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Identifying Potential Biomarkers for Cutaneous Leishmaniasis Diagnosis

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Abstract

Cutaneous Leishmaniasis represents one of the widespread epidemic disease in Iraq caused by the protozoan *Leishmania* parasite. However, There are clinical difficulties in distinguish between this disease and other skin diseases can leave permanent skin scars. This study aims to identify potential biomarkers for Cutaneous Leishmaniasis diagnosis and prognosis and to know the progression of the disease and the distribution of inflammation within the skin cells. In this review, 17 proteins have been selected using literature searching such as pubmed and web of science to identify potential biomarkers for Cutaneous Leishmaniasis diagnosis and prognosis and further studies needed to find the role of these protein in the development of the disease.

Key words: Cutaneous Leishmaniasis, biomarkers.

Introduction

Leishmaniasis is a neglected tropical parasitic disease, according to the latest global health estimates, with about 700,000-1,000,000 new cases of leishmaniasis occurring each year (1). It is a widespread epidemic disease in Iraq caused by a protozoa parasite (2). This disease is one of the diseases that leave scars for life, and it is a non-fatal disease. The disease mainly affects the poor in Asia, Africa and Latin America and has a

close relationship with population migration, malnutrition, and a weak immune system with some limited resources for treatment (3,4).

Leishmaniasis is caused by an intracellular parasite belonging to the Trypanosomatidae family, the genus *Leishmania*. The life of this parasite is obligatory within the cells of the reticuloendothelial system, either in the mucous membranes, skin, liver, spleen, and bone marrow (5). The disease is transmitted by the female sand fly (Phlebotomus (sand fly) through a blood meal. Where there are more than 30 different species of Phlebotomus, and the parasite transmits this disease either to animals or to humans through their bites (6). Three important types can be diagnosed: Cutaneous Leishmaniasis (CL), Visceral Leishmaniasis (VL), and Mucocutaneous Leishmaniasis (MCL), as these types are associated with the causative parasites of the genus *Leishmania* and its own signs (1,6), as these parasites create conditions and change the internal environment of host cells to suit their survival and reproduction (7). Some people do not show signs or symptoms, but many people do not. Patients may suffer from the appearance of one or more ulcers on the skin, and the shape and size of the ulcer may change with the passage of time until it becomes a prominent ulcer with a raised edge and usually resembles a volcano crater. Lymph nodes swell in the area of infection, depending on the location It contains ulcers, usually painless, and these lesions abound in exposed areas of the body (8).

There are clinical difficulties in distinguish between this disease and other skin diseases can leave permanent skin scars. The primary goal of identifying new biomarkers for cutaneous leishmaniasis is to improve diagnostic and prognostic accuracy. Identification of proteins that can help to detect this disease in early stage and/or to know the progression of the disease and the distribution of inflammation within the skin cells.

The first step in this process is to identify the proteins whose expression will be examined in relation to the disease which can help predict disease behavior and drug response, which leads to the correct diagnosis and is to know the location of protein distribution within the histological section (9).

Seventeen proteins have been selected using literature searching which represents the simplest method of identification the potential biomarkers for further analysis Table (1). The criteria of selection was if these proteins are related to the parasite and/or the phagocytes of the parasite or a stage of its life stages. These protein are:

1- CD1a: It consist of a heavy chain, three extracellular domains, a transmembrane domain, and a short visceral tail that is non-covalently linked to the microglobulin light chain and contains a groove for antigen binding. (10), where CD1a proteins control specific T-cell responses to Leishmania antigens (11). This protein found in the

cytoplasmic granules of a group of blood cells and muscle cells and may be used as abiomarker for identifying macrophages and monocytes that contains Leishmaniaparasites Where CD1a-restricted T cells are exposed to lipid antigens rather thanpeptide antigens (12) . The previous study showed that increased CD1a expression significantly in patients with leishmaniasis compared to the normal tissues using Immunohistochemistry (2), suggesting that increasing expression of CD1a in the tissue may play a role in development of the disease.

