Effect of Zinc and Copper on some immunological markers in Cutaneous Leishmaniasis

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Abstract

The elucidative diminished of immune function connecting t the Zinc profile is one of the major highlighted study all over the world. The aim of this study is to evaluated Zn concentration in serum of cutaneous leishmaniasis patients and efficacy on some immunological parameters.A total of 60 patients with cutaneous leishmaniasis (CL) were included in this study during the period between February /2009 to July /2009 in the out – patients clinic of the dermatology department of Azadi General Hospital in Kirkuk governorate.In vitro by using flame atomic absorption spectrophotometer .It was conclude that the mean of serum Zn level in all CL patients were significant decreased ($P< 0.05$) , since the mean concentration in patients group were 7.61± 0.03 µmol / l, 6.69 ± 0.31 µmol / l , while they were 14.8 ± 0.04 µmol / l, 12.02 ± 0.02 µmol / l in control groups respectively. Analysis of CD4+ and CD8 + T-lymphocyte percentage shows that the mean of CD4+ and CD8 + percentage in all CL patients were significant decreased ($P < 0.05$) since the mean percentage of CD4+ in patients groups were 36.5 ± 0.098 % , 36.29 ± 0.06% and it was 59.95 ± 0.16%, 58.9 ± 0.14% in control groups respectively .While CD8 + presenting in patients groups were 22.37 ± 0.05 %, 22.47 ± 0.04% , while they were 30.95 ± 0.12%, 31.15± 0.15 in control groups respectively. The mean ratio of CD4+/ CD8+ was significant decreased in male and female patients in comparison with their control groups , since the mean of CD4+ / CD8+ in patients groups were 1.63 ± 0.004, 1.62 ± 0.004 , while they were 1.94 ± 0.01 and 1.9 ± 0.01 in control groups.

INTRODUCTION

The Trepanosomatidea of the genus Leishmania is etiological agent of a variety of diseases manifestation, collectively known as Leishmaniasis. [1] Leishmanasis is prevalent through out the tropical and subtropical regions of Africa, Asia, Mediterranean, Southern Europe (old world) and South and Central America (New world) [2]. Tow stage in life cycle are known, the promasigote in sand fly and culture, amastigote in man and reservoir mammals [3]. Cutaneous leishmanasis in the Middle East is caused by parasites of species Leishmania tropica ,which is called Oriental sore [4] .In Iraq they find that there are three species of Leishmania that cause CL: L.tropica, L.major and L.infantum[5]. Leishmania parasite are transmitted by the bite of the infected female of Phlebotomus ,sand fly[6].The sand fly becomes infected when sucking blood from reservoir host, which include man ,or domestic and wild animals.Immunity of host, against CL play an important role in elimination of intracellular parasites , the cell mediated immune response(CMI), T-lymphocytes and macrophage plays role in elimination of intracellular parasites[7]. The productions of immunoglobulin like immunoglobulin M(IgM) which accounts for approximately 10% if immunoglobulin pool[8]. Zinc is an essential trace elements for all organisms ,in human subject, body growth and development strictly dependent on Zn; the nervous ,reproductive and immune system are particularly influenced by Zn deficiency[9].Zn is an essential component of thymulin hormone , thymulin hormone keep the ratio of CD markers of T-4 and T-8 to 2:1[10]. Number of CD T-cells and cytotoxic T-lymphocytes increase after Zn supplementation ; On other hand T-lymphocyte responses as delayed hypersensitivity and cytotoxic activity are suppressed during Zn deficiency[11].

Aim of the study:

1-Estimation of Zn levels in CL patients.

2- Verify the correlation among Zn serum levels, CD4+, CD8+.

3- Evaluate the possible role of CD4+/CD8+.

Materials and Methods:

A total of 60 patients infected with CL were included in this study during the period between February /2009 to July /2009 in the out – patients clinic of the dermatology department of Azadi

General Hospital in Kirkuk governorate .Thirty 30 healthy individual were used as control group in this study.A blood sample (3cm3) was collected from each patients and control groups. The collected sample was transferred immediately into 2 tubes as follow: - a- (2cm3) used for lymphocytes separation technique for detection of CD4+, CD8+. b- (1 cm3) of blood in plain tube (serum tube) for serum isolation for detection of serum Zn concentration.

