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Short Communication

Lack of Polymorphism in Human Interleukin-2 Gene among Malarial Patients from District Bannu, Pakistan

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ABSTRACT

Inflammatory cytokines play an important role in human immune responses to malarial disease. However, role of these mediators in disease pathogenesis and the relationship between host and injury remain unclear. This study was aimed at finding out polymorphism in human Interleukin-2 gene among malarial patients. Blood samples were collected from 122 patients suffering from malaria from Malaria Control Program operating in women and children District Head Quarter, Hospital at Bannu, Pakistan during summer 2007. Isolated human DNA was amplified by PCR using 3 exons of human IL-2 gene. All PCR products were of the same size as that of exon deduced from gene bank thus showing lack of polymorphism in Human Interleukin-2 gene. It was concluded that there are some invariant allele copies prevalently associated as a result of static and reduced genetic drift among population of District Bannu, Pakistan. © 2010 Friends Science Publishers

Key Words: Interleukin-2 gene; *Plasmodium faciparum*; *P. vivax*

INTRODUCTION

Malaria is present in endemic form in about 103 countries of the world. Every year more than one billion persons in the world suffer from this disease (Hayton & Su, 2004). The parasite is relatively protected from attack by the body's immune system. Four species of the parasite are involved in spread of malaria viz., *Plasmodium falciparum, P. malariae, P. vivax* and *P. ovale*. The most common and most dangerous species is *P. falciparum* (Gallup & Sachs, 2001). *P. falciparum* is the most common cause of infection and is responsible for about 80% of all malarial cases and is also responsible for about 90% of the deaths from malaria (Mendis *et al*., 2001). The pathogenesis of malaria is complex and most likely entails immunologic and nonimmunologic mechanisms (Miller *et al*., 2002).

Interleukin-2 (IL-2), described as T-cell growth factor (Morgan *et al*., 1976), is a polypeptide of an apparent relative molecular weight of 15,500. It is produced and secreted by T cells, activated natural killer cells, or large granular lymphocytes during the first hours following stimulation by a mitogen or an antigen. IL-2 is considered to play a pivotal role in the regulation of the immune response. Activated T cells (2) as well as activated B cells (Loughnan *et al*., 1987) express specific IL-2 receptors (IL-2Rs) on their surfaces. Both the cellular and humoral immune responses against malarial parasites are mainly regulated by the T-cell (Cohen, 1979). IL-2 enhances monocyte cytotoxicity, increases phagocytosis and proliferation of macrophages and stimulates natural killer cell proliferation and cytolytic activity (Anasetti *et al*., 1994).

The magnitude of a cellular immune response is dependent in part upon the amount of IL-2 secreted by T cells (Waldmann, 1991). Cellular responses to IL-2 depend upon expression of specific cell surface receptors (Goldsmith & Greene, 1994). Considerable attention recently has focused on polymorphisms and their potential subtly to alter protein function in ways that might prove biologically or clinically important. But increasing numbers of polymorphisms are also being identified in the regulatory regions of genes (Mitchison, 2000). In the former, non-coding variation predominates, as distinct from extrovert genes, in which the reverse applies. Balanced polymorphism is potentially valuable for the insight it provides into therapeutic approaches. Though malarial parasite multiplies in red blood cells but the severity of symptoms it develops are not as severe as expected especially in case of children. It could be attributed to host genetic, immune, social and geographic factors. Previous studies have shown the relationship between Cytokines and malaria with special emphasis to estimation of serum/plasma levels of different cytokines like IL-4 (Banchereau *et al*., 1994), IL-10 , IL-12, TNF-α and IFN-γ (Kern *et al*., 1989). In some cases though functional polymorphism studies have also been conducted but the role of immune response to disease still remains unknown (Atamas *et al*., 1996).

P. falciparum and *P. vivax* are the major health problems in Pakistan. At least 39 districts, mainly from the

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two southern provinces of Balochistan and Sindh have been classified as high risk areas. Studies have been conducted in different areas of Pakistan on prevalence and characterization of human malarial parasites (Nizamani *et al*., 1995; Yasinzai & Kakarsulemankhel, 2004; Khatoon *et al*., 2009) but studies host response to parasites are very scarce.

