Association of TLR Gene Polymorphisms with Susceptibility to Visceral Leishmaniasis

Sheimaa J.alsheiban

Iraq/Ministry of Education/General Directorate of Education in Wasit

Abstract

Leishmania donovani and Leishmania infantum are the two most important organisms that together are responsible for causing visceral leishmaniasis (VL). VL continues to be a significant public health burden; particularly in areas where there is a recognized endemicity for this disease. The response of the immune system by the host is critical in determining whether one will get infected or not. Toll-like receptors (TLRs), as a component of the innate immune response, detect the presence of pathogens by recognizing specific molecules and starting the sequence of events that provides the host with a defense against the pathogens they encounter. Genetic differences between people that occur at the level of the genes encoding proteins that are located within TLRs may be one of the many factors that affect whether or not a person becomes infected with a pathogen, such as VL.

This research has calculated the correlation of some selected TLR gene polymorphisms (TLR2, TLR4 and TLR9) with susceptibility to visceral leishmaniasis. The study design was a case-control study consisting of 120 VL patients with confirmed diagnosis compared with 120 healthy individuals. PCR-RFLP techniques were used for genotyping. The analytical methods used included; comparison of genotype frequencies, estimating odds ratios, and conducting one-way analyses of variance (ANOVA) to determine if there were any differences in immunologic markers.

Our findings suggest that there was a significant relationship between the polymorphism of TLR4 (Asp299Gly) and increased susceptibility to VL ($p < 0.01$). The parasite burden of those with the mutant allele were statistically significantly ($p < 0.01$) higher (average of 3.8 + 0.9 log parasites/mL) than the parasite load of those with the wild-type allele (average of 2.1 + 0.7 log parasites/mL). The polymorphism of TLR2 (Arg753Gln) also was moderately associated with susceptibility to VL ($p < 0.05$), while TLR9 polymorphism did not demonstrate any statistically significant association with susceptibility to VL ($p > 0.05$). ANOVA analysis revealed that cytokine concentrations differed significantly among genotype groups ($p<0.001$; F-value = 45.30), suggesting that TLR polymorphisms may influence how immune responses are regulated. Genetic variations in TLR genes may impact a person's risk for developing visceral leishmaniasis (VL), potentially affecting how their immune system responds to other parasitic diseases. This information may help in identifying those who are at risk for developing VL and may also help inform the development of new therapies specific to individuals affected by VL.

Keywords- Visceral leishmaniasis; Toll-like receptors; TLR polymorphism; Genetic susceptibility; Cytokines

***Tob Regul Sci.™ 2026 ;12(1):* 8 - 19**

ارتباط تعدد أشكال جينات مستقبلات تول-لايك (TLR) مع القابلية للإصابة بداء الليشمانيا الحشوي

الملخص

تُعد كل من *Leishmania donovani* و*Leishmania infantum* العاملين الرئيسيين المسؤولين عن الإصابة بداء الليشمانيا الحشوي .(VL) ولا يزال هذا المرض يمثل عبئًا صحيًا كبيرًا، خاصة في المناطق التي يُعد فيها متوطنًا. ويلعب الجهاز المناعي للمضيف دورًا حاسمًا في تحديد قابلية الإصابة بالمرض. تُعتبر مستقبلات تول-لايك (TLRs) جزءًا أساسيًا من المناعة الفطرية، حيث تقوم بالتعرف على مسببات الأمراض من خلال التعرف على جزيئات محددة، وتبدأ سلسلة من الاستجابات التي تساعد الجسم على الدفاع ضد العدوى.

قد تُعد الاختلافات الجينية بين الأفراد، خصوصًا في الجينات المشفرة لبروتينات مستقبلاتTLR ، من العوامل التي تؤثر في قابلية الإصابة بالأمراض، ومنها داء الليشمانيا الحشوي.

هدفت هذه الدراسة إلى تقييم العلاقة بين بعض تعددات أشكال جينات TLR المختارة TLR2) و TLR4و (TLR9 والقابلية للإصابة بداء الليشمانيا الحشوي. تم تصميم الدراسة على شكل دراسة حالة–شاهد، حيث شملت 120 مريضًا مصابًا بالليشمانيا الحشوية تم تأكيد إصابتهم، مقابل 120 شخصًا سليمًا. تم إجراء تحليل الأنماط الجينية باستخدام تقنية PCR-RFLP.وشملت طرق التحليل مقارنة تكرار الأنماط الجينية، وحساب نسبة الأرجحية(Odds Ratio) ، بالإضافة إلى استخدام تحليل التباين الأحادي (ANOVA) لتقييم الاختلافات في المؤشرات المناعية.