1- Measurement of Serum Zn Concentration:-

Atomic absorption /Flame Emission Spectrophotometer model (SHIMADZU A.A .6200) fitted with air –acetylene flam was used for measurement of Zn concentration. The hollow cathode lamp for Zn was used in atomic absorption spectrophotometer [12].

The Procedure:-

Frozen sample were thawed at room temperature then the level of serum Zn of all patients and control groups were measured by using (Atomic absorption spectrophotometer) as follow:-

On hundred micro liters (100µl) of serum were diluted in nine hundred micro liters (900µl) of D.W to reach total volume one milliter (1 cm3) for each sample was aspirated in to air acetylene flame and the element are measured.

The results were sorted in Part Per Million (PPM) unit and converted to µmol/L by dividing the result of PPM on molecular weight of Zn.

2- Analysis of Peripheral Blood T-Lymphocytes CD4+, CD8+:-

Special cover slid, light microscope, low power and fluorescent (40x) magnification were used.

Ficole 40(Pharmacia fine chemicals) was used for isolation T- lymphocytes which are an aqueous solution of high-density sucrose epichlohydrin polymer and sodium diatrizoate was used. The Isopaque – Ficol technique originally described by Boyum (1968) [13].

Statistical analysis:

Differences between means of two groups were compared by Student's unpaired t-test.

Results

Our results show that 57% of patients were male, while 43% were female (Fig-1)

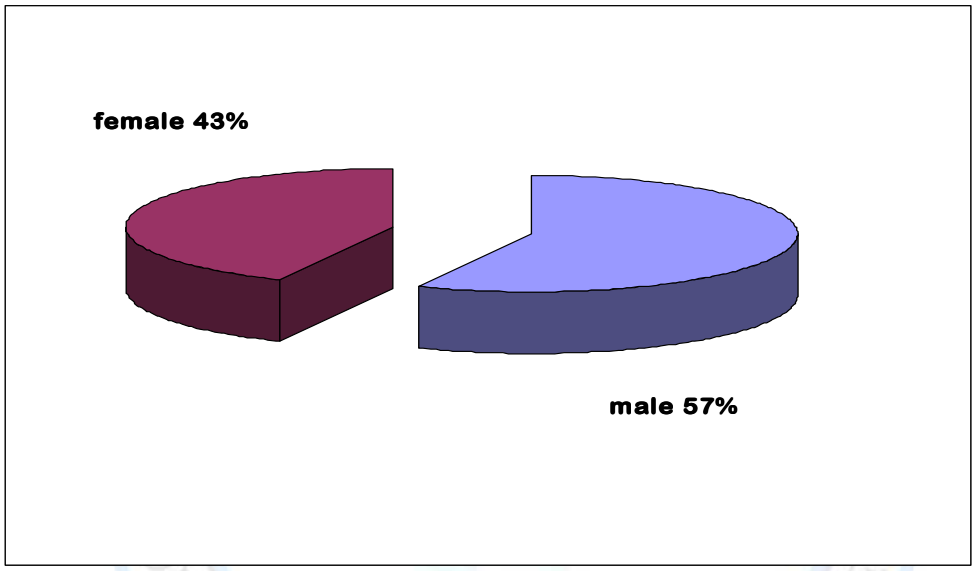


Figure (1):-Distribution of CL According to Sex

Zinc Level in serum of CL patients:-

Zinc concentrations in CL patients were estimated in the serum of CL patients by using flame atomic absorption spectrophotometer. Figure (2) shows the mean of Zn concentration in the serum of male patients and control group. It is found that the mean of Zn concentration in patients significantly decreased ($P<0.05$) since the mean of Zn concentration in patients groups was 7.61± 0.03 µmol / l , while it was 14.8 ± 0.04 µmol / l in control group . Statistical analysis revealed significant difference between tow these groups.

