Evaluation of Fc γ RIIIB-NA1/NA2 Polymorphism in Visceral Leishmaniasis

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Received: May 23, 2013; Revised: August 6, 2013; Accepted: September 15, 2013

Background: Several lines of evidence demonstrating that innate and adaptive immunity play important roles in the defense against visceral leishmaniasis (VL). A polymorphism within the Fc γ RIIIB gene can lead to the expression of three variants of NA1, NA2, and the combined one (NA1/NA2) which alters affinity of IgG to its receptor.

Objectives: The main aim of this study was to evaluate the Fc γ RIIIB-NA1/NA2 polymorphism in the Fc γ RIIIB gene of VL patients in comparison to healthy controls.

Patients and Methods: In this cross-sectional study, three groups; 54 seropositive patients with clinical presentation of VL (group 1), 104 seropositive patients without clinical presentation (group 2), and 104 healthy controls (group 3) were evaluated with respect to the Fc γ RIIIB-NA1/NA2 polymorphism using a PCR-SSP method. The titration of anti-leishmania antibodies was analyzed using an immunofluorescence technique.

Results: Our results indicated that polymorphisms within the Fc γ RIIIB gene (that lead to the expression of the NA1/NA2 isoforms) are significantly associated with VL. The results demonstrated that the genotype heterozygotic for Fc γ RIIIB-NA1/NA2 expression was significantly increased in VL patients, group 1 when compared to groups 2 and 3. Conversely, there is a decrease in homozygous NA1 and NA2 genotypes in VL patients; however, the overall frequency of NA1 and NA2 alleles appear similar across the three cohorts examined.

Conclusions: According to our results, it is likely that the increased frequency of the Fc γ RIIIB-NA1/NA2 genotype is associated with impaired immune responses against VL and its subsequent clearance from the patient.

Keywords: Polymorphism, Genetic; Leishmaniasis, Visceral; Iran

1. Background

Various Leishmania species are responsible for leishmaniasis, which is a vector-borne parasitic disease (1). Although, the sand fly is the major vector of the leishmaniasis transfer into humans (1), the parasite can also be transmitted via needle stick injury (2), blood transfusions (3) or through placenta during pregnancy (4). Several studies identified that the immune responses against leishmaniasis is dependent on genetic status, environmental factors and the infecting species (5). Clinical manifestations of Leishmaniasis are various including; cutaneous, mucocutaneous, and visceral (6) which is the most severe and lethal form if left untreated. The visceral form (VL) is also known as Kala Azar (7).

It seems that the progression and severity of the disease is based on several host factors which include a genetic component. The final clinical outcome and severity of the disease is, in part, determined by how well the host immune system can respond to the infection. Previous

studies reported that both innate or adaptive immunity play important roles in both defense against VL and the eradication of the parasite (8). Neutrophils which are important innate immunity cells play critical roles combating leishmaniasis via phagocytosis of the parasite, degranulation, and generation of reactive oxygen species (ROS).

The mechanism of internalization of the parasite functions in an antibody/Fc-gamma receptor dependent manner with Fc γ RIIIB contributes a major role in the process (9). Therefore, the efficacy between Fc γ RIIIB and its corresponding antibodies (IgG) leads to appropriate immune responses against VL. Previous studies have showed that the Fc γ RIIIB gene (NM-00570.4) contains a polymorphism including Fc γ RIIIB*1 and Fc γ RIIIB*2 alleles, which code for human neutrophil antigen (HNA)-1a (or NA1) and HNA-1b (or NA2) (10). Interestingly, studies demonstrated that Fc γ RIIIBNA1/NA1 is associated with phagocytosis of IgG1 and IgG3-coated particles more powerful than the

Implication for health policy/practice/research/medical education:

The polymorphism within Fc γ RIIIB gene may be responsible for impaired immune responses against visceral leishmaniasis and resistance to leishmaniasis. This study can be used for screening patients at risk.

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Fc γ RIIIB-NA2/NA2 genotype (11).

2. Objectives

Based on the fact that, the quality and quantity of immune responses determine the outcome of leishmaniasis, the main aim of this study was to evaluate the Fc γ RIIIB-NA1/2 polymorphism in seropositive VL patients with and without clinical presentation in comparison with healthy controls.