Research objective of this study was to find out polymorphism in human Interleukin-2 gene among malarial patients in perspective of "is there any difference in *P. falciparum* and *P. vivax* and mixed infections?

MATERIALS AND METHODS

Blood samples were collected from 114 patients suffering from malaria from Malaria Control Program operating in women and children District Head Quarter, Hospital at Bannu, during summer 2007. 114 samples found out to be positive for presence of malarial parasite were subjected to PCR identification and 22 of these having mixed infection as published earlier (Khatoon *et al*., 2009) were selected for this study. This study was approved by ethical and review committee of Quaid-i-Azam University, Islamabad. After taking written and verbal consent from the patients, blood samples were collected in vials containing EDTA (1 mg/mL) as an anticoagulant and stored at -20°C until DNA extraction.

QIAamp® DNA mini kit (Fermentas, USA CAT#51306) has been used for extraction of human DNA using whole blood. Primers were designed manually for first 3 exons of human Interleukin 2 gene sequence deduced from Ensemble Gene Location NCBI36:4:123591475:123597930:-1. Sequence of primers is as follows:

Exon I	IL-2f	5′- TACTCTTGCTCTTGTCCAC -3′
	IL-2r	5′- CTTTACCTCAGATGAGCTGC-3′
Exon II	IL-2f	5′-AGTCATAGGTACTTGAGCCC-3′
	IL-2r	5′-CTCAGTAGCTTATACTCCCC-3′
Exon III	IL-2f	5′-AGGCAACAGGCCTATAAGAC-3′
	IL-2r	5′-CTGATCAGCCCTTGAAAGGA-3′.

PCR reaction was carried out using 2 µL of 10x PCR buffer, 2.5 µL of 25 mM MgCl2, 5 µL of 2 mM dNTP mix, 2 µL of 5 mM each forward and reverse primer, 5 U/µL of Taq DNA polymerase, DNA template 1.5 µL and reaction volume was made up to 20 µL by adding PCR water. PCR conditions were 94ºC for 5min, 40 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 60 sec and finally elongation at 72ºC for 5 min. 10 µL of each PCR product was run on 1% agarose gel containing 2 µL of 10 mg/µL of Ethidium Bromide. The final PCR products were run on 1% agarose gel in 1XTBE along with 50 and 100 bp DNA ladders on each side of the gel to make a comparison. GIBCOBRL Model H5 of LIFE TECHNOLOGIES was used to run gel at 500 mA current, 70V for 90 min. BIORAD Gel documentation system was used for the photography of gel.

RESULTS

All samples were studied for the presence of malarial parasite and its prevailing species. For this purpose, microscopic and PCR based analysis was carried out. In this study, no polymorphism was found for any of the three exons of IL-2 gene in the malaria patients. As given in Fig. 1, it was found that all bands of the samples corresponded to the 800 bp band for the exon 1 of the IL-2 gene. It depicted that this region of exon 1 showed no polymorphism. Similarly in case of exon 2 of IL-2 gene, all samples resulted in amplification of fragment of 500 bp, which is indicative of lack of polymorphism (Fig. 2). Fig. 3 indicates all bands of the samples correspond to the 700 bp for exon 3 of IL-2 gene hence showing conservation of IL-2 gene at this locus.