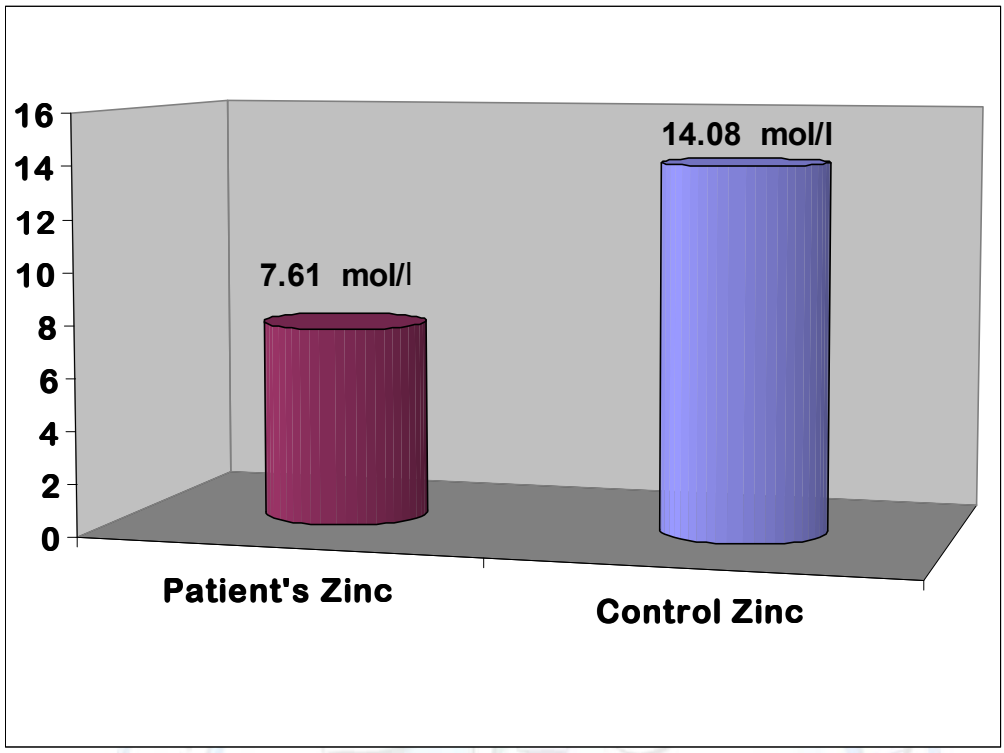


Figure (2): Zn Concentration in Male Patients and Control groups.

Figure (3) shows the mean of Zn level in serum of female patients and control group. It was found that there was significant decreased ($P<0.05$) between these groups , since the mean of Zn level in serum of patients and control group 6.69 ± 0.31 µmol / l and 12.02 ± 0.02 µmol / l respectively.

Estimation of CD4+ &CD8 + in Peripheral Blood Lymphocytes of CL Patients:-

2-1 Estimation of CD4 + %:-

The results shown that there was significant decrease in the CD4+ % ($P<0.05$) in the peripheral blood lymphocytes in male patients in comparison with control group since the mean of CD4+ % in patients group was 36.5 ± 0.098 % while the mean of CD4+% in peripheral blood of control group was 59.95 ± 0.16%.(Figure 4)

