



ISSN: 0067-2904

The role of Matrix metalloproteinase (MMP-9) and its tissue inhibitor (TIMP1) in Cutaneous Leishmaniasis patients and their role in prognosis

Ghuffran Muhammed Hassan¹, Hayder Zuhair Ali¹ , Watheq Muhammed Hussein²

¹Department. of Biology, College of Science, University of Baghdad, Al-Jaderiya Campus, Baghdad, Iraq 10071

²Dermatology department, University of Diyala college of medicine, Diyala governorate, Baquba, Iraq

Received: 25/3/2024

Accepted: 17/8/2024

Published: 30/8/2025

Abstract

Cutaneous leishmaniasis (CL) is a widespread, yet often overlooked, parasitic disease caused by the Leishmania protozoan, which is prevalent in numerous countries, including Iraq. This condition is marked by the appearance of skin lesions on various exposed areas of the body. In most old-world regions, sodium stibogluconate (SSG) is the classical widely used drug to treat CL. The progression of skin ulceration is controlled by different inflammatory modulators including cytokines and enzymes. In this study, the possible role of the enzyme Matrix metalloproteinase9 (MMP-9) and its inhibitor Metalloproteinase inhibitor-1 (TIMP-1) as immunological markers was evaluated in CL patients suffering from cutaneous leishmaniasis before and after treatment by sodium stibogluconate. A total of 161 serum samples of newly diagnosed individuals and patients undergoing Pentostam injections were collected from an endemic area of Diyala, east central Iraq. The level of MMP-9 and TIMP-1 was evaluated in the serum of recently diagnosed CL patients and previously diagnosed CL patients who were undergoing five stages of sodium stibogluconate injections. The results have shown that among the study groups, the recently infected individuals who received no treatment showed significantly elevated levels of both MMP-9 (mean: 20.864 ng/ml) and TIMP-1 (mean: 1193.748 ng/ml). Additionally, the concentration of TIMP-1 was significantly raised in the groups undergoing pentostam treatment from injection-1 to injection-4, when compared to the control individuals. The current findings highlight important insights into the studied serum markers to follow up the development of the treatment in cutaneous leishmaniasis patients.

Key words: cutaneous leishmaniasis, treatment, MMP-9, TIMP-1, SSG.

دور البروتينات المعدنية المصفوفة (MMP-9) ومثبطات الأنسجة (TIMP-1) في داء
الليشمانيا الجلدي ودورها في تقدم المرض

غفران محمد حسان¹، حيدر زهير علي¹، واثق محمد حسين²

¹ قسم علوم الحياة، كلية العلوم، جامعة بغداد، بغداد، العراق

² كلية الطب، جامعة ديالى، ديالى، العراق

الخلاصة

*Email: ghuffran.muhammed@sc.uobaghdad.edu.iq

داء الليشمانيا الجلدي هو مرض طفيلي واسع الانتشار، ولكن غالباً ما يتم تجاهله، ويسببه طفيلي الليشمانيا، وهو منتشر في العديد من البلدان، بما في ذلك العراق. يتميز هذا المرض بظهور آفات جلدية على مناطق مكشوفة مختلفة من الجسم. في معظم مناطق العالم القديم، يعتبر ستيوغلوكونات الصوديوم (SSG) الدواء الكلاسيكي المستخدم على نطاق واسع لعلاج داء الليشمانيا الجلدي. حيث يتم السيطرة على التقرحات الجلدية المتقدمة بواسطة اليات التهابية مختلفة بما في ذلك السيتوكينات والإنزيمات. في هذه الدراسة، تم تقييم الدور المحتمل لبروتينات المعدنية المصفوفة (MMP-9) ومثبطه الأنسجة (TIMP-1) كعلامات مناعية في مرضى داء الليشمانيا الجلدي قبل وبعد العلاج بستيوغلوكونات الصوديوم. تم جمع ما مجموعه 161 عينة مصل من الأفراد الذين تم تشخيصهم حديثاً والمرضى الذين خضعوا لحقن بننوستام من منطقة موبوءة في ديالى، شرق وسط العراق. ثم تم تقييم مستوى MMP-9 و TIMP-1 في مصل مرضى CL الذين تم تشخيصهم مؤخراً ومرضى CL الذين تم تشخيصهم سابقاً والذين خضعوا لخمس مراحل من حقن ستيوغلوكونات الصوديوم. أظهرت النتائج أنه بين مجموعات الدراسة، أظهر الأفراد المصابون حديثاً والذين لم يتلقوا أي علاج مستويات مرتفعة بشكل ملحوظ لكل من MMP-9 20.864ng/ml و TIMP-1 1193.748ng/ml بالإضافة إلى ذلك، ارتفع تركيز TIMP-1 بشكل ملحوظ في المجموعات التي خضعت لعلاج بننوستام من الجرعة الأولى إلى الجرعة الرابعة، مقارنة بالأفراد الذين لم يتلقوا العلاج. تسلط النتائج الحالية الضوء على رؤية مهمة حول علامات المصل المدروسة لمتابعة تطور العلاج في مرضى داء الليشمانيا الجلدي.

