# The Expression of Chemokine Genes in Neutrophiles Exposed to Leishmania

Naji T, Samadi S, Makoyi M*, Esmailiyan E, Tofighiyan Z, and Hematzadeh A

***Abstract*—** Chemokines are low-molecular-weight proteins that stimulate recruitment of leukocyte. Studies show that the chemokine gene expression in blood cells have an impact on parasitic diseases. Our study is about checking effect of chemokine gene in neutrophil exposed to leishmania. In this examination at the beginning of the work we put leishmania parasite sample in nitrogen tank at the pastor institute Department of Immunology. Then we transfered leishmania to NNN medium.We controlled parasite medium in 3th and 7th days. Parasites of 7th day in logarithmic phase was used for passage. RPMI was added to the medium for our job. With this method after 2-3 passages, parasites proliferate to reach the intended amount. Then parasites were used to dealing with neutrophils. The data was analyzed using ANOVA. Our findings show that**.** Our results showed that leishmania parasite has not stimulated the ccl4 gen production.

***Index Terms***— Chemokine Genes, Neutrophiles, Leishmania

## I. INTRODUCTION

Neutrophils or occasionally neutrocytes ) are the most abundant type of granulocytes and the most abundant (40% to 75%) type of white blood cells in most mammals they from an essential part of innate immune system[1]. Its functionality varies in different animals [2]. Neutrophils are a type of phagocyte and are normally found in the blood stream during the beginning (acute) phase of inflammation, particularly as a result of bacterial infection, environmental exposure and some cancers, neutrophils are one of the first-responders of inflammatory cells to migrate towards the site of inflammation.[3-4] Neutrophils have a variety of specific receptors, including ones for complement, cytokines like interleukins and IFN-Y, chemokines, and other proteins[5]. Chemokines are low-molecular-weight proteins that stimulate recruitment of leukocyte. [6]

They are secondary pro-inflammatory mediators that are induced by primary pro-inflammatory mediators such as interleukin-1 (IL-1) or tumor necrosis factor (TNF). [7]

Tahereh Naji (PhD) is with the Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

Soheil Samadi is with Department of Medicine, Tabriz Branch , Islamic Azad University, Tabriz, Iran

Mahsa Makoyi (*corresponding author) is with Department of Medicine, Tabriz Branch , Islamic Azad University, Tabriz, Iran.

Ehsan Esmailiyan is with Department of Medicine, Tabriz Branch , Islamic Azad University, Tabriz, Iran.

Zahra Tofighiyan is with Department of Medicine, Tabriz Branch , Islamic Azad University, Tabriz, Iran.

Arsalan Hematzadeh is with the Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

Leishmania is a genus of trypanosomes that are responsible for the disease leishmaniasis.[8-10] They are spread by sandflies of the genus Phlebotomus in the Old World, and of the genus Lutzomyia in the New World. At least 93 sandfly species are proven or probable vectors worldwide [11]. Their primary hosts are vertebrates;Leishmania commonly infects hyraxes, canids, rodents, and humans.[11] According to pervious studies shown that, neutrophils in concert with macrophages play a previously unrecognized leishmanicidal effect on L. (L.) amazonensis [12]. Accordind to last studies, During human infection, increased CCL2 expression has been reported in lesions from patients with localized self-healing CL caused by L. mexicana, compared to patients with nonhealing diffuse cutaneous disease (a chronic but hyporeactive presentation of CL), in which CCL3 is upregulated [13]. Previous evidence suggests that *Leishmania* virulence, based on lesion sizes induced in mice, may be linked to the differential expression of chemokines in murine macrophages. Infection of BALB/c mice with a highly virulent strain of *L. braziliensis* induced high expression levels of CCL3, CCL2, CCL11, and CXCL1/KC in footpad lesions and correlated with enhanced inflammation compared to infection with a less virulent *L. braziliensis* strain [14]. Previous studies have shown that, TLR9 plays a critical role in neutrophil recruitment during the protective response against *L. infantum* infection that could be associated with DC activation [15]. Last studies have shown that, that the circulating neutrophils during CL are not necessarily more microbicidal, but they have a more pro-inflammatory profile after parasite restimulation than neutrophils from healthy subjects [16]. Leishmania parasites have evolved intricate mechanisms to evade macrophage antimicrobial functions [17]. Leishmania-induced macrophage dysfunctions have been correlated mainly with depletion of microbicidal molecules [17]-[19]. According to last studie, Macrophages and neutrophils had cooperate in immune responses to Leishmania infection [20].