أظهرت النتائج وجود علاقة ذات دلالة إحصائية بين تعدد أشكال جين TLR4 (Asp299Gly) وزيادة القابلية للإصابة بالمرض .($p < 0.01$) كما تبين أن الأفراد الحاملين للأليل الطافر لديهم حمولة طفيلية أعلى بشكل معنوي($p < 0.01$) ، حيث بلغ المتوسط (3.8 ± 0.9 لوغاريتم طفيليات/مل) مقارنة بالأفراد ذوي النمط البري (2.1 ± 0.7 لوغاريتم طفيليات/مل). كما أظهر تعدد أشكال جين TLR2 (Arg753Gln) ارتباطًا متوسطًا بالقابلية للإصابة($p < 0.05$) ، في حين لم يُظهر تعدد أشكال TLR9 أي ارتباط معنوي.($p > 0.05$)

كما كشف تحليل التباين (ANOVA) عن وجود فروق معنوية في مستويات السيتوكينات بين المجموعات الجينية المختلفة ($p < 0.001$)؛ قيمة($F = 45.30$ ، مما يشير إلى أن تعدد أشكال جينات TLR قد يؤثر على تنظيم الاستجابة المناعية.

تشير هذه النتائج إلى أن الاختلافات الجينية في جينات TLR قد تؤثر على خطر الإصابة بداء الليشمانيا الحشوي، وربما تلعب دورًا في كيفية استجابة الجهاز المناعي للأمراض الطفيلية. وقد تسهم هذه النتائج في تحديد الأفراد الأكثر عرضة للإصابة، كما قد تساعد في تطوير استراتيجيات علاجية موجهة تعتمد على الخصائص الفردية للمرضى.

الكلمات المفتاحية -: داء الليشمانيا الحشوي؛ مستقبلات تول-لايك؛ تعدد أشكالTLR ؛ القابلية الجينية؛ السيتوكينات

Introduction

Visceral leishmaniasis (VL) is one of the most serious parasitic diseases in humans, especially in low- and middle-income countries. VL is caused mainly by two protozoan intracellular parasites: Leishmania donovani and Leishmania infantum. These parasites infect macrophages and lead to impaired immune function (1). VL is transmitted via the bite of an infected female sandfly. After VL has become established after initial infection, it can lead to a systemic infection that is marked by the following signs: fever greater than one month in duration, hepatosplenomegaly (enlargement of the liver and spleen), pancytopenia (decrease in blood cell count), and, if left untreated, can lead to very high mortality (2). In spite of efforts internationally to reduce morbidity and mortality from this disease, VL continues to be a significant burden in areas where it occurs, including parts of South Asia, East Africa and the Mediterranean basin (3).

Clinical outcomes due to viral infection vary significantly between individual patients, making it challenging to develop an accurate clinical picture for all patients affected. Some patients will

present with a severe type of VL, or may even die from VL, while other patients will have asymptomatic VL or mild symptoms (4). The variability of the clinical outcome suggests the influence of host factors on disease susceptibility, disease progression, and other virus-host relationships. Specifically, host-related factors such as genetic and immune status can impact the clinical outcome of VL. In recent years, there has been a growing interest in elucidating how host genetics influence immunological responses to Leishmania (5).

Leishmania's immune response is multifaceted and is a delicate balancing act of both pro and anti-inflammatory actions. Macrophage activation and production of the cytokines interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), which facilitate the elimination of the parasite, are two important elements in effective control of the parasite (6). On the contrary, excessive amounts of the anti-inflammatory cytokine interleukin-10 (IL-10) will inhibit protective immunity and allow for survival of the parasite. This equilibrium can oftentimes be unstable and is subject to numerous influences and genetic diversity related to immune genes. Toll-like receptors (TLRs) created a very strong response in initial immune responses and are part of the innate immune system. The very important role of TLRs is to act as pattern recognition receptors (PRRs) for pathogens through pathogen-associated molecular patterns (PAMPs) and to lead to a cascade of immune activation through the activation of cell signaling by TLRs (7). After TLR activation, the TLRs activate an intracellular signaling pathway of adaptor proteins such as MyD88, which eventually leads to the activation of transcription factors such as NF-κB and the secretion of cytokines and chemokines. These early immune responses are very important because they provide a large portion of the initial activity and dictate the subsequent adaptive immune response (8).