Introduction

Cutaneous leishmaniasis (CL) is a protozoan infection caused by the flagellated form of the parasite *Leishmania*. Leishmaniasis is considered a zoonotic disease spread world-wide through the vector of sandfly *Phlebotomus* spp. in the old-world or *Lutzomyia* in the new-world [1]. More than 20 different species of the genus *Leishmania* are known producing different symptoms and pathology, all of which are classified under the family Trypanosomatida [2, 3]. Leishmaniasis is endemic in more than 98 countries of tropical and Mediterranean regions [4]. The most clinical forms of leishmaniasis are classified into three medical manifestations, cutaneous leishmaniasis (CL), mucosal mucocutaneous leishmaniasis (MCL), and visceral leishmaniasis (VL). Infection by any *Leishmania* species is influenced by factors such as parasite virulence, the immunological response of the host, and the location of the lesions [5, 6]. The intracellular amastigotes exploit some circulatory molecules in order to properly establish an infection [7, 8].

Matrix metalloproteinase-9 (MMP-9) plays a crucial role in facilitating the breakdown of the extracellular matrix (ECM) during the vector *Phlebotomus* spp.'s feeding process on the victim. This breakdown allows the vector to successfully introduce the parasite into the skin of the vertebrate host. Subsequently, the promastigote migrates through the compromised extracellular matrix at the site of the lesion to start invasion [9]. The Metalloproteinases belong to a family of endopeptidases with a total of 23 members. These substances are known as Calcium dependent and zinc endopeptidases, they also have the ability to breakdown and modify the proteins of the extracellular matrix (ECM) [10, 11]. These enzymes are necessary for the degradation of extracellular matrix which guide defense-cells migration [12]. It is known that MMPs contribution is normally constant withing normal tissue state, while the rapid synthesis was observed during tissue renovation [13, 14]. Additionally, numerous factors may regulate the expression of these markers, such as cytokines, hormones and growth factors [15]. Tissue Inhibitors of Metalloteinase (TIMPs) consist of 1-4 proteins that comprise (184 -194) amino acids and have been considered as endogenous protein regulators with inhibitory effect on the enzyme Matrix metalloproteinase-9 (MMP-9) [16]. TIMPs were proved to have a crucial role in the extra-

cellular activation and subsequent clearance of MMPs [17]. Previous studies found the role of TIMPs in utilization of the extracellular matrix. Additionally, MMP-9 influences the expression levels of various chemokines, cytokines, growth factors and cell adhesion molecules. It plays a role in regulating the production of these immune signaling molecules and mediators of cell-cell interaction [17, 18]. Any regulatory failure in the matrix metalloproteinases and tissue inhibitors of metalloproteinases may influence various clinical disorders, including inflammation, abnormal blood vessels formation, tissue deformation, fibrosis and development of cancer [19]. During macrophages and neutrophils infiltration, metalloproteinases are stimulated as extracellular matrix for blood vessel walls reformation and aneurysm [20, 21]. A previous study on post kala-azar dermal leishmaniasis (PKDL) found that MMP-9 regulates collagen breakdown and facilitates basement membrane modeling, whereas TIMP inhibits activated MMP, indicating the likely healing process [1]. Furthermore, the process of wound healing is facilitated through re-epithelialization, which involves two fundamental functions: cell migration and proliferation [22]. Wound healing is regulated by intricate interactions between proteins and the extracellular matrix (ECM) [23, 24]. In this cross-sectional investigation, serum levels of the enzyme Matrix metalloproteinase and its cytokine modulator TIMP-1 were monitored in CL patients suffering from acute lesions and in patients undergoing sodium stibogluconate treatment.

Materials and Methods

Ethics consideration:

This cross-sectional study was conducted over a six-month period, from January 2022 to June 2022. Before collecting any sample, an approved statement was obtained from ethical committee at the Department of Biology, College of Science, University of Baghdad on December 1, 2021, No. CSFC/1221/0088.

Collection of samples:

A total of 161 blood samples were collected at Baquba Teaching Hospital in Diyala governorate, located northeast of Baghdad, the capital city, between January and June 2022. All patients were first clinically identified by the resident dermatologist for papules and lesion examination, suspected cases of cutaneous leishmaniasis were examined in the hospital laboratory for microscopic direct observation of collected skin scraping samples under light microscope, each sample was stained with Giemsa and inspected under light microscope for amastigote observation (1000 x) [25], (Figure 1). About five ml of venous blood was collected using a vacuum blood collection tube and serum was stored at -20°C for later measurement of enzymes. Patients were divided into 6 groups: the first group of individuals with new infection non-treated and the next five groups of patients were undergoing treatment of sodium stibogluconate (SSG) from dose-1 to dose-5 (10 mg/kg), at one week interval between each SSG injection. The total numbers of patients was 161, included 84 females and 77 males, were investigated within age ranged from 10 to 65 years old. The control group consisted of 50 healthy individuals recruited from the same target area as the study subjects.

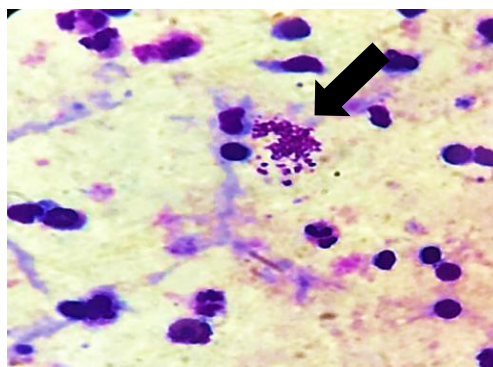


Figure 1: A lesion scraping stained with Giemsa showing amastigotes under light microscope at 1000x, black arrow→ amastigotes aggregation.