## II. MATERIAL AND METHODS

This study, performed in conjunction with the Leishmania infantum parasite. Stored parasite sample existence in nitrogen tanks at the Pastor Institute Department of Immunology. Parasites was transferred to the NNN medium. In the third and seventh days after the start of culture, environment with the view of parasite growth and lack of microbial and fungal was controlled and parasites of the seventh day (in logarithmic phase)was used for passage. RPMI was added to the medium for this job. Usually, each time adding medium is a passage. With this method, after 2-3 passages, parasites proliferate to reach the intended amount. Parasites cultivated in a static or stationary

phase is removed and were used to dealing with neutrophils. To achieve this phase of the seventh day after microscopic examination and confirmation of the presence of this phase parasites was used in the shape of promastigotes spindle shaped.

Perform real time PCR for samples: gene expression evaluated by SYBR Premix EX TaqII ( TAKARA company) use color SYBR green 1 to detection of proliferation of PCR products .DNA polymerase Takara EX $Taq^{TM}$ HS enzyme in the master mix addition to the feature Hot start, which causes unwanted products not to proliferate needs activate temperature of 95 ° C for 30 seconds. Polymerization property of this enzyme at 60 to 66 ° C, which allows the attachment and expansion PCR cycle integration and thus the reaction time of PCR be reduced.

Survey of Expression of ccl4 (MIP1-β): stage product was electrophoresed on agarose gel and it was determined that there was just a band on the size of 112 bp and primer dimer is not present in the product. Efficiency was evaluated at a later stage in the ccl4 efficiency was 0.987.

## III. Results

Figure I shows the standard curve slope CCL4

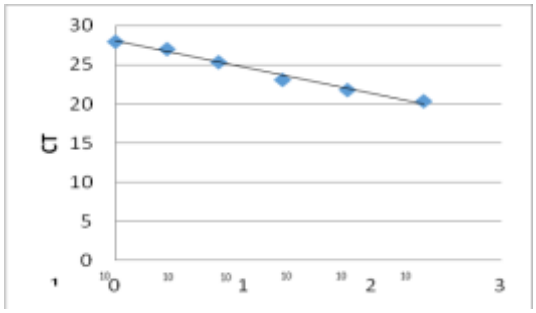


Fig. 1. the standard curve slope CCL4

Figure II shows the melting standard curve

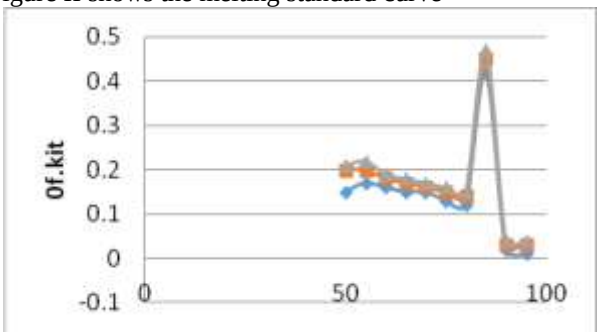


Fig. 2. the melting standard curve

Our findings showed that the average of stimulated samples is 6.1 and of not stimulated samples is 5.6.

This means that leishmania parasite has not stimulated the ccl4 production.