3. Patients and Methods

3.1. Subjects

This cross-sectional study was performed on 3 groups; 54 patients with clinical presentation of VL who were seropositive for the *Leishmania* (group 1), 104 seropositive patients without clinical presentation (group 2) and finally, 144 healthy controls whose age, sex, nationality and race were matched (group 3). The patients were selected from East Azarbaijan (a city located in northwest of Iran) where the *Leishmania infantum* is endemic (12-14), while, other species were not reported. Visceral leishmaniasis was diagnosed by an expert specialist according to medical history, laboratory findings, and clinical presentations. The study protocol was approved by local Ethical Committee of the Ardabil University of Medical Sciences. An informed consent form was filled out and signed by participants prior to entrance in the study.

3.2. DNA Extraction

Peripheral blood was obtained from the participants in EDTA pre-treated tubes and genomic DNA was purified using a commercial kit (Bioneer, Korea) according to the manufacturer's guidelines. The purified DNA was aliquoted and stored at -20°C for use in PCR-SSP analysis.

3.3. Polymorphism Detection

The Fc γ RIIIB gene polymorphism (within exon 3) was evaluated using PCR-SSP method. PCR was performed in a final volume of 20 μ L containing 2 μ L of Taq DNA polymerase buffer (10X), 0.3 μ L of Taq DNA polymerase (five units), 1 μ L of each dNTP [(dATP, dCTP, dGTP, dTTP) at a concentration of 10 mM, 2 μ L of each primer (25 ng/ μ L), 2 μ L of MgCl₂ (stock concentration 1.5 mM), 1 μ L of prepared DNA and sterile double distilled DNase free water.

The sequences of the forward and reverse primers for amplification of the NA1 allele were 5'-CAGTGGTTTCA-CATGTGAA-3' and 5'-CATGGACTTCTAGCTGCACCG-3' and for the NA2 allele were 5'-CTCAATGGTACAGCGTGCTT-3' and 5'-CTGTACTCTCCACTGTCGTT-3'. The PCR product sizes of NA1 and NA2 genes were 142 and 169 bp, respectively. The amplification was performed using the following procedure: 95°C for 5 minutes (denaturation) followed by 35 cycles of 30 seconds at 95°C, 53°C for 30 seconds

and 72°C for 40 seconds using a thermal cycler (Bioneer, South Korea). Electrophoresis was performed on a 2.5% pretreated ethidium bromide agarose gel (Cinnaclon-Iran) for the detection of PCR products.

3.4. Immunofluorescence Assay

The titration of the anti-leishmania antibody was performed using a commercial kit (Qiagen, USA) according to the manufacturer's guidelines.

3.5. Statistical Analysis

Hardy-Weinberg equilibrium analysis was used to check the validity of the raw data. The χ^2 and One-way analysis of variance (ANOVA) tests were used from the SPSS software version 13 to determine the differences between groups and a P value less than 0.05 was considered as significant.

4. Results

Our results indicated that the Fc γ RIIIB-NA1/NA2 polymorphisms were significantly associated with VL (Table 1). The results demonstrated that the Fc γ RIIIB-NA1/NA2 genotype was significantly higher in group 1 compared to groups 2 and 3 (Table 1). The statistical analysis revealed that the difference between groups regarding the genotypes were significant ($P = 0.02$). However, the results showed that the groups were not different regarding the frequency of the Fc γ RIIIB-NA1 and NA2 alleles (Table 1) ($P = 0.17$). The current results revealed that age was not associated with antibody production against leishmania ($P = 0.07$). Our results also demonstrated that women had higher antibody titers against leishmania than men ($P = 0.006$) (Table 2). The current results also demonstrated that the VL patients (group 1) carrying the Fc γ RIIIB-NA1/NA1, NA2/NA2 and NA1/NA2 genotypes had mean anti-leishmania antibody titrations values to the 2.75 ± 0.41 , 2.47 ± 1.63 , and 2.34 ± 0.43 respectively, which were not statistically significant ($P = 0.82$). Our results revealed that participants in group 2 carrying the Fc γ RIIIB-NA1/NA1, NA2/NA2 and NA1/NA2 genotypes had mean anti-leishmania antibody titrations values to the 3.5 ± 0.17 , 2.9 ± 0.09 , and 3.2 ± 0.06 , respectively. The statistical analysis demonstrated that the difference between group 2 carrying the Fc γ RIIIB-NA1/NA1 and NA2/NA2 was significant ($P = 0.003$).