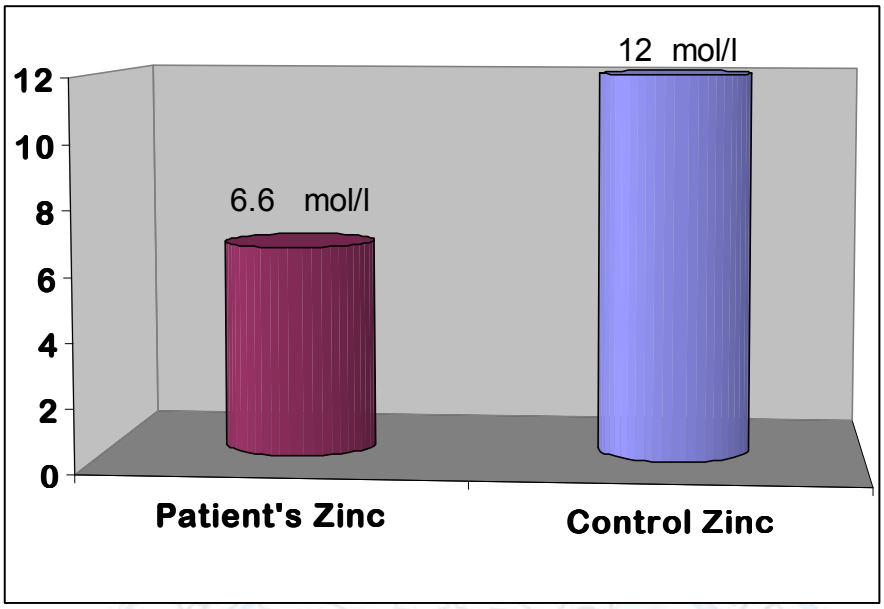


Figure (3): Zn Concentration in Female Patients and Control Group.

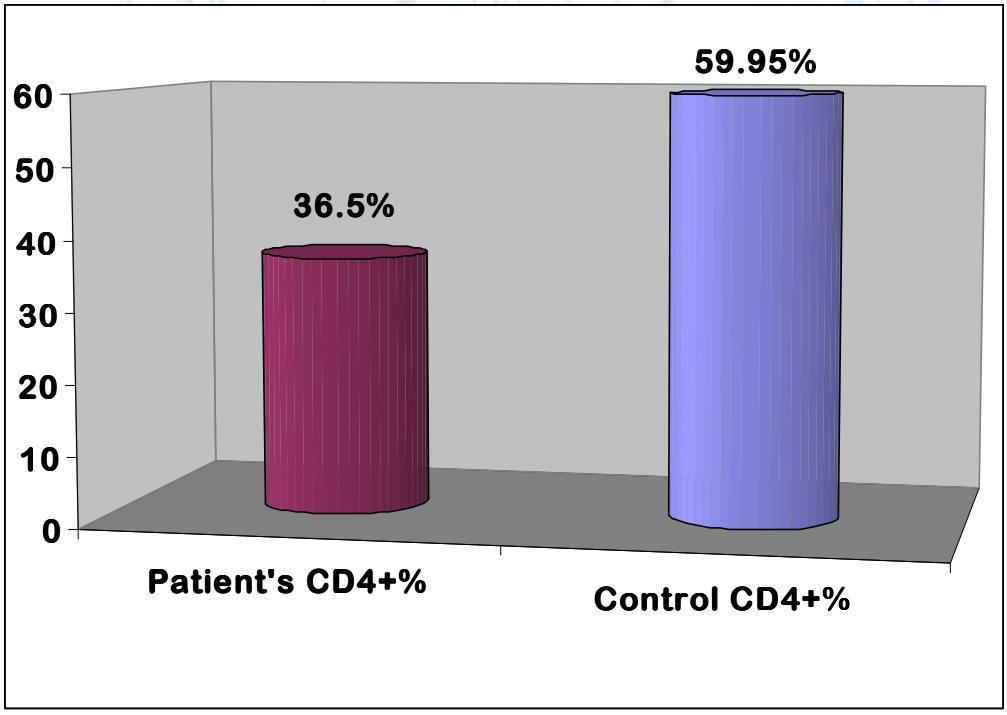


Figure (4): CD4+% in Male Patients and Control Groups

The same was seen in CL female group since the mean of CD4+% in patients group was 36.29 ± 0.06% , while the mean of CD4+% in control group was 58.9 ± 0.14%, statistical analysis revealed that a significant difference between two these groups since P<0.05. (Figure 5)