Figure III shows the reproduction curve and preliminary line

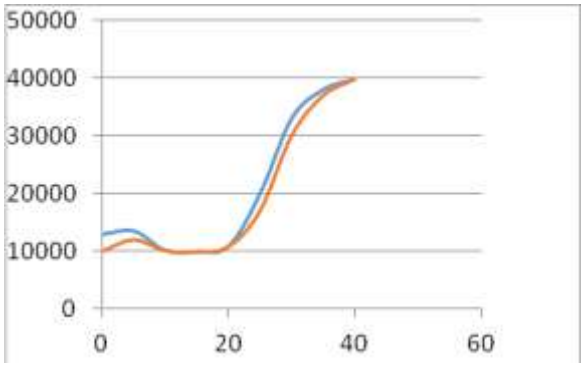


Fig. 3. the reproduction curve and preliminary line

Figure IV shows the CT of ccl4 gene in stimulated and non stimulated samples

| No | Colour | name | type | Ct |
|---|---|---|---|---|
| 5 | | Mip c | unknown | 25.40 |
| 6 | | Pip s | Unknown | 24.12 |
| 13 | | Mip c | Unknown | 25.41 |
| 14 | | Mip s | unknown | 23.86 |

Figure IV the CT of ccl4 gene in stimulated and non stimulated samples

Figure V shows the samples stimulated and unstimulated for CCL4

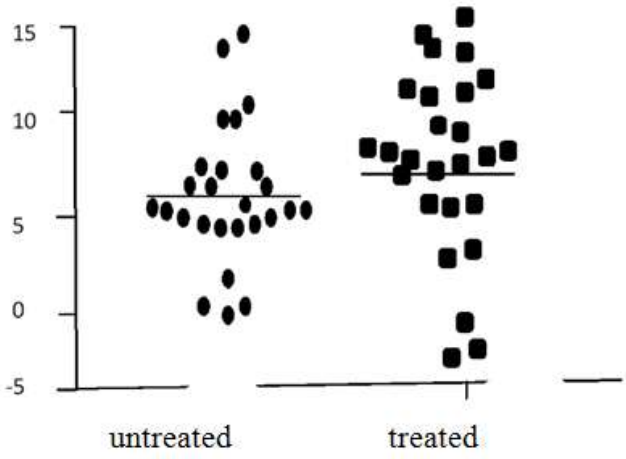


Fig.5. the samples stimulated and unstimulated for CCL4

Our findings showed that the average of stimulated samples is 6.1 and of not stimulated samples is 5.6.

This means that leishmania parasite has not stimulated the ccl4 production.

## IV. Discussion

Our findings show that Expression of Chemokine Genes in Neutrophiles Exposed to Leishmania. Last studies have shown that, the activation of antimicrobial effector functions in macrophages and, as a consequence, resistance or susceptibility of the host to Leishmania infections correlate with distinct patterns of cytokine production in the infected skin [21-22] The