2- CD68: It is an intracellular membrane glycoprotein with a molecular weight of 110 kDa. It is also called macrosialin and consists of 354 amino acids. Its location is within the cytoplasmic granules of endosomes. It is a member of the family of lysosomes associated membrane proteins, and its appearance is restricted to cells. Macrophages and mononuclear cells as well as CD68 protein shows clear expression in T cells and natural killer cells (13). The CD68 protein consists of two lamp-like domian domains, separated by a prolin-rich hinge, and contains a terminal cytosolic tail (14). It can also bind with low-density lipoproteins and because of its dense location within the lysosomes and endosymbionts. It contributes to protecting the membranes of these bodies from the effect of acidic hydrolysis, and then it quickly moves to the plasma membrane, which explains its role in cell adhesion and antigen processing, as it is a distinctive sign of phagocytic cells (15). It has major effects on the outcome of the disease. This proteins may play a significant role in the intracellular immune response to Leishmaniasis infections (16). The previous studies demonstrated that the Leishmania parasite is characterized by its ability to survive inside macrophage cells to avoid attacking them, and in turn prevents natural killer cells from recognizing parasite-infected macrophage cells (17). A research done by us showed increased CD68 expression significantly in patients with Leishmaniasis compared to the normal tissues using immunohistochemistry (2), suggesting it may play a significant role in this disease formation and progression.

3- KDNA: (Kinetoplast DNA) Molecular tests are widely used for epidemiological and diagnostic purposes at the level of genetic makeup by analyzing the molecular sequence of the KDNA gene. This was previously used to distinguish between the two types of Leishmania parasite *L.tropica , L.major* (18).

4- ILL (Iranian Lizard Leishmania) is a protein that has an effective role in immunizing against cutaneous leishmaniasis, as no lesion was developed at the vaccination site (19). Haghoust and his colleagues found that ILL is an effective vaccine against cutaneous leishmaniasis in mice and is able to induce an immune response which can prevent lesion formation (19).

5- TLR2: It is a protein used to identify functional signals to recruit neutrophils during infection. Parasites use neutrophil cells to establish disease, as they activate several

cellular receptors to release signals from the cell surface to participate in the inflammatory response (20). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

6- rK39: It is an immunological test used in the diagnosis of leishmaniasis using serum. This test has multiple benefits because it is easy to use and does not require expensive and complex equipment compared to other tests. It may help in the early diagnosis of Leishmaniasis (21).

7- TIMP-1: It is one of the metalloproteins that activates cellular signals. It was found that there is a correlation between the serum level of TIMP-1 and genetic polymorphisms between patients with cutaneous leishmaniasis and a control group (22). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

8- ITS1: ITS1 genes were used with Giemsa slides and showed a clear indication of the usefulness of slides for Leishmania parasites in retrospective epidemiological diagnoses. It was used to amplify the ribosomal genes and determine Leishmania species (23). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

9- CCL7 Chemokin: They are proteins that have a significant role in the negative regulation of skin infections, as suppression by early immune cells in the skin may limit the ability of the Leishmania parasite to spread (24). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

10- HSP70 (Heat shock proteins): They are proteins that have a major role in the immune response and are used as cellular signals in the host body when infected with the Leishmania parasite. It is present in the blood circulation as a signal of danger to the host. (25). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

11- IL-10 Interleukin: It is a protein that has the ability to respond to the immune system to determine the successful immune response and regulate immunity in infections. (26). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

12- Microsatelite: It is a protein used for phylogenetic analysis of a specific type of Leishmania and cannot be used in general (27). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

13- Lb NMT (N-misristoy Itran): They are proteins that have the ability to stabilize the enzyme and stabilize the form of the link so that the specific molecule has the ability to meet the target. (28). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

14- C-MET: It is a protein that has a role in controlling the skin lesion during infection as it promotes the migration of neutrophils to the sites of infection (29). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

15- Kinase-4: are important protein regulators of many biological processes that play an important role in the immune response by determining the binding sites and partial function of the mitogen-activated protein of Leishmania parasite (30).