The acknowledgement of Leishmania parasites relies on various TLRs and their various receptor/action mechanisms. TLR2 is widely known to recognize lipophosphoglycan (LPG), which is primarily found on the surface of many different Leishmania species. Some studies have suggested that TLR4 or similar receptors may interact with other components of the parasite's cellular structure (9). Finally, TLR9 recognizes unmethylated CpG motifs present within all bacteria, thus providing another mechanism for triggering an immune response subsequent to parasite infection. The three primary Leishmania-affiliated TLRs described here do not act independently but are part of a complex, integrated signalling network that ultimately produces a combined immune response to an infectious agent (10).

Genetic variants of TLR genes have been shown to lead to varying degrees of susceptibility to many infectious agents, due to their key role in immune activation and response. SNPs in TLR genes may affect the receptor's structure, binding of ligand, or the efficiency of downstream signalling (11). An example of a polymorphism that affects immune function, the TLR4 Asp299Gly (A/D plot indicates location of the polymorphism in the TLR4 gene), has been shown to drastically alter the ability of the immune system to respond to LPS and further

increase the likelihood of acquiring an infection. Similarly, variants in TLR2 and TLR9 have been linked to other infectious diseases including TB, malaria and viruses (12).

The role of TLR polymorphisms in visceral leishmaniasis is still being examined and the evidence to date is unclear. There have been some studies that report associations of some TLR variants with greater susceptibility to infection, which can be explained by either the presence of defective recognition by the host immune system, or a reduction in the activation of protective immune responses (13). However, other studies do not show any association at all. Differences in study populations or the different strains of Leishmania might account for these discrepancies, as well as other potential environmental factors. These variations could reflect the intricate nature of how pathogens and hosts interact; thus means that the influence of TLR polymorphisms will likely vary based upon the patient(s) you are investigating. There are many complexities of TLR signaling that arise from interactions among TLR types (14). The activation of TLR from one type can result in the activation of another type, such as TLR2 activating TLR9, or the activation of another immune receptor will change how the TLR response overall will be. TLR2 and TLR9 exhibit different cytokine profiles when activated simultaneously than when either one is activated alone, and there are a number of regulatory mechanisms, for example, negative feedback loops and cytokine-mediated inhibition, that will further alter the TLR activation response (15). Because of this, if scientists only consider individual polymorphisms in isolation without considering their interactions, then they are very likely to miss functional effects of those individual polymorphisms. Clinically, the clinical implications of understanding how genetic risk factors are associated with VL risk will help to inform strategies for managing patients who present with VL. Knowing which patients have genetic risk for severe VL can be used to develop specific criteria for screening and initiating early intervention treatment options (16). Furthermore, the understanding of TLR induced immune response may also lay a foundation for developing new treatment modalities including immunomodulatory therapy and vaccinations. In addition to this limitation, another limitation is the relatively small sample size (and therefore lack of power) and geographic homogeneity of most studies of TLR polymorphisms in VL, which have been conducted primarily in a small number (usually two) of developing countries (17). There is still much more to learn about how TLR polymorphisms may affect clinical disease outcomes through a combination of immunology, clinical medicine and genetics. Potential for improving our understanding of the role that TLR polymorphisms play in the pathogenesis of VL could be achieved through the integration of genomic (TLR) genotyping data with functional immune markers (cytokine levels) (18).

In this research study, we are looking at how variations (polymorphisms) in different genes (specifically, the TLR2, TLR4 and TLR9) might affect a person's chance of getting visceral leishmaniasis. Previous studies have looked only at how the genes are involved in visceral leishmaniasis, while we will perform not only genetic testing on participants but also take their immune systems into consideration by measuring various cytokines to fully explain the role of

genetics in visceral leishmaniasis. To analyze our data and evaluate our findings, we will use statistical methods such as ANOVA and average/standard deviation comparisons.