present study provides the first evidence that self-healing LCL and progressive DCL, in addition to differences in Iymphokine expression, have differential expression of monocyte-macrophage attractant chemokines. Studies of the pathogenesis of human CL have revealed a fine balance between type 1adaptive immune responses leading to parasite clearance and exaggerated inflammatory responses leading to tissue damage [2,5].As an illustration, IFN-γ and TNF, which are required for cure of infection in mouse models, do not prevent ulceration in humans and actually correlate with the development of disease [5].Levels of IFN-γ and TNF directly correlate with lesion size [5] and the levels fall after successful therapy[4]. Moreover, immunomodulators that downmodulate the immune response and decrease TNF production, such as GM-CSF or pentoxyfilline, are more effective than antimony alone at reducing the time to healing and promoting cure of patients who are refractory to treatment with antimony alone[36,37].Furthermore, peripheral blood cells from individuals with subclinical infection, detected by a positive delayed type hypersensitivity test(DTH) to soluble leishmanial antigen (SLA) with no history of symptomatic disease, produce lower levels of these cytokines than CL patients [6]. Although neutrophils have been observed in CL lesions [23], a role for these cells in the pathogenesis of L.braziliensis disease pathogenesis has not been defined. Neutrophils are generally thought to be short-lived hematopoietic cells that migrate quickly to sites of infection. In mice, neutrophils migrate in large numbers to tissues infected with L.braziliensis [12],[24].Neutrophils are also found in tissues of CL patients albeit usually in small numbers [25,26].In contrast, macrophages and lymphocytes are the main hematopoietic cells at the site of inflammation in patients with CL, after several weeks to months of infection when biopsies are usually performed [14[,[27].The current study was based on the hypothesis that neutrophils contribute to the inflammation observed in human CL. Neutrophils can migrate to the site of infection and may produce inflammatory mediators in response to L.braziliensis infection triggering adaptive immune response, and thus could have an impact on the outcome of disease. However, we observed increased parasite loads in CL patient neutrophils during increased lengths of parasite exposure. Previous studies have been demonstrated that blocking neutrophil CR3 reduces the uptake of L.braziliensis [13] andTLR2 expression increases after L. braziliensis infection [15]. It is possible that neutrophils fromCL patients may increase their expression of these receptors associated with parasite uptake, and this may influences parasite burden. Following infection, neutrophils from both CL patients and healthy subjects presented a similar pattern of activation characterized by increased CD66b and decreased CD62L expression. CD66b is endogenous in specific granules and its increased appearance on the PMN surface indicates exocytosis from specific granules [28]. CD62L, also called L-selectin, is a homing receptor that is cleaved from the neutrophil surface upon activation, and its loss facilitates migration out of the circulation [29].The combined changes in both surface markers is indicative of activated phenotype [13,30]. Similarly, activated neutrophils were observed in a murine model of L. braziliensis infection [11] and studies of L.amazonensis-infected human neutrophils [14] showed that neutrophils from patients with CL due to a different organism a decrease inCD62L after exposure to the parasite. We also observed that, like infected cells, bystander neutrophils also presented an activated phenotype. This could have occurred due to exposure to infected neutrophils, and/or to transient contact with parasites. Alternatively, it has been demonstrated that exosomes, released from Leishmania spp. parasites have prionflammatory properties [31] and can activate resting neutrophils [32] or dendritic cells [33]. Furthermore, bystander dendritic cells express high levels of class II, CD80 andCD86after exposure to L.braziliensis, and their activation has been shown to require both, and host TNF [33].

## V. Conclusion

Our results showed that leishmania parasite has not stimulated the ccl4 gen production.

## Acknowledgment

We appreciate all who helped us to exert the present study. This paper reports a part of results selected from research work carried out by Arsalan Hematzadeh supervised by Dr. Ajdari and consulted by Dr. Tahereh Naji and Dr. Saeed Reza Pakzad at Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology , Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran (IAUPS).

## References

[1] BeilWJ, Meinardus HagerG, NeugebauerDC, SorgC Differencesintheonsetoftheinflammatoryresponsetocutaneousleishmania sisinresistantandsusceptiblemice. JLeukocBiol.1992.

[2] NauseefWM Isolation of human neutrophils from venous blood. Methods Mol Biol.2007. http://dx.doi.org/10.1007/978-1-59745-467-4_2

[3] Gueirard P, Laplante A, Rondeau C, Milon G, Desjardins M. Trafficking of Leishmania donovani promastigotes in non-lytic compartments in neutrophils enables the subsequent transfer of parasites to macrophages. Cell Microbiol .2008.