16- LdMPK4: They are cellular proteins that play an important role in signal transduction in cells, where they are a natural inhibitor of Leishmania parasite, as well as potential targets for drugs due to their important role in various cellular processes (31). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

17- D1-D9: They are cellular proteins used in the diagnosis of leishmaniasis, as well as the evaluation of DNA integrity and identification of Leishmania species in clinical samples. (32). This protein may play a significant role in the cutaneous leishmaniasis diagnosis and / or prognosis.

Table (1) shows the selected proteins, their type and their role in the Leishmania parasite

No	Potential Biomarker	Function	Result	References
1	CD1a	An Antigen-Presenting Protein That Binds Lipid And Glycolipid Antigens And Presents Them To T-Cell Receptors And Is Structurally Related To MHC Histocompatibility Complex Proteins	Diagnosis Of Leishmaniasis With And Without The Body Of A Leishmaniasis. Participates In The Immune Response Against Leishmania Antigens	(11)
2	CD68	Promotes Phagocytosis And The Recruitment And Activation Of	Presentation Of Exogenous Antigens To T Cells That	(33)

		Macrophages	**Control The Outcome Of Infection After Initial Uptake Of The Mastigot By Macrophages In The Phagocytosis**	
3	**KDNA**	**Distinguish Between The Two Types Of Leishmania Parasite *L.Tropica , L.Major***	**This Method Was Used To Determine The Sex Of Leishmania**	**(18)**
4	**ILL**	**It Has A Preventive Role In Immunization**	**An Effective Vaccine Against Cutaneous Leishmaniasis In Mice Is Able To Induce An Immune Response That Prevents Lesion Formation**	**(19)**
5	**TLR2**	**It Forms Activating Groups Of Several Receptors Depending On The Binding And These Groups Release Signals From The Cell Surface And Participate In The Inflammatory Response And To Upregulate Signaling Pathways By PAMPS To Modulate The Inflammatory Response Of The Host And Promote Apoptosis**	**Identification Of Functional Signals By TLR2 To Recruit Neutrophils To The Skin In Response To Infection**	**(20)**
6	**Rk39**	**It Is Commonly Used In Serological Testing For Initial Verification**	**The Results Confirm The Accuracy Of The Tape Test In The Early Diagnosis Of Leishmaniasis**	**)21(**
7	**TIMP-1**	**A Metalloprotein That Acts As A Growth Factor That Regulates Cell Differentiation,**	**It Plays A Role In Vessel Wall Degeneration And Aneurysm**	**(22)**

		Migration, Activation Of Cellular Signaling, And Has A Natural Inhibitory Role Involved In Extracellular Matrix Degradation		
8	Use Of Giemsa Slides And ITS1 Genes	Amplification Of Ribosomal Genes And Determination Of Leishmania Species	A Positive Comparison Showed That The Best Modern Slides Were Used In The PCR Reaction	(23)
9	CCL7	It Has A Role In The Negative Regulation Of Dermatitis	It Specifically Decreases The Flow Of Neutrophils Into The Affected Skin	(24)
10	HSP70 Protein IFN-Y TGB-BI	It Plays An Important Role In The Immune Response And Maintains The Functions Of A Newly Formed Protein In The Body And Is Present In The Circulatory System As A Danger Signal To The Host	The Current Study Showed That There Is A Difference Between The Rate Of Heat Shock Protein Concentration In Cutaneous Leishmaniasis Compared With The Control Group	(25)
11	IL-10 IFN-Y	A Cytokine Produced Mainly By Monocytes And To A Lesser Extent By Lymphocytes. It Has Multi-Wave Effects In Regulating Immunity And Inflammation	An Increase In The Rates Of Immunoglobulins, Igg, Igm, Compared With The Control Group	(26)
12	Microsatelite	For A Phylogenetic Analysis Of Leishmania Strains	It Proved To Provide A Powerful Tool For Epidemiological Studies Due To Its Ability To Differentiate Strains From Other Types Of Leishmania	(27)