5. Discussion

Previous studies demonstrated that Fc γ RIIIB binds to IgG subclasses and its affinity to IgG is regulated by the Fc γ RIIIB isoforms (i.e. NA1 or NA2) which are in turn determined by polymorphisms within the gene (9). Our results showed that Fc γ RIIIB-NA1/NA2 genotype was significantly higher in group 1 (VL and seropositive) compared to group 2 and 3, while, Fc γ RIIIB-NA1/NA1 and NA2/NA2 genotypes in group 2 were higher than group 1. Therefore, it appears that Fc γ RIIIB-NA1/

NA2 genotype is associated with VL and may be considered as a risk factor for developing VL in Iranian patients infected with *Leishmania*. Based on the fact that the FcγRIIIB-NA1 isoform for human IgG2 and IgG3 has a higher affinity than the FcγRIIIB-NA2 isoform (9) and our results which revealed that the FcγRIIIB-NA2/

NA2 genotype was more frequent in group 2, it may be concluded that the rate of FcγRIIIB expression but not its affinity to IgG2 and IgG3 can determine the outcome of leishmaniasis. Thus, it seems that the prevalence of FcγRIIIB-NA1/NA2 genotype in VL patients may be a risk factor for *Leishmania*.

Table 1. The Prevalence of FcγRIIIB Polymorphisms Among Patients ^a

	Group 1 ^b	Group 2 ^b	Group 3 ^b	P value ^c
Genotypes				0.024
NA1/NA1	8 (14.8)	21 (20.2)	13 (9)	
NA2/NA2	23 (42.6)	58 (55.8)	79 (54.9)	
NA1/NA2	23 (42.6)	25 (24)	52 (36.1)	
Alleles				0.176
NA1	39 (36.1)	67 (32.2)	78 (27.1)	
NA2	69 (63.9)	141 (67.8)	210 (72.9)	

^a Data in table are presented as No. (%).

^b Group 1: Patients with clinical presentation of VL and seropositive for the leishmania, Group 2: Without clinical presentation but seropositive, Group 3: Healthy controls.

^c Significance (P values) is shown for the comparison between groups 1 and 2.

Table 2. The Anti-Leishmania Antibody Titration in Women and Men ^{a, b}

Titration of Anti-Leishmania Antibody	Sex		Total
	Female	Male	
0	89 (47.6)	62 (53.9)	151 (50)
1/10	2 (1.1)	9 (7.8)	11 (3.6)
1/20	6 (3.2)	5 (4.3)	11 (3.6)
1/40	65 (34.8)	27 (23.5)	92 (30.5)
1/80	19 (10.2)	6 (5.2)	25 (8.3)
1/160	4 (2.1)	6 (5.2)	10 (3.3)
1/320	2 (1.1)	0	2 (0.7)
Total	187 (100.0)	115 (100.0)	302 (100.0)

^a Data are presented in No. (%).

^b The table illustrates that the percent of women who have a high titer (1/40, 1/80 and 1/320) of anti-Leishmania antibodies were more than men.

Our results may propose a mechanism for inadequate immune responses against *Leishmania* in VL patients. Accordingly, previous investigations demonstrated that FcγRIIIB is expressed on the natural killer (NK) cells and participates in antibody dependent cell-mediated cytotoxicity (ADCC) against infected cells (15, 16). Therefore, it appears that the polymorphisms within FcγRIIIB gene can affect functions of NK cells which is reported by previous studies (17, 18). To the best of our knowledge this is the first study which evaluated the FcγRIIIB-NA1/NA2 polymorphisms in patients with VL.