Materials and Methods

Study Design and Population

In order to explore the association of genetic differences in the Toll-like receptors (TLR) 2, TLR3, and TLR4 with susceptibility to visceral leishmaniasis (VL), we conducted a case-control study of 240 people (120 with confirmed VL by clinical symptoms, serologic testing, and identification of Leishmania amastigotes via microscopy, and 120 matched controls). The matched controls did not have a history of VL or any other long-term infectious disease, and controls were matched with VL patients for age, sex, and location (19).

Sample Collection and DNA Extraction

Peripheral Blood Samples were collected from all subjects through the use of EDTA tubes containing 5 ml of whole blood, obtained in a sterile environment. Genomic DNA extracted from the whole blood was performed by either a standard phenol-chloroform extraction or by using a commercial kit according to the manufacturer's protocol. DNA purity and concentration were determined using a spectrophotometer and all samples were stored at -20 °C until analysed (20).

Genotyping of TLR Polymorphisms

Polymorphisms at the chosen TLR genes including TLR2-Arg753Gln; TLR4-Asp299Gly and TLR9-rs352140 are evaluated by use of a polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP). Primers were made from previously published sequences and PCR amplification was done in 25µl reaction volume, using specific enzymes for digestion of the resulting PCR product. The fragments then separated using agarose gel electrophoresis and visualized with UV light (21).

Cytokine Measurement The concentrations of TNF-α and IL-10 in the serum were determined through ELISA, as specified by the manufacturer's instructions . The absorbance of the samples was measured with a microplate reader, and the concentrations of the cytokines were calculated using standard curves. Statistical Analysis Chi-square tests were used to compare genotype and allele frequencies on a case/control basis. To assess the strength of the relationship, odds ratios (OR) and 95% confidence intervals were calculated. Continuous variables (parasite load, cytokine levels) were analyzed by one-way analysis of variance (ANOVA) and expressed as mean ± standard deviation (SD). Statistical significance was defined as $p < 0.05$. All analyses were performed with statistical software (SPSS Version 25) (22).

Table 1. Demographic characteristics of study participants

Parameter	VL Patients (n=120)	Controls (n=120)
Age (years)	34.5 ± 8.2	33.8 ± 7.9
Male (%)	60	58
Female (%)	40	42

Table 2. PCR conditions and primer sequences

Gene	Primer Sequence (5'–3')	Annealing Temp (°C)	Product Size (bp)
TLR2	F: AGCTCAGCTTCTGGCTCTG	60	412
	R: TTGAGTTCTCCAGCTCCTG		
TLR4	F: GGTTGCTGTTCTCAAAGTGATTTT	58	249
	R: ACCTGAAGACTGGAGAGTGAGTTAA		
TLR9	F: GAGGCTGGTTTGTCTGTGTG	60	360
	R: CTGTTGGGAGGTAGGAGGAA		

Table 3. ELISA measurement parameters

Cytokine	Detection Range (pg/mL)	Sensitivity (pg/mL)
TNF-α	5–500	2
IL-10	2–300	1

Table 4. Statistical analysis framework

Variable	Statistical Test	Output
Genotype frequency	Chi-square	p-value
Disease risk	Odds ratio (OR)	95% CI
Cytokine levels	ANOVA	Mean ± SD, F-value, p-value
Parasite load	ANOVA	Mean ± SD

Results and Discussion

Distribution of TLR gene variants differ significantly in VL patients as compared to healthy controls. The TLR4 polymorphism has significantly higher mutant allele frequency in VL patients as compared to controls (TLR4 (Asp299Gly) — 55% in VL patients and 30% in healthy controls), which suggests a strong relationship between the mutant allele and a person's susceptibility for developing disease (23). Using an odds ratio (OR) of 2.5 (95% CI: 1.5 - 4.2, p = 0.001), this finding shows that individuals who carry this variant would have more than double the risk of developing VL (24). There was also some evidence of a variant genotype associated with TLR2 (Arg753Gln) and its occurrence among cases versus controls (p = 0.03), but the difference in numbers was not as large as for the TNF polymorphism mentioned above. Similarly, there was no significant association between the TLR9 polymorphism evaluated and risk of developing disease (p > 0.05), indicating that TLR9's effects depend on other environmental factors (25).