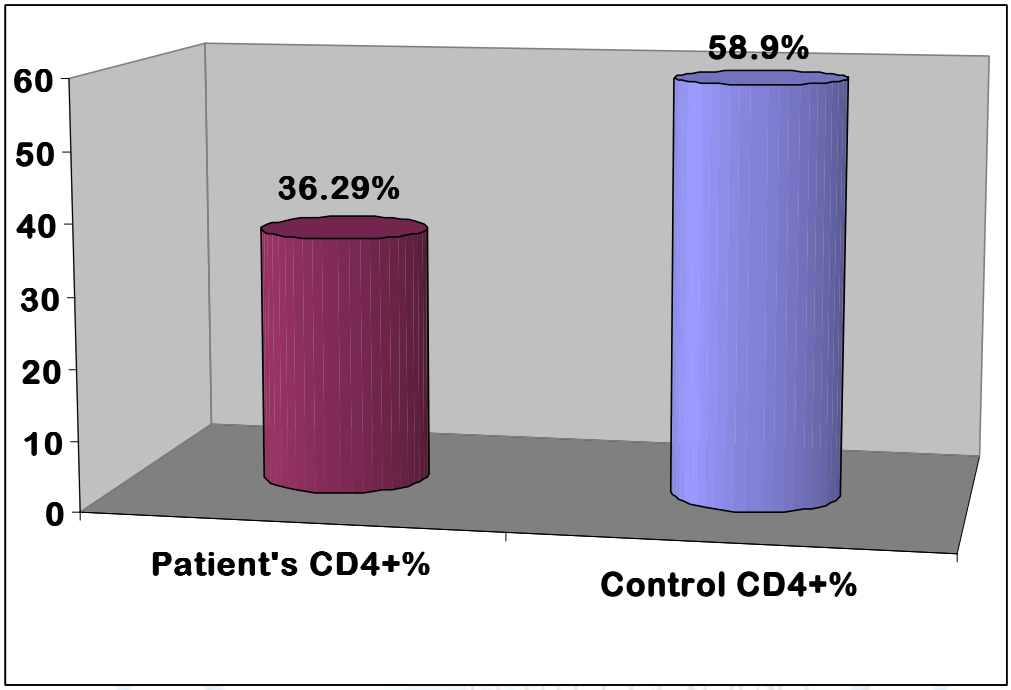
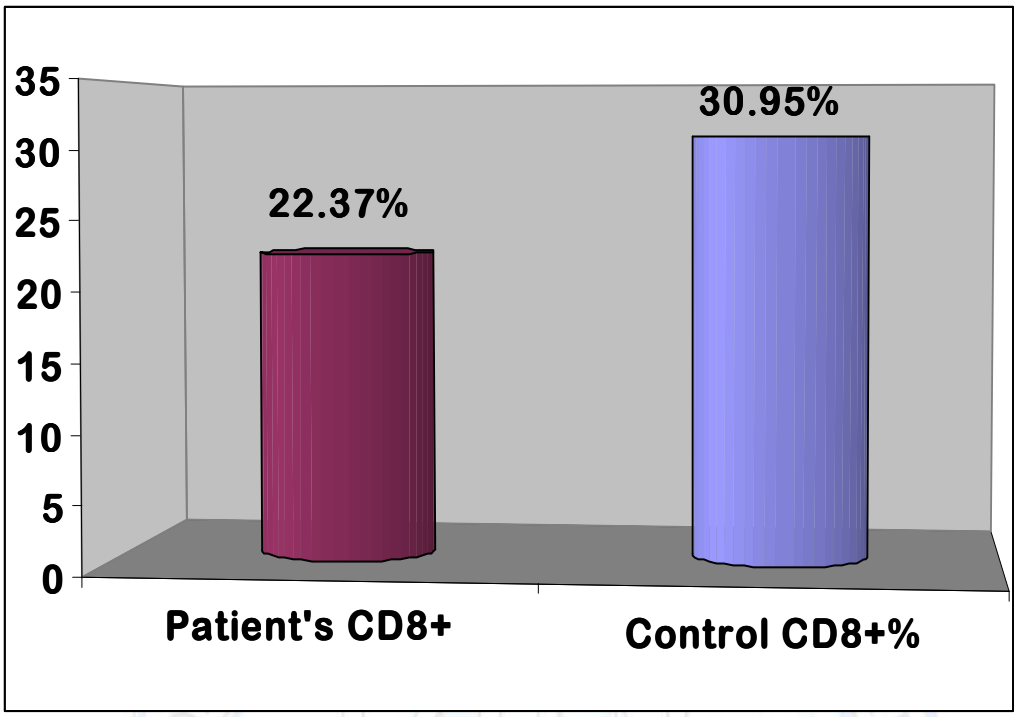