But several studies are conducted on the malaria which is another parasitic infection (19). For example, Adu et al. revealed that FcγRIIIB-NA2 allele was significantly associated with clinical malaria in Ghanaian children (20). Ouma et al. reported that FcγRIIIB-NA1 allele was associ-

ated with reduced risk of severe malarial anemia (SMA), while, the patients carrying FcγRIIIB-NA2 allele had increased susceptibility to SMA (21). Also, Omi et al. also identified that FcγRIIIB-NA1/NA2 polymorphism have interactive effects on immune responses against malaria infection (22). Therefore, based on our data and the aforementioned studies it may be concluded that the FcγRIIIB-NA1/NA2 genotype is significantly associated with parasite infections including leishmaniasis and malaria.

In contrast with our results the polymorphisms in other FcγRs including FcγRIIA were not associated with VL. For example, our previous study revealed that polymorphism in the FcγRIIA gene was not associated with Iranian VL patients (unpublished data). Similarly, Oliveira et al. also reported that the polymorphism in the FcγRIIIB gene was not associated with American tegumentary leishmaniasis (ATL) (9). Their results also demonstrated

that the polymorphism was not related to the development of various clinical forms of ALT (9).

According to our results and those of Oliveira et al. (9) it may be concluded that the FcγRIIIB (but not other FcγRs) polymorphisms play important roles in VL pathogenesis. Our results also identified that age was not associated with antibody production against *Leishmania*. Therefore, it may be concluded that age does not influence humoral immunity against leishmaniasis. Additionally, our results showed that the FcγRIIIB-NA1/NA2 polymorphism was not associated with anti-*Leishmania* antibody titration in VL patients but FcγRIIIB-NA1/NA1 genotype was associated with higher antibody production in seropositive participants without clinical presentation. Hence, based on our results and previous studies it may be concluded that FcγRIIIB-NA1/NA1 genotype can affect either IgG/receptor affinity or antibody production against the *Leishmania* parasite in Iranian population. Furthermore, the results showed that women produced higher antibody against *Leishmania* than men. Based on the different hormonal status, the immune system of women shift to the Th2 system (23), so, this population has stronger humoral immunity than men. Therefore, the higher antibody titration against *Leishmania* in women may be related to the different hormonal status.

Finally, based on our results it appears that the FcγRIIIB-NA1/NA2 genotype is significantly associated with Iranian VL patients and more studies which evaluate other FcγRs are recommended for future studies.

Acknowledgements

There is no acknowledgment.

Authors' Contribution

All of authors involved in all steps of the manuscript preparation.

Financial Disclosure

There was no financial disclosure

Funding/Support

This project was supported by a grant from Hamadan University of Medical Sciences.