Table 5. Genotype frequencies of TLR polymorphisms

Gene	Genotype	Patients (%)	Controls (%)
TLR2	Wild	48	65
	Mutant	52	35
TLR4	Wild	45	70
	Mutant	55	30
TLR9	Wild	60	63
	Mutant	40	37

The number of parasites present in people with mutant TLR4 genes is very high compared to those who have normal functioning TLR4 genes, with an average of 38 times more parasites present in the blood of people with a mutant TLR4 gene compared to a person who has normal TLR4 gene function (21 times higher) (26).

Table 6. Parasite load by genotype (mean ± SD)

Genotype	Parasite Load (log parasites/mL)
Wild type	2.1 ± 0.7
Mutant type	3.8 ± 0.9

ANOVA: $F = 67.5$, $p < 0.001$

A large F-value indicates an effect of genotype on parasite burden and suggests that polymorphisms in TLR4 might reduce one's ability to effectively clear parasites via altered TLR4-mediated signalling pathways leading to macrophage activation (27). Cytokine measurements showed that individuals with mutant genotypes (ranging from 28 ± 5 pg/mL for TNF-α and 35

± 6 pg/mL for IL-10) have lower levels of TNF-α ($p < 0.01$) than each of their wild-type counterparts (45 ± 6 pg/mL for TNF-α), but higher levels of IL-10 than each of their wild-type counterparts ($p < 0.01$) than each of their wild-type counterparts (18 ± 4 pg/mL) (28).

Table 7. Cytokine levels (mean ± SD)

Group	TNF-α (pg/mL)	IL-10 (pg/mL)
Wild type	45 ± 6	18 ± 4
Mutant type	28 ± 5	35 ± 6

ANOVA: $F = 45.3$, $p < 0.001$

It has been demonstrated by these results that the immune system of persons with mutation-induced changes has undergone a transformation in its response to inflammation. Higher levels of IL-10 will result in the deactivation of macrophages, thereby limiting their ability to kill parasites (29). Lower levels of TNF-α will reduce the pro-inflammatory response essential for the establishment of sufficient immune protection. The risk analysis further confirmed the association between TLR polymorphisms and VL. Susceptibility refers to the likelihood or predisposition of an individual, group, or system to be affected or influenced by a particular factor, condition, or disease (30).

Table 8. Association between TLR polymorphisms and VL risk

Gene	OR	95% CI	p-value
TLR2	1.8	1.1–2.9	0.03
TLR4	2.5	1.5–4.2	0.001
TLR9	1.1	0.7–1.8	0.60

The results from this study suggest there are significant associations between polymorphisms of the TLR gene, primarily TLR4 alleles, and the risk of developing visceral leishmaniasis. In addition, evidence of association between mutations associated with increased parasite burden in the host and alterations in the cytokine profile suggest that these mutations may confer a disadvantage to the host in its ability to mount a strong immune response (31). It's especially remarkable that mutant carriers have lower levels of TNF-α because this cytokine plays a critical role in activating macrophages to kill intracellular parasites. Lower TNF-α levels may lead to a more permissive environment for Leishmania to survive and reproduce. Additionally, greater levels of IL-10 may act to further inhibit immune responses, stimulating the progression of the disease (32).

Our finding are also provided by TLR signalling as described in the literature referenced above. TLR9 has not been shown to contribute significantly to Leishmania infection; therefore, it can be

deduced that not all TLRs contribute equally to Leishmania infection, and that the effect of each TLR is likely dependent on the specific host-pathogen relationship studied (33). The data are characterised by low intra-genotype variance (low SD), indicating that the results produced from each individual are similar and that there is no outlier bias; thus, increasing the validity of the study's conclusions, and these results have applicability to biology (34, 35). Furthermore, evidence demonstrates that TLR polymorphisms are not independent and experiencing variability as a result of other gene polymorphisms as well as from non-genetic influences (such as exposure to a number of pathogens) and other infections (36, 37). Therefore, what is reported represents only a small part of much larger interactive system. Genetic variability in innate immune receptors appear to play an important role in susceptibility to VL, and understanding how these receptors operate may help identify populations at high risk and develop effective treatment and/or prevention methods.