Immunoassay of MMP-9 and TIMP-1: The enzyme-linked immunosorbent assay (ELISA) was followed according to the manufacturer's procedure (Wuhan, Fine Biotech, China) to measure the level of matrix metalloproteinases (MMP-9) and tissue inhibitors of metalloproteinases (TIMP-1) for all the collected serum samples of patients and controls. Results were examined using an ELISA reader (Promega Instrument) of the 96 well-plates (two kits). The absorbency at 450 nm wavelength was measured. With 0.188 ng/ml sensitivity of MMP-9 and 18.75 pg/ml of TIMP-1.

Data analysis

Concentrations of each parameter was calculated against the controls, student t test was used for assigning significant differences, p value ≤ 0.05 was considered significant [26].

Results

Patients

The present study surveyed 161 individuals who were diagnosed with cutaneous leishmaniasis, in the endemic city of Baquba, a place known for its high prevalence of cutaneous leishmaniasis infection [27].

Study was conducted on individuals who were newly infected and had not received a treatment, as well as five groups of individuals who had previously been diagnosed and were undergoing weekly pentostam therapy and follow-up. Some patients were suffering from multiple ulcers located in different parts of exposed body. The data showed that 64 patients had a single cutaneous ulcer, 52 patients had between 2-4 ulcers, and 45 patients exhibited more than 4 ulcers. In terms of ulcer burden, the patient population comprised of 64 individuals with a solitary ulcer, 52 with multiple (2-4) ulcers, and 45 patients with greater than 4 skin lesions. Furthermore, infected individuals within the same family were documented, (Figure-2).

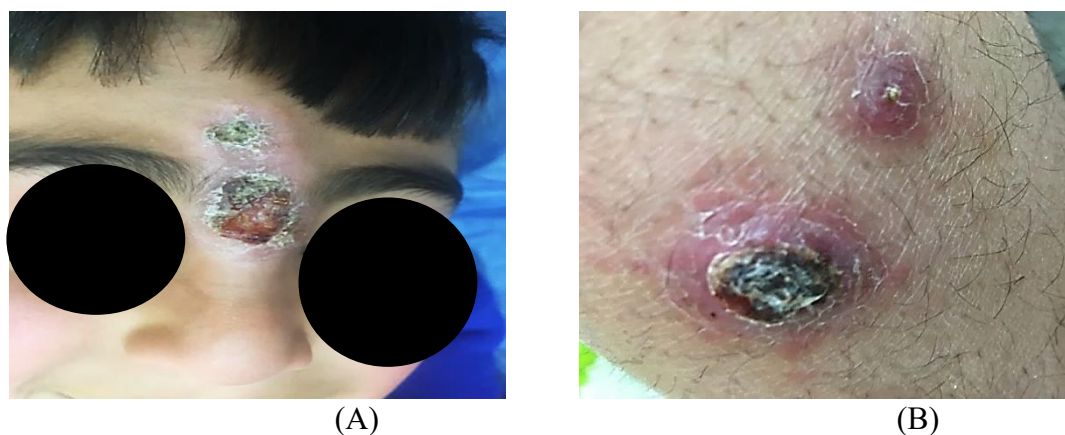


Figure 2: Cutaneous lesions of collected samples, A= non-treated ulcer between the eyes, B= ulcer after dose-2 treatment on the elbow.

Gender and age distribution: The values displayed in Table 1, demonstrating the mean concentrations of MMP-9 and TIMP-1 in males and females for each studied group. Gender difference was not statistically significant ($p > 0.05$) in the average values of the studied parameters between females and males at all untreated or treated groups.

Table 1: Comparison of serum mean levels of MMP-9 and TIMP-1 (p value ≤ 0.05) among the studied groups according to genders.

Group	New infection non treated (47 samples)		Treated group-1 (38 samples)		Treated group- 2 (27 samples)		Treated group- 3 (26 samples)		Treated group- 4 (16 samples)		Treated group 5 (7 samples)	
Gender	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
MMP-9	20.13 ± 1.98	21.64 ± 4.45	19.71 ± 2.19	20.86 ± 3.25	19.97 ± 4.52	19.02 ± 3.67	17.79 ± 2.89	18.64 ± 5.49	16.31 ± 1.40	16.79 ± 1.67	14.85 ± 1.83	17.35 ± 1.90
P value	0.0685		0.11		0.277		0.313		0.274		0.0691	
TIMP-1	1193.75 ± 161.14	1193.74 ± 103.22	1194.49 ± 68.93	1167.12 ± 107.25	1176.35 ± 114.96	1230.90 ± 149.33	1197.72 ± 139.87	1251.78 ± 150.36	1148.02 ± 41.97	1161.44 ± 82.26	1130.58 ± 72.68	1100.92 ± 62.68
P value	0.499		0.184		0.15		0.18		0.35		0.298	

Table two showing the average values of MMP-9 and TIMP-1 for all untreated and treated groups according to the age distribution. The only statistical difference (p value ≤ 0.05) was observed in the average value of MMP-9 among patient age group receiving Pentostam dose-1 ($p = 0.028$), however the significant value remained higher than that of the control group.

Table 2: Comparison of serum mean levels of MMP-9 and TIMP-1 (p value ≤ 0.05) among the studied groups according to age.

