4) :1840-8.
[18] Woodruff, A.W. Mechanism involved in anaemia associated with infection and splenomegaly in the tropics Trans.Roy. Soc. Trop. Med. Hyg. 67.1973. pp.313-328.