13	N-Misristoy Itran Sferase (Lb NMT)	It Has The Ability To Determine And Stabilize The Enzyme APO And HOLO And To Evaluate The Stability Of The Protein Complex	Stability Of HOLO-Binding Protein, APO The Results Showed The Binding Form To Stabilize The Enzyme	(28)
14	C-MET Tyrosin Kinase	Plays A Role In Cell Migration And Invasion	Promote Migration Of Neutrophils To Inflamed Sites	(29)
15	Kinase-4	It Plays A Role In The Response To Environmental Stress And In Determining The Pathway Of Cellular Signaling	ERK2 Inhibitors Have Been Obtained, As They Work To Prevent The Reproduction Of Parasites And Treat Diseases Caused By Leishmania	(30)
16	Ldmpk4	Plays A Role In The Signal Transduction Cascade By Dephosphorylating Kinase Enzymes And Thus Controlling The Expression Of Protein Molecules Required For Cell Activity	A Natural Inhibitor Of Leishmania Parasite	(31)
17	D1-D9 B5B9	Determination Of Leishmania Species In Clinical Samples By Qpcr-ML, Qpcr-Ama (KDNA)	The Evolutionary Relationships Were Inferred Using The Maximum Likelihood Method As Well As DNA Integrity Assessment Under The Same Conditions	(32)

Conclusion

Leishmaniasis is a widespread epidemic disease in Iraq caused by a protozoa parasite (2). This disease is one of the diseases that leave scars for life, and it is a non-fatal disease. There are clinical difficulties in distinguish between this disease and other skin diseases can leave permanent skin scars. The primary goal of identifying new biomarkers for cutaneous leishmaniasis is to improve diagnostic and prognostic accuracy.

The present review selected 17 proteins which may be used as a potential biomarkers in the future for the diagnosis and prognosis of Leishmaniasis. Many studies showed increased CD1a and CD68 protien level in pateints with Leishmaniasis compared to the normal people using different methods (2, 43, 35, 36, 37). However, the study of the researcher (38), it did not agree with previous studies, and the reason for this may be attributed to the biopsy site, where biopsies were taken from the bone marrow, where it is treated with nitric acid as a desiccant in bone biopsy samples, which spoiled the nature of CD1a and caused Amastigote reactions Negative immunohistochemistry despite the presence of the parasite, or it may be due to the number of samples, histological mode of action, or the type of antibody. The researcher (39) when using the IHC technique on skin diseases (scabies, lichen, and cutaneous leishmaniasis) reported the presence of helper T cells in all studied skin diseases. It was low except in the case of cutaneous leishmaniasis, as it showed an increase in the numbers of phagocytic cells and a clear response to the biomarker CD68.

The researcher's study (2) showed the presence of T cells associated with CD1a in the skin, and this is a clear indication of their participation in skin diseases (cutaneous leishmaniasis), as they have a functional ability in cases of infection and skin inflammation, as CD1a can interact with Amastigote after phagocytosis (38). These result suggest that these protein may play a role in the disease diagnosis and prognosis as well as treatment.

Research now concentrates on protein and/or genomic factors. For example, CD1a and CD68 has been validated for the prognosis of aggressive leishmaniasis (2) and it is suggested that detection of these protein may advance the diagnosis of the disease through early identification of high-risk patients. Understanding the biochemical and genetic aspects of leishmaniasis biomarkers not only has the ability to more efficiently detect the disease, but it may also provide an insight into how and why the disease arises, and may suggest a method to manage or even cure it.

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