Group	New infection non treated (47 samples)		Treated group-1 (38 samples)		Treated group- 2 (27 samples)		Treated group- 3 (26 samples)		Treated group- 4 (16 samples)		Treated group 5 (7 samples)	
Age range	10–18	19–49	10–18	19–49	10–18	19–49	10–18	19–49	10–18	19–49	10–18	19–49
MMP9	20.25 ± 2.76	21.83 ± 3.40	21.22 ± 3.05	19.4 ± 2.4	21.25 ± 4.83	19.05 ± 2.57	16.77 ± 4.65	19.62 ± 3.73	17.20 ± 1.71	16.43 ± 1.43	14.26 ± 1.85	17.58 ± 1.49
P value	0.178		0.028		0.0631		0.07		0.145		0.08	
TIMP1	1214.23 ± 161.14	1173.17 ± 117.84	1157.5 ± 95.15	1201.2 ± 85.64	1148.3 ± 104.63	1225.31 ± 135.43	1232.2 ± 152.54	1216.92 ± 143.46	1184.8 ± 68.10	1142.2 ± 63.73	1103.1 ± 56.84	1128.9 ± 76.73
P value	0.167		0.072		0.075		0.257		1.22		0.32	

Serum levels of MMP-9:

Serum concentration of MMP-9 was determined in all the studied groups of untreated and five treated groups. The results revealed a significant increase ($p \leq 0.05$) in the serum of untreated new infection group and the first treated group, in which serum concentration was raised significantly to 20.57 ng/ml ($p < 0.0029$) and 19.58.35 ng/ml ($p < 0.025$), respectively, when compared to the mean concentration of controls ($17.61 \pm$ ng/ml). Interestingly, the mean level of MMP-9 started decreasing, when lesions began responding to treatment, where no significance (p value ≥ 0.05) was observed after treatment with 2- 5 doses, which were 19.41, 18.20, 16.58, and 15.9 ng/ml, respectively (Figures 3 and 4; Table 3).



Figure 3: Recovering arm ulcer after week five of pentostam treatment.

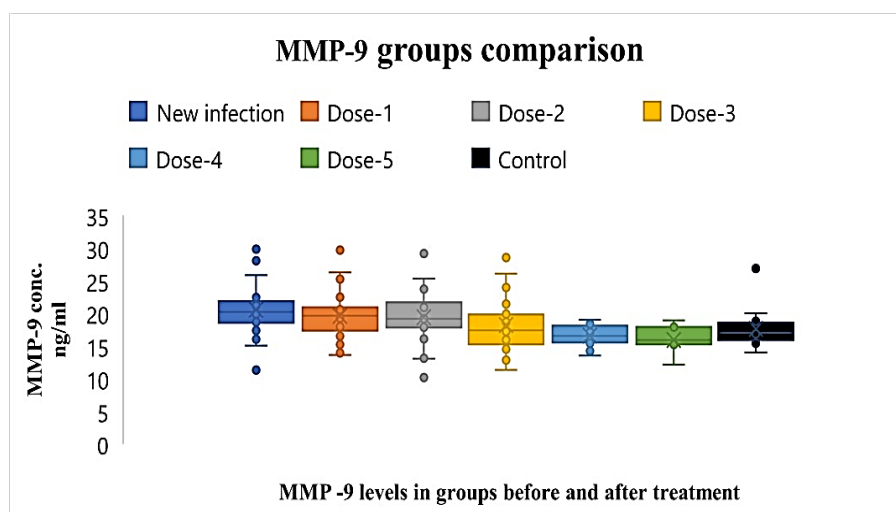


Figure 4: The mean level serum of MMP-9 concentration in all studied groups.

Serum level of tissue inhibitor (TIMP-1)

The TIMP-1 levels were found to be significantly elevated ($p \leq 0.05$) in the newly infected untreated group, with a mean concentration of 1193.74 ng/ml. Subsequently, a statistical difference in the TIMP-1 level was also calculated in the treated groups from doses one to four (1179.36, 1208.68, 1166.54, and 1155.57) ng/ml in comparison to the control mean level, which was 1028.12 ng/ml (Table 3 and Figure 5).

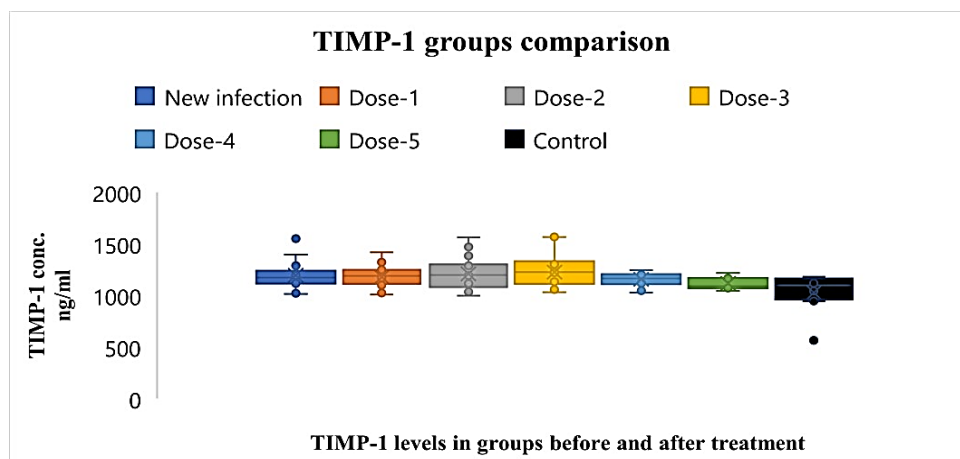


Figure 5: The mean level serum of TIMP-1 concentration in all studied groups.