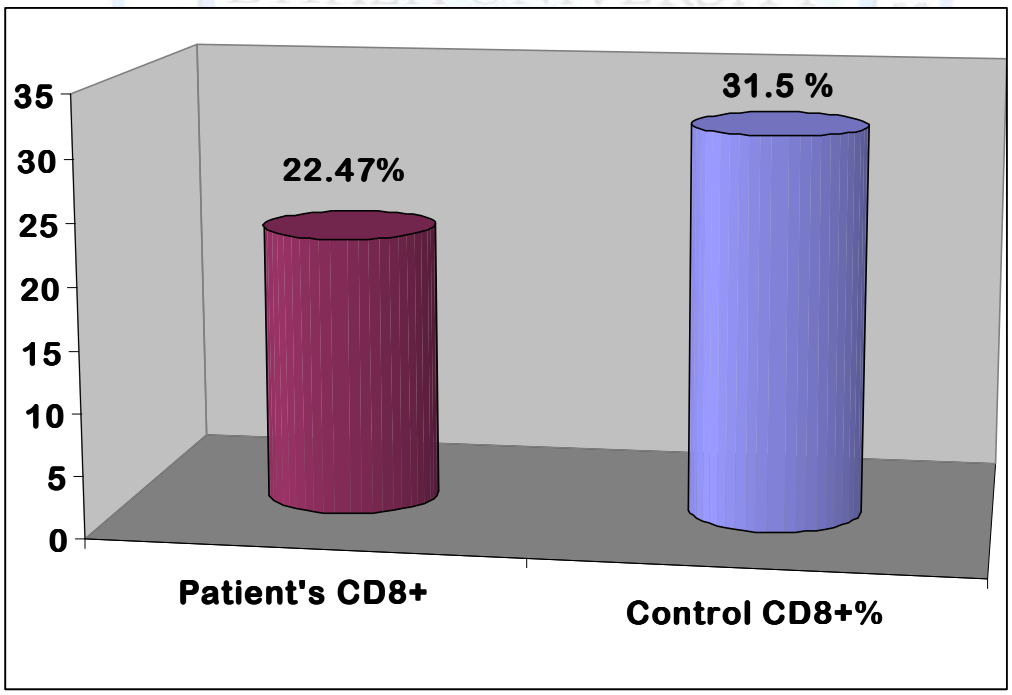
Figure (5): CD4+% in Female Patients and Control Groups

2-2 Estimation of CD8 + %:-

The results shown that there was significant decrease in the CD8+ % (P<0.05) in the peripheral blood lymphocytes in male patients in comparison to that observed in control group since the mean of CD8+ % in patients group was 22.37 ± 0.05 % while the mean of CD8+% in peripheral blood of control group was 30.95 ± 0.12%.(Figure 6). The same was seen in female patients group since the mean of CD8+ % was 22.47 ± 0.04% and 31.15± 0.15 in patients and control groups respectively, statistical analysis revealed that a significant difference between two these groups since P<0.05. (Figure 7)



Figure(6): CD8+% in Male Patients and Control Groups



Figure(7): CD8+% in Female Patients and Control Groups

2-3 CD4+ / CD8+ Ratio

Figure (8) show the CD4+: CD8+ ratio in CL male patients and control group. It was found that the mean of ratio in patients group was 1.63 ± 0.004,while it was 1.94 ± 0.01 in control group, statistical analysis revealed a significant differences between two these groups since ($P < 0.05$) . The same results was observed in female patients and control groups. Figure(9) show the mean ratio of CD4+ / CD8+ in patients and control groups, since the mean ratio in patients group was 1.6 ± 0.004, while it was 1.89± 0.01 in control group, statistical analysis differences between two these groups since ($P< 0.05$).