DISCUSSION

Three exons of human IL-2 gene were used to study polymorphism in malarial disease. In this study, *P. vivax* and *P. falciparum* samples were used since these are the principle species, which are responsible for malaria in Pakistan (Gallup & Sachs, 2001). In our research work we selected Interleukin-2, because it plays an important part in immune response to infectious diseases, in particular, where T-helper cell defense is required. There are indications that polymorphisms in cytokine and cytokine receptor genes could influence genetic disease resistance and susceptibility (Atac *et al.*, 1995). We found that there was no polymorphism in three exons of IL-2 gene. This observation is opposite to our hypothesis. This gene is highly conserved with in the population of the Bannu. It may be related with their socio-economic trends. In Bannu, people strictly observe their traditions and intermarriages to close relatives might keep the gene conservancy and no polymorphism. Malarial patients are equally susceptible to the malarial parasite. Polymorphism may provide an insight in understanding the diseases and their etiological responses to the altered response of a gene (s) and its expression. A mutation in the α-chain of the interleukin-3 receptor is reported to the susceptibility to *Listeria* infections in mice (Nadeau *et al*., 1995). The magnitude of a cellular immune response is dependent in part upon the amount of IL-2 secreted by T cells (Waldmann, 1991). Cellular responses to IL-2 depend upon expression of specific cell surface receptors. Because of therapeutic potential, efforts have been made to inhibit IL-2 function. These efforts include creation of genetically engineered mutant IL-2 molecules 9 (Heaton *et al*., 1993) and use of monoclonal antibodies to block IL-2 binding to α chain of high affinity receptor (Waldmann *et al*., 1992). Increased IL-2 activity is thought to contribute to pathology in certain infectious diseases (Yamamura *et al*., 1992), hence it can be concluded that expression of IL-2 might increase during malarial infection but at genetic level no polymorphism has been detected.

Fig. 1: Agarose gel electrophoresis (1%) for exon1 of IL-2. (M1=50bp, M2= 100bp)

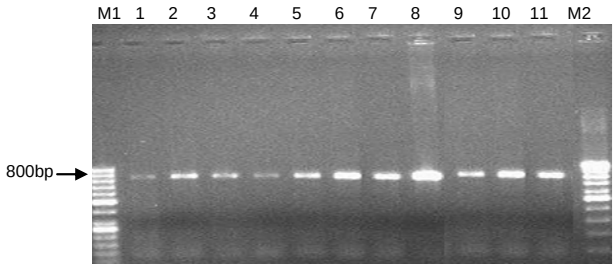


Fig. 2: Agarose gel electrophoresis (1%) for exon2 of IL-2 (M1= 100bp)

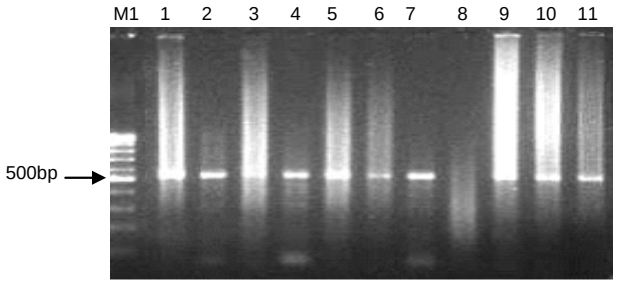
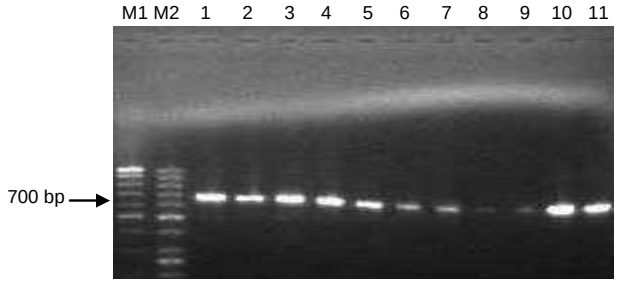


Fig. 3: Agarose gel electrophoresis (1%) for exon 3 of IL-2 gene (M1=50bp, M2=100bp)



CONCLUSION

This study shows lack of polymorphism at IL-2 gene locus in malarial patients, which is an indicative of the fact that gene sequence does not play role in susceptibility to *Plasmodium* infection. However, studies need to be done on expression of this gene if it is enhanced or reduced during the course of infection.

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REFERENCES

Anasetti, C., J.A. Hansen, T.A. Waldmann, F.R. Appelbaum, J. Davis, H.J. Deeg, K. Doney, P.J. Martin, R. Nash, R. Storb, K. Sullivan, R. Witherspoon, M.H. Binger, R. Chizonite, J. Hakimi, D. Mould, H. Satoh and S. Light, 1994. Treatment of acute graft-versus-host disease with humanized anti-Tc: an antibody that binds to the interleukin-2 receptor. *Blood.*, 84: 1320–1327

Atac, E.L., B. Schook, M.S. Rutherford and M.B. Wheeler, 1995. *In*: Myers, M.J. and M.P. Murtaugh (eds.), *Cytokines in Animal Health and Disease,* pp: 421–447. Marcel Dekker Inc. New York