[4] CharmoyM,Brunner AgtenS, AebischerD, AudersetF,LaunoisP, et al. Neutrophil-derive CL3 is essential for the rapid recruitment of dendritic cells to the site of Leishmaniama jorinoculation in resistant mice. PLoSPathog.2010. http://dx.doi.org/10.1371/journal.ppat.1000755

[5] Mollinedo F, Janssen H, de la Iglesia-Vicente J, Villa-Pulgarin JA, Calafat J Selective fusion of azurophilic granules with Leishmania-containing phagosomes in human neutrophils. .2010

[6] Ribeiro-de-Jesus A, Almeida RP, Lessa H, Bacellar O, Carvalho EM. Cytokine profile and pathology in human leishmaniasis. Braz J Med Biol Res.1998. http://dx.doi.org/10.1590/S0100-879X1998000100020

[7] FalcaoSA, WeinkopffT, HurrellBP, CelesFS CurveloRP,etal ExposuretoLeishmaniabraziliensistriggersneutrophilactivationandapoptosis. PLoSNeglTropDis.2015.

[8] Ryan KJ; Ray CG (editors) (2004). Sherris Medical Microbiology (4th ed.). McGraw Hill. pp. 749–54.

[9] Myler P; Fasel N (editors) (2008). Leishmania: After The Genome. Caister Academic Press. .

[10] Ansari MY, Equbal A, Dikhit MR, Mansuri R, Rana S, Ali V, Sahoo GC, Das P (Nov 2015). "Establishment of Correlation between In-Silico &In-Vitro Test Analysis against Leishmania HGPRT to inhibitors". International Journal of Biological Macromolecules 83: 78–96. http://dx.doi.org/10.1016/j.ijbiomac.2015.11.051

[11] WHO (2010) Annual report. Geneva.

[12] de souza carmo EV, Katz S, baebieri CL neutrophils reduce the parasite burden in leishmania (leishmania) amazonensis infected macrophages. 2010.

[13] Ritter U, Moll H, Laskay T, Brocker E, Velazco O, Becker I, Gillitzer R. 1996. Differential expression of chemokines in patients with localized and diffuse cutaneous American leishmaniasis. J. Infect. Dis. 173:699–709.
http://dx.doi.org/10.1093/infdis/173.3.699

[14] Teixeira MJ, Fernandes JD, Teixeira CR, Andrade BB, Pompeu ML, Santana da Silva J, Brodskyn CI, Barral-Netto M, Barral A. 2005. Distinct Leishmania braziliensis isolates induce different paces of chemokine expression patterns. Infect. Immun. 73:1191–1195.
http://dx.doi.org/10.1128/IAI.73.2.1191-1195.2005

[15] Sacramento L, Trevelin SC, Nascimento MS, Lima-Jùnior DS, Costa DL, Almeida RP, Cunha FQ, Silva JS, Carregaro V.Toll-like receptor 9 signaling in dendritic cells regulates neutrophil recruitment to inflammatory foci following Leishmania infantum infection. fect Immun. 2015 Dec;83(12):4604-16.

[16] Conceição J, Davis R, Carneiro PP, Giudice A, Muniz AC, Wilson ME, Carvalho EM, Bacellar O.Characterization of Neutrophil Function in Human Cutaneous Leishmaniasis Caused by Leishmania braziliensis. PLoS Negl Trop Dis. 2016 May 11;10(5):e0004715.
http://dx.doi.org/10.1371/journal.pntd.0004715

[17] Olivier M, Gregory DJ, Forget G. 2005. Subversion mechanisms by which Leishmania parasites can escape the host immune response: a signaling point of view. Clin Microbiol Rev 18:293–305.
http://dx.doi.org/10.1128/CMR.18.2.293-305.2005

[18] Liew FY, Millott S, Parkinson C, Palmer RM, Moncada S. 1990. Macrophage killing of Leishmania parasite in vivo is mediated by nitric oxide from L-arginine. J Immunol 144:4794–4797.

[19] Murray HW. 1982. Cell-mediated immune response in experimental visceral leishmaniasis. II. Oxygen-dependent killing of intracellular Leishmania donovani amastigotes. J Immunol 129:351–357.