Table 3: Mean of MMP-9 and TIMP-1 concentrations in the serum of all collected groups; p value ≤ 0.05 (*), p ≤ 0.01 (**), p value ≤ 0.001 (***)

Group	MMP-9 ng/ml		Mean TIMP-1 ng/ml	
	Mean value	P value	Mean value	P value
New infected untreated	20.57 ± 3.78	0.0029 **	1193.75 ± 134.4	0.00019 ***
Dose-1 mean	19.58 ± 3.45	0.025 *	1179.369 ± 91.9	0.00015 ***
Dose-2 mean	19.41 ± 3.98	0.08	1208.68 ± 136.7	0.00049 ***
Dose-3 mean	18.20 ± 4.2	0.09	1166.548 ± 147.1	0.0004 ***
Dose-4 mean	16.85 ± 1.5	0.1	1155.572 ± 66	0.0099 **
Dose-5 mean	15.92 ± 2.1	0.14	1117.869 ± 64.9	0.8
Control means	17.60 ± 2.9		1028.127 ± 196	

Discussion

A total of over 161 individuals diagnosed with CL were examined to identify potential serum markers in both untreated newly infected patients and those receiving multiple weekly doses of pentostam treatment. The studied area of Diyala government, where all samples were collected, was previously investigated in some researches and found to be an endemic region with a cutaneous leishmaniasis. It was also considered as a source of CL spreading to neighbor cities that are not typically affected by the disease [4, 27, 28].

As documented earlier in the results, males and females of wide range of age from 9 to over 60 years old have been confirmed to have CL in the endemic studied area, although similar surveys concluded that men are usually more prone to have CL infection due to the outdoor activities [29]. Cutaneous leishmaniasis (CL) is characterized by a visible ulceration with lifted borders that appears after the transmission of the parasite by sandflies in a few weeks [30]. Lymphadenopathy is one of the early symptoms of the disease, and 2–3 weeks later, a papule or exo-ulcerative lesion will appear at the bite site before the inflammatory ulcer is finally manifest [30, 31]. The evaluation of the immune response during the phase in which patients have not yet developed the cutaneous ulcer is important to determine factors contributing to disease progression [32].

Although macrophages are the primary attackers of infection to eradicate the parasite, *Leishmania* developed certain mechanisms to escape the immune defense leading to survive

and spread. Such mechanisms can be affected by the production of cytokines and other immune mediators [33]. A previous study showed that the lesions of cutaneous leishmaniasis by *Leishmania braziliensis* exhibit a variety of cytokine patterns regulating the infection at the tissue level, it triggers an inflammatory reaction driven by T helper type 1 (Th1) cytokines [34]. When many cutaneous *Leishmania* species infects a vertebrate host, the immune system produces significant quantities of MMP-9, primarily from activated macrophages [35]. Such enzyme is thought to have a role in leukocyte recruitment, microvascular permeability regulation, and the breakdown of extracellular matrix components during the first inflammatory response [36]. Treatment with antimonials can greatly shorten the healing time for cutaneous lesions, even if complete cure of the condition may take longer than six months [37]. Roughly three weeks following the completion of treatment, the majority of patients are clinically cured, although 15% of cases require multiple courses of medication [38]. Generally, the MMP-2 and MMP-9 are the most types of metalloproteinases produced by activated macrophages in response to tegumentary leishmaniasis [17]. Furthermore, it has been found that matrix metalloproteinases-9 (MMP-9) can serve as indicators of the disease development and as target proteins for therapeutic interventions [39]. It was also found that MMP-9 may serve as a biomarker to prognosticate an increased risk for parasite dispersion and the development of cutaneous leishmaniasis [40, 41], in addition to the other type of visceral leishmaniasis [42]. MMP-9 activity level was observed to increase in *L. braziliensis* infected *ex vivo* macrophage cultures, which demonstrates how the metalloprotease is essential for host defense against leishmaniasis progression [42, 43].

Another cross-sectional study proved that mucosal ulcers were distinctly progressed when high levels of MMP-9 were detected, concluding that infected macrophages produce more MMP-9, which in turn, lead to breakdown of cellular matrix in the damaged skin and promoting the spread of the invading parasites to other sites [44].

Previous studies proved that macrophages from individuals with reduced MMP-9 activity may have the ulcer infection to be controlled at the entry site [42, 45]. As reported in the results of this research where MMP-9 levels were higher in the untreated patients and only in the beginning of treatment, MMP-9 levels in the pre-ulcerative stage of the disease were found to be lower than those in the ulcerative stage, indicating that the development of the abscessed lesion of the disease is accompanied by elevated levels of MMP-9 as well as an imbalance between MMP-9 and its inhibitor metalloproteinase inhibitor-1 (TIMP-1). On the other hand, tumor necrosis factor (TNF) regulates MMP-9 in cutaneous leishmaniasis (CL) because exogenous TNF stimulates MMP-9 production in healthy patients, whereas negative neutralization of this cytokine regulates MMP-9 synthesis in patients with the cutaneous form of the disease [46].

Another study of mouse model infected with *L. chagasi* found that increased MMP-9 by macrophages was associated with the tissue damage of the infected mice [47]. In addition, high levels of mRNA for MMP-2 were found in ulcers of CL patients infected with the new world *L.*

braziliensis [42].

The current finding of elevated TIMP-1 is in the line of a previous study which reported a significantly higher amount of TIMP-1 in the patient of post-kala-azar dermal leishmaniasis group compared to the healthy control group [5]. Upregulating TIMP-1 is critical in reducing the potential effects of MMPs on tissue integrity, since TIMPs are major inhibitors of MMPs and efficiently reduce their enzymatic activity [48]. Furthermore, TIMP-1 was found to have

significant role in many parasitic infections either by protozoa or helminths [49]. Interferon-gamma (IFN- γ), interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β), found to stimulate over production of metalloprotease 9 (MMP-9) and tissue inhibitor of metalloproteinases (TIMP), both of which contribute to the inhibition of macrophages and the migration of cells to different tissues. Consequently, this process facilitates the persistence of infection and the progression of disease [45].