Atamas S.P., J. Choi, V.V. Yurovsky and B. White, 1996. Interleukin 4 in Systemic Sclerosis: Not just an Increase. *J. Immunol*., 156: 435–441

Banchereau, J., F. Briere, J. Galizzi, P. Miossec and F. Rousset, 1994. Human interleukin 4. *J. Lipid. Mediat. Cell. Signal.*, 9: 43–53

Cohen, S., 1979. Immunity to malaria. *Proc. R. Soc. London B. Biol. Sci.*, 203: 333–345

Gallup, J. and J.D. Sachs, 2001. The economic burden of malaria. *American J. Trop. Med. Hyg*., 64: 85–96

Goldsmith, M.A. and W.C. Greene, 1994. *In*: *The Cytokine Handbook*, pp: 14–18. Academic Press Ltd London

Hayton, K. and X.Z. Su, 2004. Genetic and biochemical aspects of drug resistance in malarial parasites. *Current. Drug. Target. Infe. Dis.,* 4: 319–324

Heaton, K.M., G. Ju, D.K. Morris, K. Delisio, P. Bailon and E.A. Grimm, 1993. Characterization of Lymphokine-Activated Killing by Human Peripheral Blood Mononuclear Cells Stimulated with Interleukin 2 (IL-2) Analogs Specific for the Intermediate Affinity IL-2 Receptor. *Cell. Immunol.,* 147: 167–179

Kern, P., C. Hemmer, J. Van Damme, H. Gruss and M. Dietrich, 1989. Elevated tumor necrosis factor alpha and interleukin-6 serum levels as markers for complicated *Plasmodium falciparum* malaria. *American J. Med*., 87: 139–143

Khatoon, L., F.N. Baliraine, M. Bonizzoni, S.A. Malik and G. Yan, 2009. Short Report: Prevalence of Antimalarial Drug Resistance Mutations in *Plasmodium vivax* and *P. falciparum* from a Malaria-Endemic Area of Pakistan. *American J. Trop. Med. Hyg.,* 81: 525–528

Loughnan, M.S., K. Takatsu, N. Harada and G. Nossai, 1987. T-cell replacing factor (interleukin 5) induces expression of interleukin 2 receptors on murine splenic B-cells. *Proc. Natl. Acad. Sci. USA.*, 84: 5399–5403

Mendis, K., B. Sina, P. Marchesini and R. Carter, 2001. The neglected burden of *Plasmodium vivax* malaria. *American J. Trop. Med. Hyg.,* 64: 97–106

Miller, L.H., D.I. Baruch, K. Marsh and O.K. Doumbo, 2002. The pathogenic basis of malaria. *Nature*, 41: 673–679

Mitchison, N.A., 2000. Polymorphism in regulatory gene sequences. *Genome Biol*., 7: 110–117

Morgan, D.A., F.W. Ruscetti and R.C. Gallo, 1976. Selective *in vitro* growth of T lymphocytes from normal human bone marrow. *Science*, 193: 1007–1008

Nadeau, J.H., L.D. Arbuckle and E. Skamene, 1995. Genetic dissection of inflammatory responses. *Inflamm. J*., 45: 27–48

Nizamani, M.A., M. Hassan and A. Memon, 1995. Cerebral malaria in children: a study of children a study of 50 cases. *Pakistan Paed. J*., 19: 1–7

Waldmann, T.A., I.H. Pastan, O.A. Gansow and R.P. Junghans, 1992. The multichain inter-leukin-2 receptor a target for immunotherapy. *Annl. Int. Med*., 116: 148–160

Waldmann, T.A., 1991. The interleukin-2 receptor. *J. Biol. Chem*., 266: 2681–2684

Yamamura, M., X.H. Wang, J.D. Ohmen, K. Uyemura, T.H. Rea, B.R. Bloom and R.L. Modlin, 1992. Cytokine patterns of immunologically mediated tissue damage. *J. Immunol*., 149: 1470–1475

Yasinzai, M.I. and J.K. Kakarsulemankhel, 2004. A study of prevalence of malaria infection in urban areas of district Quetta, Pakistan. *Pakistan J. Zool.,* 36: 75–79

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