[20] Filardy AA1, Pires DR, DosReis GA.Macrophages and neutrophils cooperate in immune responses to Leishmania infection. Cell Mol Life Sci. 2011 Jun;68(11):1863-70.
http://dx.doi.org/10.1007/s00018-011-0653-2

[21] Pirmez C, Yamamura M, Uyemura K, Paes-Oliveira M, Conceicao-Silva F. Cytokine patterns in the pathogenesis ofhuman leishmaniasis. J Clin Invest 1993;91:1390-5.
http://dx.doi.org/10.1172/JCI116341

[22] Melby PC, Andrade-Narvaez FJ, Darnell BJ, Valencia Pcheco G, Tryon VV, Paloma-Cetina A. Increased expression of proinflammatory cytokines in chronic lesions of human cutaneous leishmaniasis. Infect Immun 1994;62:837-42.

[23] Dantas ML, deOliveira JM, Carvalho L ,Passos ST, Queiroz A, et al.(2014)Comparative analysis of the tissue inflammatory response in human cutaneous and disseminated leishmaniasis. Mem Inst OswaldoCruz 109:202–209.
http://dx.doi.org/10.1590/0074-0276130312

[24] de Moura TR, Novais FO, Oliveira F, Clarencio J, Noronha A, et al.(2005)Toward a novel experimental model of infection to study American cutaneous leishmaniasis caused by Leishmania braziliensis. Infect Immun 73:5827–5834.
http://dx.doi.org/10.1128/IAI.73.9.5827-5834.2005

[25] Dantas ML, Oliveira JC, Carvalho L, Passos ST, Queiroz A, et al.(2013) CD8+T cells in situ in different clinical forms of human cutaneous leishmaniasis. Rev Soc Bras Med Trop 46:728–734.
http://dx.doi.org/10.1590/0037-8682-0174-2013

[26] Bittencourt AL, Barral A (1991) Evaluation of the histopathological classifications of American cutaneous and mucocutaneous leishmaniasis.Mem Inst Oswaldo Cruz 86:51–56.
http://dx.doi.org/10.1590/S0074-02761991000100009

[27] Machado P, Araujo C, DaSilva AT, Almeida RP, D'Oliveira AJr,et al.(2002) Failure of early treatment of cutaneous leishmaniasis in preventing the development of an ulcer. Clin Infect Dis 34:E69–73.
http://dx.doi.org/10.1086/340526

[28] Uriarte SM ,Rane MJ, Luerman GC, Barati MT, Ward RA,et al. (2011) Granule exocytosis contributes to priming and activation of the human neutrophil respiratory burst. J Immunol 187:391–400.
http://dx.doi.org/10.4049/jimmunol.1003112

[29] Hafezi-Moghadam A, Thomas KL, Prorock AJ, Huo Y, Ley K (2001)L-selectin shedding regulates leukocyte recruitment. J Exp Med 193:863–872.
http://dx.doi.org/10.1084/jem.193.7.863

[30] Bezerra CA, CardosoTM, Giudice A, Porto AF, Santos SB, et al. (2011) Evaluation of the microbicidal activity and cytokines/chemokines profile released by neutrophils from HTLV-1-infected individuals. Scand J Immunol 74:310–317.
http://dx.doi.org/10.1111/j.1365-3083.2011.02579.x

[31] Hassani K, Shio MT, Martel C, Faubert D, Olivier M(2014) Absence of metalloprotease GP63 alters the protein content of Leishmania exosomes. PLoS One 9:e95007.
http://dx.doi.org/10.1371/journal.pone.0095007

[32] Majumdar R, Tavakoli Tameh A, Parent CA (2016) Exosomes Mediate LTB4 Release during Neutrophil Chemotaxis. PLoS Biol 14:e1002336.
http://dx.doi.org/10.1371/journal.pbio.1002336

[33] Carvalho LP, Pearce EJ, ScottP (2008) Functional dichotomy of dendritic cells following interaction with Leishmania braziliensis: infected cells produce high levels of TNF-alpha, whereas bystander dendritic cells are activated to promote T cell responses. J Immunol 181:6473–6480.
http://dx.doi.org/10.4049/jimmunol.181.9.6473