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ESTIMATION OF INTERLEUKIN (17, 22) AND HEAT SHOCK PROTEIN-20 AMONG LEISHMANIASIS PATIENTS IN AL-NAJAF PROVINCE

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ABSTRACT : The present study deals with the Cutaneous leishmaniasis the most common form of the disease, it usually produces ulcers on the exposed parts of the body, such as the face, arms and legs. Cutaneous leishmaniasis (also known as oriental sore, tropical sore, chiclero ulcer, chiclero's ulcer or Aleppo boil) is the most common form of leishmaniasis affecting humans. It is a skin infection caused by a single-celled parasite that is transmitted by the bite of a phlebotomine sand fly. There are about twenty species of Leishmania that may cause cutaneous leishmaniasis. To estimate serum levels cytokines (IL17 and IL-22) among leishmania patients in Al-Najaf province as immunological markers and to estimate serum level heat shock protein 20 in leishmania patient and investigate the relationship of HSP 20 with IL-17 and IL-22. This study was conducted with (80) patients whose age ranges from (6-46) years, divided among 40 patients with cutaneous leishmaniasis (males 26) and (females 14), who have visited the dermatology unite at Al-Sader Teaching Hospital and Central Health Laboratory in Al–Najaf province, Iraq, for managing the disease and 40 (males 26) and (females 14) voluntary healthy as a control group. The study was carried out from November to April 2019. This study was performed to estimate serum levels of interleukin 17, interleukin 22 and Heat shock protein-20 in leishmania patients and control. It also estimate the level of IL-17, IL-22 and heat shock protein-20 for both male and female patients and control. The results showed a highly significant differences at (P<0.001) in all study parameters in comparison with the control. The result also showed the variances in the means of IL 17, 22 and HSP-20 among patients as compare with the control. All study groups show a highly significant variances (P<0.001) in the means of IL 17, 22 and HSP-20 among leishmanisis patients as compare with control. As a conclusion from this study, the importance of interleukin 17, 22 and heat shock protein-20 in Leishmaniasis patients can be used to detect the complications related to the disease and immunological effect among these patients.

Key words : Interleukin, heat shock protein, leishmaniasis.

INTRODUCTION

Leishmaniasis is caused by vector-borne protozoan parasites of the genus Leishmania and transmitted via infected female sandflies (*Phlebotomus* and *Lutzomyia*). The disease is endemic in more than 98 countries and an estimated 350 million people are at risk. The overall prevalence is 12 million cases and the annual incidence is 2–2.5 million cases. In most countries, the incidence numbers are probably underestimated because cases are not recognized and reporting is not mandatory (McGwire and Satoskar, 2014). Depending on the infecting species, an infection with Leishmania parasites can give rise to three clinical manifestations. The first is localized cutaneous leishmaniasis (CL) with single to multiple skin ulcers, satellite lesions, or nodular lymphangitis. The second is CL with mucosal involvement (MCL) and the third is systemic visceral leishmaniasis (VL) with involvement of internal organs, such as the liver, spleen, and bone marrow, which is lethal if not appropriately treated (Bailey and Lockwood, 2007). IL-17 is one of the best-studied cytokines in immunology, at least in part owing to its involvement in inflammatory pathology (Miossec *et al*, 2012; Kumar *et al*, 2019). Interleukin 17 (IL-17) and its closest relative, IL-17F, have recently drawn much attention in the field of immunology. IL-17 and IL-17F are expressed by a distinct type of T cells, T helper 17 cells and certain other lymphocytes. These cytokines play key regulatory roles in host defense and inflammatory diseases (Jin and Dong, 2013). Interleukin-22 (IL-22) has important functions in host defense at mucosal surfaces as well as in tissue repair. It is unique as a cytokine that is produced by immune cells, including T-helper (Th) cell subsets and innate lymphocytes, but acts only on non-hematopoietic stromal cells, in particular epithelial cells, keratinocytes, and hepatocytes (Rutz *et al*, 2013). It is one member of a family of cytokines termed IL-10–related cytokines that also includes IL-19, IL-20, IL-24 and IL-26 and was originally called IL-TIF, for IL-10–related T cell–derived inducible factor (Ouyang *et al*, 2011; Banerjee *et al*, 2018). Heat shock protein 20 (HSPB6) is a member of the small HSP family (HSPB)

and is ubiquitously expressed in many tissues including liver (Mymrikov *et al*, 2011). Heat shock proteins (HSP) are highly conserved molecules with many immunological functions. They are highly immunogenic with important role in cancer immunotherapy and in vaccine development against infectious diseases (Holakuyee *et al*, 2012). They act as chaperon in peptide folding and under stress conditions such as temperature shock will increase and bind to the cellular proteins to sustain the folding of the proteins, moreover, HSP have many immunelogical functions such as stimulation of innate immunity also HSP induce dendritic cells to produce proinflammatory cytokines such as IL-1, IL-6, TNF-α and IL-12 (Holakuyee *et al*, 2012).

MATERIALS AND METHODS

Patients

A case control study was conducted in the laboratory of Dermatology Unite at Al-Sader Teaching Hospital and Central Health Laboratory in Al-Najaf province, Iraq, during the period of November 2018 to April 2019. A total (40) cutaneous leishmaniasis Iraqi patients divided into (Male 26 and Female14) were enrolled in this study, their ages range from (6-46) years. They were diagnosed depending on the clinical picture (*i.e.*, size, number, location and type of lesion) and laboratory diagnosis achieved by physicians' works in these health associations. All patients gave their informed consent to participate in the study.