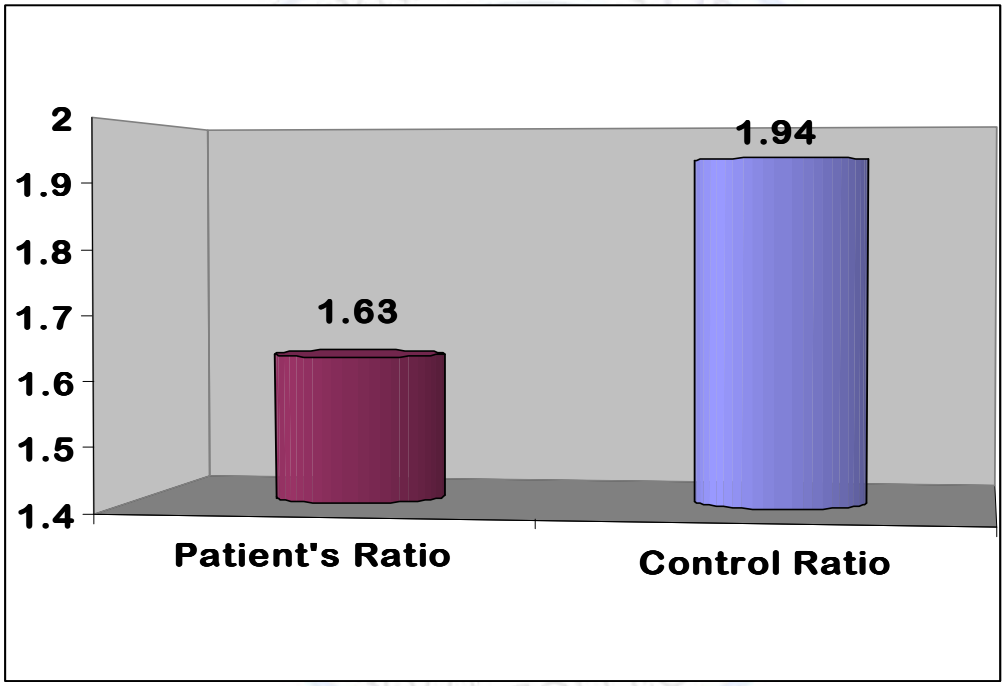


Figure (8): CD4 +/ CD8+ Ratio in Male Patients and Control Groups

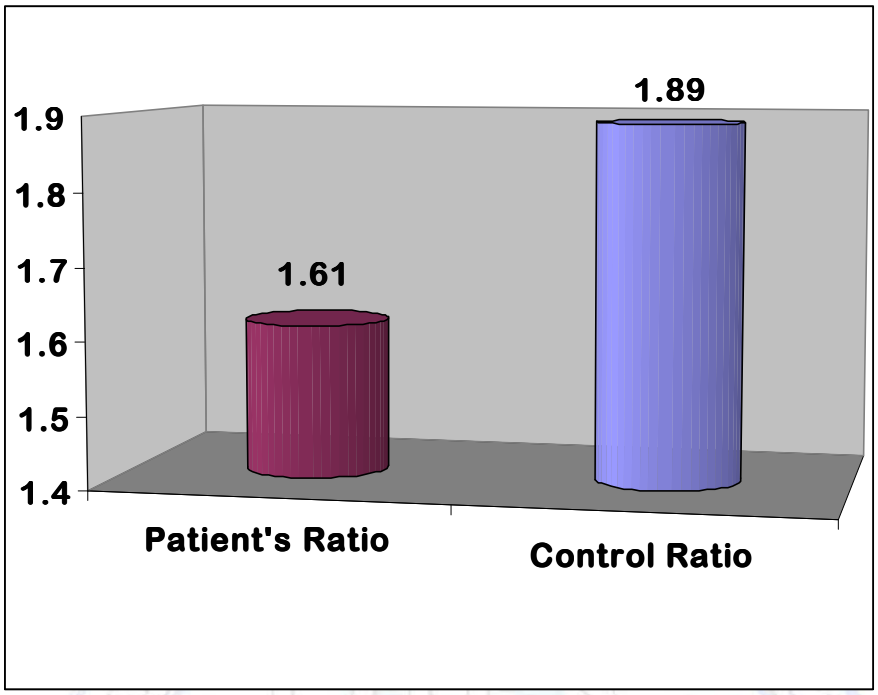


Figure (9): CD4 +/ CD8+ Ratio in Female Patients and Control Groups

Discussion

1- Zinc Concentration in Serum of CL Patients:-

Zn is an essential trace elements tat important for body growth, immunity, nervous and reproduction systems, especially in male reproduction system. Zn is involved in high concentrations in male reproduction system especially in testosterone hormone that responsible for maturation and development of sperms. Zn is an important for growth and multiplication of cells; therefore requirements of Zn is different for all ages groups[14].

Our result reveled that serum Zn concentration in all CL patients is significantly decreased since ($P<0.05$) (Fig. 2, 3). CL disease is regarded as infectious disease [3] and Zn deficient people are more susceptible to infectious disease [15] , this result could be explained that CL patients might be already have Zn deficiency therefore were infected by CL disease . Even that the decreased in Zn level in serum might be due to defense mechanism. This is in agreement with that found by [16] they found that Zn is required by human being and pathogens for proliferation and decreasing in plasma Zn concentration during an acute phase in infection is a defense mechanism of human organism.

Furthermore we found that our results revealed that Zn concentration in serum of all CL patients was less than the normal value in those are represented by Scotland Nutrition Society (12- 18) µmol / l [17] , Germany Nutrition Society (12-16) µmol / l [18] and less than(11-22) µmol / l [19].

The Percentage of CD4+% & CD8+% in Peripheral Blood Lymphocytes of Zn Deficient CL Patients :-

3-1 The Percentage of CD4+% in Peripheral Blood Lymphocytes of Zn Deficient CL Patients:-

According to our result that sorted out through analysis of peripheral blood lymphocytes of CL patients by using fluorescent microscope we found that there was a significant decreased in the percentage of CD4+ % in male and female in comparison to that observed in their control groups since($p<0.05$)(Fig.4,5).