Conclusion

This study aimed to examine MMP-9 and TIMP-1 as potential serum biomarkers for ulcerative leishmaniasis. The results focused on comparing the levels of MMP-9 and TIMP-1 in the serum of patients with untreated skin lesions versus those undergoing treatment. Such markers found to be higher in the acute stage of the disease and at the beginning of treatment. Our findings indicate that the studied enzymes can be selected to predict the disease stage and therapy follow up in cutaneous leishmaniasis.

Reference

- [1] S. Islam, E. Kenah, M.A.A. Bhuiyan, K.K. Rahman, B. Goodhew, C.M. Ghalib, M.M. Zahid, M. Ozaki, M.W. Rahman, R. Haque, and S.P. Luby, "Clinical and immunological aspects of post-kala-azar dermal leishmaniasis in Bangladesh," *The American journal of tropical medicine and hygiene*, vol. 89, no. 2, p. 345, 2013.
- [2] F.T. Silveira, T.V. dos Santos, G.V.A. Flores, C.M.S. Pacheco, W.H.S. Ochoa, E.C. Sousa, C.Z. Valeriano, V.L.R. Da Matta, C.M.S. Gomes, P.K. Ramos, and L.V. Lima, "An integrative taxonomic analysis of Honduran Central America parasite isolated from non-ulcerated cutaneous leishmaniasis points to *Leishmania* (*Leishmania*) *poncei* n. sp.(Kinetoplastea: Trypanosomatidae)," 2023.
- [3] H.S. Al-Warid, I.M. Al-Saqur, S.B. Al-Tuwaijari, and K.A.A. Zadawi, "The distribution of cutaneous leishmaniasis in Iraq: demographic and climate aspects," *Asian Biomedicine*, vol. 11, no. 3, pp. 255-260, 2017.
- [4] A.M. Hussein, and H.Z. Ali, "Detection of TNF Alpha Level as Biomarker in Different Stages of Cutaneous Leishmaniasis Infection," *Iraqi Journal of Science*, pp. 3313-3321, 2022.
- [5] N.A. Ansari, G.K. Katara, V. Ramesh, and P. Salotra, "Evidence for involvement of TNFR1 and TIMPs in pathogenesis of post-kala-azar dermal leishmaniasis," *Clinical and Experimental Immunology*, vol. 154, no. 3, pp. 391-398, 2008.
- [6] M.M. Al-Halbosi, H.Z. Ali, G.M. Hassan, and F. Ghaffarifar, "Artemisinin efficacy against old world *Leishmania donovani*: In Vitro and ex vivo study," *Annals of Parasitology*, vol. 66, no. 3, 2020.
- [7] M.N.C. Soeiro, K. Werbovetz, D.W. Boykin, W.D. Wilson, M.Z. Wang, and A. Hemphill, "Novel amidines and analogues as promising agents against intracellular parasites: a systematic review," *Parasitology*, vol. 140, no. 8, pp. 929-951, 2013.
- [8] D.M. Bayram, G.M. Hassan, and H.Z. Ali, "Detection of some CC Chemokine Ligands in Patients with Cutaneous Leishmaniasis," *Iraqi Journal of Science*, pp. 1431-1437, 2022.
- [9] H. Nagase, R. Visse, and G. Murphy, "Structure and function of matrix metalloproteinases and TIMPs," *Cardiovascular research*, vol. 69, no. 3, pp. 562-573, 2006.
- [10] M.W. Thompson, "Regulation of zinc-dependent enzymes by metal carrier proteins," *Biometals*, vol. 35, no. 2, pp. 187-213, 2022.
- [11] O. Zítka, J. Kukacka, S. Krizkov, D. Húska, V. Adam, M. Masarik, R. Prusa, and R. Kizek, "Matrix metalloproteinases," *Current medicinal chemistry*, vol. 17, no. 31, pp. 3751-3768, 2010.
- [12] P.T.G. Elkington, C.M. O'kane, and J.S. Friedland, "The paradox of matrix metalloproteinases in infectious disease," *Clinical and Experimental Immunology*, vol. 142, no. 1, pp. 12-20, 2005.
- [13] W.C. Parks, C.L. Wilson, and Y.S. López-Boado, "Matrix metalloproteinases as modulators of inflammation and innate immunity," *Nature Reviews Immunology*, vol. 4, no. 8, pp. 617-629, 2004.