Conclusion

Research results show that the genetic variation of Toll-like receptors, particularly TLR4 and TLR2, is associated with a higher chance for someone to contract visceral leishmaniasis. Individuals with mutated alleles tend to have a heavier parasite load along with an improper immune response marked by lower levels of pro-inflammatory cytokines and higher levels of anti-inflammatory cytokines. Changes in how TLRs respond within the host may influence how well the host can control the Leishmania infection. There were statistically significant differences in measured parasite loads and cytokine expression levels among Genotype (G) groups. The relatively low variability in mean parasite load and cytokine expression indicates that these results are biologically meaningful and not due to random chance. This analysis has shown no significant association with TLR9 polymorphism however additional future research may yield different results if future studies incorporate other environmental factors not included in the current study. Overall, this speculation corroborates the likely explanation that the risk of developing VL is due to several factors and that genetics and immunity combine to contribute to an individual's chance of developing VL. From a practical standpoint, it would be beneficial to understand these results in order to better identify which at-risk populations may experience an increased potential to progress to severe disease. Future work may require adding information about TLR gene variation into current risk assessment models for the future, especially in endemic regions. In addition, developing targeted therapies or immunomodulatory approaches would also be possible since this information could detail how the immune response to pathogens is affected by polymorphisms in host TLRs. Despite the significance of the findings, there are limitations that merit attention. First, the sample used for this study only consists of individuals from one area. Therefore, it cannot be assumed that these data will apply to individuals living in other areas that may have different mutations present in the population or strains of parasites responsible for infecting individuals. To establish stronger conclusions about the differences among parasites and the effects of parasite infection, further studies need to occur

throughout multiple locations with more representative sample sizes and also examine many more strains of parasites.

References

1. Mandal, A., Kumar, M., Kumar, A., Sen, A., Das, P., & Das, S. (2021). TLR4 and TLR9 polymorphism: Probable role in susceptibility to visceral leishmaniasis. *Innate Immunity*, 27(6), 493–500.
2. Ejghal, R., Hida, M., Bennani, M. L., Meziane, M., Aurag, R., & Lemrani, M. (2016). The TLR2 and TLR4 gene polymorphisms in Moroccan visceral leishmaniasis patients. *Acta Tropica*, 158, 77–82.
3. Rasouli, M., Moravej, A., Kalani, M., & Mansoori, Y. (2012). Toll-like receptor 4 polymorphisms in Iranian visceral leishmaniasis patients. *Molecular Biology Reports*, 40(6), 4009–4014.
4. Fakiola, M., Strange, A., Cordell, H. J., et al. (2013). Common variants in the HLA-DRB1–HLA-DQA1 region are associated with susceptibility to visceral leishmaniasis. *Nature Genetics*, 45(2), 208–213.
5. Blackwell, J. M., Fakiola, M., Ibrahim, M. E., et al. (2009). Genetics and visceral leishmaniasis: Of mice and man. *Parasite Immunology*, 31(5), 254–266.
6. Kaye, P., & Scott, P. (2011). Leishmaniasis: Complexity at the host–pathogen interface. *Nature Reviews Microbiology*, 9(8), 604–615.
7. Sacks, D., & Noben-Trauth, N. (2002). The immunology of susceptibility and resistance to leishmania major in mice. *Nature Reviews Immunology*, 2(11), 845–858.
8. Akira, S., Uematsu, S., & Takeuchi, O. (2006). Pathogen recognition and innate immunity. *Cell*, 124(4), 783–801.
9. Medzhitov, R. (2001). Toll-like receptors and innate immunity. *Nature Reviews Immunology*, 1(2), 135–145.
10. Becker, I., Salaiza, N., Aguirre, M., et al. (2003). Leishmania lipophosphoglycan (LPG) activates NK cells via TLR2. *Journal of Immunology*, 170(9), 4319–4327.
11. de Veer, M. J., Curtis, J. M., Baldwin, T. M., et al. (2003). MyD88 is essential for clearance of Leishmania major. *Journal of Immunology*, 170(10), 5332–5338.
12. Liese, J., Schleicher, U., & Bogdan, C. (2008). TLR9 signaling is essential for resistance to Leishmania major infection. *Infection and Immunity*, 76(6), 2715–2723.
13. Arbour, N. C., Lorenz, E., Schutte, B. C., et al. (2000). TLR4 mutations are associated with endotoxin hyporesponsiveness. *Nature Genetics*, 25(2), 187–191.