References

- Hartemink N, Vanwambeke SO, Heesterbeek H, Rogers D, Morley D, Pesson B, et al. Integrated mapping of establishment risk for emerging vector-borne infections: a case study of canine leishmaniasis in southwest France. *PLoS One*. 2011;**6**(8).
- Herwaldt BL, Juranek DD. Laboratory-acquired malaria, leishmaniasis, trypanosomiasis, and toxoplasmosis. *Am J Trop Med Hyg*. 1993;**48**(3):313-23.
- Wiwanitkit V. Bone marrow leishmaniasis: a review of situation in Thailand. *Asian Pac J Trop Biomed*. 2011;**4**(10):757-9.
- Meinecke CK, Schottelius J, Oskam L, Fleischer B. Congenital transmission of visceral leishmaniasis (Kala Azar) from an asymptomatic mother to her child. *Pediatrics*. 1999;**104**(5).
- Roberts MT. Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment. *Br Med Bull*. 2005;**75**:76:115-30.
- Newlove T, Robinson M, Meehan SA, Pomerantz R. Old World cutaneous leishmaniasis. *Dermatol Online J*. 2012;**18**(12):32.
- Wilson ME, Jeronimo SM, Pearson RD. Immunopathogenesis of infection with the visceralizing *Leishmania* species. *Microb Pathog*. 2005;**38**(4):147-60.
- Ali N, Mekuria AH, Requena JM, Engwerda C. Immunity to visceral leishmaniasis. *J Trop Med*. 2012;**2012**:780809.
- Oliveira CR, Pereira LI, Pereira AJ, Ferreira AA, Crespo AM, Silveira LA. Allelic polymorphism of human FcγRIIIA-H/R131 receptor in American tegumentary leishmaniasis. *Int J Immunogenet*. 2011;**38**(3):225-31.
- Salviati M, Ortona E, Colasanti T, Francia A, Pontecorvo S, Delle Chiaie R. Olanzapine-induced neutropenia in a patient with systemic lupus erythematosus: a role of FcγRIIIB polymorphism? *Lupus*. 2012;**21**(1):97-9.
- Bredius RG, de Vries CE, Troelstra A, van Alphen L, Weening RS, van de Winkel JG, et al. Phagocytosis of *Staphylococcus aureus* and *Haemophilus influenzae* type B opsonized with polyclonal human IgG1 and IgG2 antibodies. Functional hFcγRIIIa polymorphism to IgG2. *J Immunol*. 1993;**151**(3):1463-72.
- Oshaghi MA, Ravasan NM, Javadian EA, Mohebbi M, Hajjarian H, Zare Z, et al. Vector incrimination of sand flies in the most important visceral leishmaniasis focus in Iran. *Am J Trop Med Hyg*. 2009;**81**(4):572-7.
- Oshaghi MA, Ravasan NM, Hide M, Javadian EA, Rassi Y, Sadraei J, et al. *Phlebotomus perfiliewi* transcaucasicus is circulating both *Leishmania donovani* and *L. infantum* in northwest Iran. *Exp Parasitol*. 2009;**123**(3):218-25.
- Mohebbi M, Javadian E, Yaghoobi-Ershadi MR, Akhavan AA, Hajjarian H, Abaei MR. Characterization of *Leishmania* infection in rodents from endemic areas of the Islamic Republic of Iran. *East Mediterr Health J*. 2004;**10**(4-5):591-9.
- Smith HR, Idris AH, Yokoyama WM. Murine natural killer cell activation receptors. *Immunol Rev*. 2001;**181**:115-25.
- Siberil S, Dutertre CA, Fridman WH, Teillaud JL. FcγRIIIb: The key to optimize therapeutic antibodies? *Crit Rev Oncol Hematol*. 2007;**62**(1):26-33.
- Lieke T, Nylen S, Eidsmo L, Schmetz C, Berg L, Akuffo H. The interplay between *Leishmania* promastigotes and human Natural Killer cells in vitro leads to direct lysis of *Leishmania* by NK cells and modulation of NK cell activity by *Leishmania* promastigotes. *Parasitology*. 2011:1-12.
- Prajeeth CK, Haeblerlein S, Sebald H, Schleicher U, Bogdan C. *Leishmania*-infected macrophages are targets of NK cell-derived cytokines but not of NK cell cytotoxicity. *Infect Immun*. 2011;**79**(7):2699-708.
- Braga EM, Scopel KK, Komatsu NT, da Silva-Nunes M, Ferreira MU. Polymorphism of the FcγRIIIb receptor IIA and malaria morbidity. *J Mol Genet Med*. 2005;**1**(1):5-10.
- Adu B, Dodoo D, Adukpo S, Hedley PL, Arthur FK, Gerds TA, et al. FcγRIIIb polymorphisms are associated with clinical malaria in Ghanaian children. *PLoS One*. 2012;**7**(9).
- Ouma C, Davenport GC, Garcia S, Kempaiah P, Chaudhary A, Were T, et al. Functional haplotypes of FcγRIIIb predict risk to repeated episodes of severe malarial anemia and mortality in Kenyan children. *Hum Genet*. 2012;**131**(2):289-99.
- Omi K, Ohashi J, Patarapotikul J, Hananantachai H, Naka I, Looareesuwan S, et al. FcγRIIIb polymorphisms are associated with susceptibility to cerebral malaria. *Parasitol Int*. 2002;**51**(4):361-6.
- Gonzalez DA, Diaz BB, Rodriguez Perez Mdel C, Hernandez AG, Chico BN, de Leon AC. Sex hormones and autoimmunity. *Immunol Lett*. 2010;**133**(1):6-13.