- [14] J. Liu, and R.A. Khalil, "Matrix metalloproteinase inhibitors as investigational and therapeutic tools in unrestrained tissue remodeling and pathological disorders," *Progress in molecular biology and translational science*, vol. 148, pp. 355-420, 2017.
- [15] C. Kapoor, S. Vaidya, V. Wadhwan, G. Kaur, and A. Pathak, "Seesaw of matrix metalloproteinases (MMPs)," *Journal of cancer research and therapeutics*, vol. 12, no. 1, pp. 28-35, 2016.
- [16] H.W. Jackson, V. Defamie, P. Waterhouse, and R. Khokha, "TIMPs: versatile extracellular regulators in cancer," *Nature Reviews Cancer*, vol. 17, no. 1, pp. 38-53, 2017.
- [17] X. Wang, and R.A. Khalil, "Matrix metalloproteinases, vascular remodeling, and vascular disease," *Advances in pharmacology*, vol. 81, pp. 241-330, 2018.
- [18] O. Zakiyanov, M. Kalousová, T. Zima, and V. Tesař, "Matrix metalloproteinases in renal diseases: a critical appraisal," *Kidney and Blood Pressure Research*, vol. 44, no. 3, pp. 298-330, 2019.
- [19] C. Amălinei, I.D. Căruntu, S.E. Giușcă, and R.A. Bălan, "Matrix metalloproteinases involvement in pathologic conditions," *Rom J Morphol Embryol*, vol. 51, no. 2, pp. 215-228, 2010.
- [20] T. Aoki, H. Kataoka, M. Morimoto, K. Nozaki, and N. Hashimoto, "Macrophage-derived matrix metalloproteinase-2 and-9 promote the progression of cerebral aneurysms in rats," *Stroke*, vol. 38, no. 1, pp. 162-169, 2007.
- [21] S. Swarnakar, S. Paul, L.P. Singh, and R.J. Reiter, "Matrix metalloproteinases in health and disease: regulation by melatonin," *Journal of Pineal Research*, vol. 50, no. 1, pp. 8-20, 2011.
- [22] I. Pastar, O. Stojadinovic, N.C. Yin, H. Ramirez, A.G. Nusbaum, A. Sawaya, S.B. Patel, L. Khalid, R.R. Isseroff, and M. Tomic-Canic, "Epithelialization in wound healing: a comprehensive review," *Advances in wound care*, vol. 3, no. 7, pp. 445-464, 2014.
- [23] P. Olczyk, Ł. Mencner, and K. Komosinska-Vassev, "The role of the extracellular matrix components in cutaneous wound healing," *BioMed research international*, vol. 2014, 2014.
- [24] O. Yamanaka, T. Sumioka, and S. Saika, "The role of extracellular matrix in corneal wound healing," *Cornea*, vol. 32, pp. S43-S45, 2013.
- [25] J.R. Ramírez, S. Agudelo, C. Muskus, J.F. Alzate, C. Berberich, D. Barker, and I.D. Velez, "Diagnosis of cutaneous leishmaniasis in Colombia: the sampling site within lesions influences the sensitivity of parasitologic diagnosis," *Journal of clinical microbiology*, vol. 38, no. 10, pp. 3768-3773, 2000.
- [26] N. Cary, "Statistical analysis system, User's guide. Statistical. Version 9," *SAS. Inst. Inc. USA*, 2012.
- [27] M.M. Al-Bajalan, S.M. Al-Jaf, S.S. Niranji, D.R. Abdulkareem, K.K. Al-Kayali, and H. Kato, "An outbreak of Leishmania major from an endemic to a non-endemic region posed a public health threat in Iraq from 2014-2017: Epidemiological, molecular and phylogenetic studies," *PLoS neglected tropical diseases*, vol. 12, no. 3, p. e0006255, 2018.
- [28] E.S. Khamaes, N.Y. Al-Bayati, and A.H. Abbas, "A Demographic and Epidemiological Investigation of Patients Afflicted with Cutaneous Leishmaniasis in The Diyala Government Region of Iraq," *Basrah Journal of Sciences*, vol. 41, no. 2, pp. 324-336, 2023.
- [29] E. Shiferaw, F. Murad, M. Tigabie, M. Abebaw, T. Alemu, S. Abate, R. Mohammed, A. Yeshanew, and F. Tajebe, "Hematological profiles of visceral leishmaniasis patients before and after treatment of anti-leishmanial drugs at University of Gondar Hospital; Leishmania Research and Treatment Center Northwest, Ethiopia," *BMC Infectious Diseases*, vol. 21, no. 1, pp. 1-7, 2021.
- [30] A. Unger, S. O'Neal, P.R. Machado, L.H. Guimarães, D.J. Morgan, A. Schrieffer, O. Bacellar, M.J. Glesby, and E.M. Carvalho, "Association of treatment of American cutaneous leishmaniasis prior to ulcer development with high rate of failure in northeastern Brazil," *The American journal of tropical medicine and hygiene*, vol. 80, no. 4, p. 574, 2009.
- [31] A. Barral, J. Guerreiro, G. Bomfim, D. Correia, M. Barral-Netto, and E.M. Carvalho, "Lymphadenopathy as the first sign of human cutaneous infection by Leishmania braziliensis," *The American journal of tropical medicine and hygiene*, vol. 53, no. 3, pp. 256-259, 1995.
- [32] A. D'Oliveira Junior, P. Machado, O. Bacellar, L.H. Cheng, R.P. Almeida, and E.M. Carvalho, "Evaluation of IFN-gamma and TNF-alpha as immunological markers of clinical outcome in