14. Schroder, N. W., & Schumann, R. R. (2005). Single nucleotide polymorphisms of TLR genes and susceptibility to infectious disease. *The Lancet Infectious Diseases*, 5(3), 156–164.

15. Newport, M. J., Allen, A., Awomoyi, A. A., et al. (2004). The toll-like receptor 2 polymorphism and susceptibility to tuberculosis. *The Lancet*, 364(9434), 1667–1671.

16. Nylén, S., & Sacks, D. (2007). Interleukin-10 and the pathogenesis of human visceral leishmaniasis. *Trends in Immunology*, 28(9), 378–384.

17. Goto, H., & Prianti, M. G. (2009). Immunoactivation and immunopathogeny during active visceral leishmaniasis. *Revista do Instituto de Medicina Tropical de São Paulo*, 51(5), 241–246.

18. Carvalho, E. M., Bacellar, O., Barral, A., et al. (1989). Antigen-specific immunosuppression in visceral leishmaniasis. *Journal of Clinical Investigation*, 83(3), 860–864.

19. Nylen, S., Maurya, R., Eidsmo, L., et al. (2007). Splenic accumulation of IL-10 mRNA in visceral leishmaniasis. *Journal of Immunology*, 178(7), 4648–4655.

20. Oliveira, I. B. N., et al. (2022). Single nucleotide polymorphisms in immune genes and leishmaniasis susceptibility. *Acta Tropica*, 231, 106450.

21. Chargui, N., et al. (2025). TLR4 and TLR9 polymorphisms in visceral leishmaniasis. *Parasite Immunology*.

22. da Silva, R. R., et al. (2022). Interleukin-10 polymorphisms and leishmaniasis progression. *Scientific Reports*, 12, 11136.

23. Kropf, P., Freudenberg, M. A., & Modolell, M. (2004). Toll-like receptor 4 contributes to effective immunity against Leishmania major. *Infection and Immunity*, 72(4), 1920–1928.

24. Schleicher, U., Liese, J., & Bogdan, C. (2007). TLR signaling in leishmaniasis. *Trends in Parasitology*, 23(11), 519–527.

25. Faria, M. S., Reis, F. C., & Lima, A. P. (2012). Toll-like receptors in leishmania infections. *Journal of Parasitology Research*, 2012, 1–7.

26. Murray, H. W., Berman, J. D., Davies, C. R., & Saravia, N. G. (2005). Advances in leishmaniasis. *The Lancet*, 366(9496), 1561–1577.

27. Sundar, S., & Rai, M. (2002). Laboratory diagnosis of visceral leishmaniasis. *Clinical and Diagnostic Laboratory Immunology*, 9(5), 951–958.

28. Singh, O. P., Sundar, S., & Singh, B. (2012). Molecular diagnosis of visceral leishmaniasis. *Expert Review of Molecular Diagnostics*, 12(5), 563–573.

29. Boelaert, M., et al. (2014). Visceral leishmaniasis control: A public health perspective. *Lancet Infectious Diseases*, 14(6), 581–590.

30. WHO. (2020). Control of the leishmaniases. *World Health Organization Technical Report Series*.

31. Chappuis, F., Sundar, S., Hailu, A., et al. (2007). Visceral leishmaniasis: What are the needs for diagnosis, treatment and control? *Nature Reviews Microbiology*, 5(11), 873–882.

32. Alvar, J., Vélez, I. D., Bern, C., et al. (2012). Leishmaniasis worldwide and global estimates. *PLoS One*, 7(5), e35671.

33. Reithinger, R., Dujardin, J. C., Louzir, H., et al. (2007). Cutaneous leishmaniasis. *The Lancet Infectious Diseases*, 7(9), 581–596.

34. Banuls, A. L., Hide, M., & Prugnolle, F. (2007). Leishmania and the genetic diversity of parasites. *Trends in Parasitology*, 23(10), 511–517.

35. Kamhawi, S. (2006). Phlebotomine sand flies and leishmania parasites. *PLoS Pathogens*, 2(7), e41.

36. McCall, L. I., Zhang, W. W., & Matlashewski, G. (2013). Determinants of virulence in Leishmania. *PLoS Pathogens*, 9(3), e1003053.

37. O'Neill, L. A., Golenbock, D., & Bowie, A. G. (2013). The history of toll-like receptors. *Nature Reviews Immunology*, 13(6), 453–460.