Control

Fourty apparently healthy were selected as the control, non of these had any systemic diseases the patient and controls (N:80) were age and sex matched.

Sample collection

Five ml of blood are withdrawn by vein puncture from Leishmania patients and same volume of blood collected from healthy control. A tourniquet was applied directly on the skin around the arm, the skin over the vein was sterilized with 70j ethyl alcohol, then collected samples were centrifuged and the serum was collected and dispensed in plastic eppendorf tubes then stored at -20^{0}C until used for serological tests.

Immunological assays

The study parameters estimated by ELISA kit ,the biochemical kits used in the study for IL-17 and 22 performed by Cusabio company (China), Heat shock protein -20 levels estimated by ELISA kit performed by Mybiosource company (USA).

Statistical analysis

The data was collected and transfer to statistical analysis using SPSS program (V. 23) in including the test of two independent -t- test to find the variances between study group. The results expressed as (Mean ± SD). P value at <0.05 was considered statistically significant and highly significant at P<0.001.

RESULTS AND DISCUSSION

The levels of IL-17, IL-22 and Heat Shock Protein-20 among leishmaniasis patients as compare with control

Table 1 shows a highly significance increases (P<0.001) in the levels of IL-17, 22 and heat shock protein-20 in the sera of leishmania patients in comparison with healthy control. Interleukin 17 is an important cytokine for protective immunity against extracellular pathogens (Rudner *et al*, 2007; Hombach-Barrigah, 2019) and also for the clearance of intracellular pathogens (Kuwabara *et al*, 2017). In addition to its important role in protective immunity, IL-17 plays a critical role in the pathogenesis of various autoimmune inflammatory diseases. Interleukin-17 (IL-17A) is a cytokine critical for the acute defense against extracellular bacterial and fungal infections. Excess production during chronic inflammation has been associated with many inflammatory and autoimmune disorders. The present review describes the key molecules of the IL-17 pathway, which are or could be targeted for treatment. Since targeting of IL-17A may affect defense mechanisms, the pathogenesis of such possible adverse events is analyzed. Then the contributions of IL-17 to bone changes in various forms of arthritis are discussed (Miossec, 2017). In our study, the levels of IL-17 increased this result in accordance with other studies by Bacellar *et al* (2009), they found increasing in the level of IL-17 in cutaneous leishmaniasis patients in comparison with control. Only a few studies have assessed the role of IL-17 in human infectious diseases and it is not known whether this cytokine participates as a defense mechanism or in the pathology of these diseases (Bacellar *et al*, 2009). IL-17 may contribute to

Table 1 : Levels of IL 17, 22 and heat shock protein-20 in leishmania patients as compare with control.

Parameter	Patients N = 40 Mean ± SD	Control N=40 Mean ± SD	P-value
IL-17 pg/ml	93.51 ± 26.60	22.61 ± 5.47	<0.001***
IL-22 pg/ml	121.92 ± 40.54	14.97 ± 3.20	<0.001***
HSP-20 pg/ml	4.91 ± 1.57	1.05 ± 0.62	<0.001***

***: Statistically highly significance, IL: Interleukin, HSP-20: heat shock protein-20, SD: standard deviation, N (80), two independent -t- test.

pathogenesis through several mechanisms, including neutrophil activation, tissue injury, and osteoclast activation and the regulated production of IL-17 contributes to infection control, while excessive IL-17 can promote neutrophil influx and tissue damage (Hussein *et al*, 2015). This result This results agree with many studies (Anderson *et al*, 2009 and Gonzalez-Lombana *et al*, 2013).

Interleukin 22 (IL-22) is a member of the IL-10 family of cytokines that was originally identified as an IL-9-inducible gene produced by mouse T cells (Gimeno *et al*, 2016; Rastrojo *et al*, 2019). The role of IL-22 in tumor development has been reported in several types of cancers, including gastric, lung, colon, hepatocellular and pancreatic carcinoma, where studies have shown up regulation of IL-22 by tumor-infiltrating lymphocytes in the tumor microenvironment, in addition to the expression of its receptor on cancerous cells (Fukui *et al*, 2014). The present study show highly significance increase in the level of IL-22 in leishmaniasis patients as compare with the control (Table 1).

The result of our study that represented in Table 1, shows a statistically highly significant increase ($P<0.001$) in the levels of heat shock protein-20 in leishmaniasis patients as compare with control. Heat shock protein 20 (HSP20/ HSPB6) is one of the small HSP family (HSPB) with monomer molecular mass in the range from 15 to 30 kDa (Bakthisaran *et al*, 2015). Small heat shock proteins (HSPs) are reported to play an important role in the regulation of a variety of cancer cell functions, and the functions of small HSPs are regulated by post-translational modifications such as phosphorylation (Mymrikov *et al*, 2011). Holakuyee *et al* (2012), concluded that the HSP direct the immune system towards Th2 pattern and does not have protective role in *L. major* infection. Heat shock protein act as chaperon in peptide folding and under stress conditions such as temperature shock will increase and bind to the cellular proteins to sustain the folding of the proteins (Holakuyee *et al*, 2012). Moreover, HSP have many immune-logical functions such as stimulation of innate immunity. HSP induce dendritic cells to produce pro-inflammatory cytokines such as IL-1, IL-6, TNF-α and IL-12 (Holakuyee *et al*, 2012). The investigators suggested the use of HSP20 as a useful serodiagnostic marker for active leishmaniasis (Montalvo-Álvarez *et al*, 2008).

CONCLUSION

The importance of interleukin 17, 22 and heat shock protein-20 in Leishmaniasis patients can be used to detect the complications related to the disease and immunological effect among these patients.

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