- cutaneous leishmaniasis," *Revista da Sociedade Brasileira de Medicina Tropical*, vol. 35, pp. 7-10, 2002.
- [33] W.N. Oliveira, L.E. Ribeiro, A. Schrieffer, P. Machado, E.M. Carvalho, and O. Bacellar, "The role of inflammatory and anti-inflammatory cytokines in the pathogenesis of human tegumentary leishmaniasis," *Cytokine*, vol. 66, no. 2, pp. 127-132, 2014.
- [34] C. Pirmez, M. Yamamura, K. Uyemura, M. Paes-Oliveira, Conceicao-Silva, F. and Modlin, R., "Cytokine patterns in the pathogenesis of human leishmaniasis," *The Journal of clinical investigation*, vol. 91, no. 4, pp. 1390-1395, 1993.
- [35] R. Choudhury, P. Das, T. De, and T. Chakraborti, "115 kDa serine protease confers sustained protection to visceral leishmaniasis caused by *Leishmania donovani* via IFN- γ induced down-regulation of TNF- α mediated MMP-9 activity," *Immunobiology*, vol. 218, no. 1, pp. 114-126, 2013.
- [36] C.A. Reichel, M. Rehberg, P. Bihari, C.M. Moser, S. Linder, A. Khandoga, and F. Krombach, "Gelatinases mediate neutrophil recruitment in vivo: evidence for stimulus specificity and a critical role in collagen IV remodeling," *Journal of Leucocyte Biology*, vol. 83, no. 4, pp. 864-874, 2008.
- [37] C. Oliveira-Ribeiro, M.I.F. Pimentel, L.D.F.A. Oliveira, É.D.C.F.E. Vasconcellos, F. Conceição-Silva, A.D.O. Schubach, A. Fagundes, C.X. de Mello, E. Mouta-Confort, L.D.F.C. Miranda, and C.M. Valet-Rosalino, "An old drug and different ways to treat cutaneous leishmaniasis: intralesional and intramuscular meglumine antimoniate in a reference center, Rio de Janeiro, Brazil," *PLoS Neglected Tropical Diseases*, vol. 15, no. 9, p. e0009734, 2021.
- [38] M.P. De Oliveira-Neto, M.S. phd, Mattos, M.A. Perez, A.M. Da-Cruz, O. phd, Fernandes, J. phd, Moreira, S.C. Gonçalves-Costa, and phd., "American tegumentary leishmaniasis (ATL) in Rio de Janeiro State, Brazil: main clinical and epidemiologic characteristics," *International journal of dermatology*, vol. 39, no. 7, pp. 506-514, 2000.
- [39] B.F. Costa, T.N. de Queiroz Filho, A.L. da Cruz Carneiro, A.S.C. Falcão, M.S. da Silva Kataoka, J.D.J.V. Pinheiro, and A.P.D. Rodrigues, "Detection and activity of MMP-2 and MMP-9 in *Leishmania amazonensis* and *Leishmania braziliensis* promastigotes," *BMC microbiology*, vol. 23, no. 1, p. 223, 2023.
- [40] F.A. de Oliveira, C. Vanessa Oliveira Silva, N.P. Damascena, R.O. Passos, M.S. Duthie, J.A. Guderian, A. Bhatia, T.R. de Moura, S.G. Reed, R.P. de Almeida, A.R. and de Jesus, "High levels of soluble CD40 ligand and matrix metalloproteinase-9 in serum are associated with favorable clinical evolution in human visceral leishmaniasis," *BMC infectious diseases*, vol. 13, no. 1, pp. 1-9, 2013.
- [41] E. Gadisa, G. Tasew, A. Abera, W. Gelaye, M. Chanyalew, M. Abebe, T. Laskay, and A. Aseffa, "Serological signatures of clinical cure following successful treatment with sodium stibogluconate in Ethiopian visceral leishmaniasis," *Cytokine*, vol. 91, pp. 6-9, 2017.
- [42] A.C. Maretti-Mira, K.M. de Pinho Rodrigues, M.P. de Oliveira-Neto, C. Pirmez, and N. Craft, "MMP-9 activity is induced by *Leishmania braziliensis* infection and correlates with mucosal leishmaniasis," *Acta tropica*, vol. 119, no. 2-3, pp. 160-164, 2011.
- [43] E.K. Elmahallawy, and A.A. Alkhaldi, "Insights into *Leishmania* molecules and their potential contribution to the virulence of the parasite," *Veterinary Sciences*, vol. 8, no. 2, p. 33, 2021.
- [44] N.O. Carragher, and M.C. Frame, "Focal adhesion and actin dynamics: a place where kinases and proteases meet to promote invasion," *Trends in cell biology*, vol. 14, no. 5, pp. 241-249, 2004.
- [45] L.S. Murase, J.V.P. de Souza, Q.A. de Lima Neto, T.F.P. de Mello, B.M. Cardoso, D.S.S.L. Lera-Nonose, J.J.V. Teixeira, M.V.C. Lonardoni, and I.G. Demarchi, "The role of metalloproteases in *Leishmania* species infection in the New World: A systematic review," *Parasitology*, vol. 145, no. 12, pp. 1499-1509, 2018.
- [46] T.M. Campos, S.T. Passos, F.O. Novais, D.P. Beiting, R.S. Costa, A. Queiroz, D. Mosser, P. Scott, E.M. Carvalho, and L.P. Carvalho, "Matrix metalloproteinase 9 production by monocytes is enhanced by TNF and participates in the pathology of human cutaneous Leishmaniasis," *PLoS Neglected Tropical Diseases*, vol. 8, no. 11, p. e3282, 2014.
- [47] J.D. Osta, A.C.N. de Melo, A.B. Vermelho, M. de Nazareth Meirelles, and R. Porrozzì, "In vitro evidence for metalloproteinase participation in hepatocyte damage induced by *Leishmania chagasi*-infected macrophages," *Acta tropica*, vol. 106, no. 3, pp. 175-183, 2008.

- [48] A.C. Maretti-Mira, M.P. de Oliveira-Neto, A.M. Da-Cruz, M.P. De Oliveira, N. Craft, and C. Pirmez, "Therapeutic failure in American cutaneous leishmaniasis is associated with gelatinase activity and cytokine expression," *Clinical & Experimental Immunology*, vol. 163, no. 2, pp. 207-214, 2011.
- [49] N. Geurts, G. Opdenakker, and P.E. Van den Steen, "Matrix metalloproteinases as therapeutic targets in protozoan parasitic infections," *Pharmacology & therapeutics*, vol. 133, no. 3, pp. 257-279, 2012.