This finding is in agreement with that found by [20], they found that the peripheral lymphoid organs, T-lymphocytes were progressively depleted from the spleen, lymph nodes and peripheral in Zn-deficient animals, Further more that is in agreement with those of [21] in that the process of blast transformation and the number of T-lymphocytes in peripheral blood decreased in Zn deficiency human.

This could be explained that the decreased in mean of CD4+ % might be related to Zn deficiency and inability of CL patients were included in our study to eliminate the infection.The Percentage of CD8+% in Peripheral Blood Lymphocytes of Zn Deficient CL Patients:- Our data revealed that the percentage of CD8+ % in male and female patients was significant decreased in comparison to that in their control groups ($P< 0.05$) (Fig. 6,7).

Our finding is in agreement with [22] in that Zn deficiency was effect on proliferation of CD8+ T- lymphocytes and is in agreement with that found by [23] in that Zn deficiency is associated with decreased T-cell proliferation after mitogen stimulation .

3-3 The CD4+ / CD8+ Ratio in Peripheral Blood Lymphocytes of Zn Deficient CL Patient :-

Our data revealed that CD4+ / CD8+ ratio in male and female patients were significant decreased in comparison to that observed in their control groups since($P < 0.05$) (Fig.8,9)

The ratio of CD4+/ CD8+ was significant decreased ($p< 0.05$) decreased in male and female CL patients this might be related to decreased in count of CD4% and lead to decreased the ratio of CD4/CD8 or might be related to conformation change in their thymulin hormone and decreased in activity of thymulin hormone due to Zn deficiency therefore their immune power were decreased and they were infected by CL.

This finding is in agreement with that found by [10] they found that Zn is bound to thymulin hormone in a 1:1 stiochiometry structure and thymulin activiy, in vitro and vivo in both animals and humans is dependent on plasma Zn concentration and thymulin hormone responsible to keep the ratio of CD4+/CD8+ in normal range, and is in agreement with that found by [24] , they were found that CD4+:CD8+ ratio decreased due to Zn deficiency in child infected with shigellosis.

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