ejpmr, 2017,4(8), 143-147

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

IMMUNOLOGICAL CHARACTERISTICS OF HYPERREACTIVE MALARIAL SPLENOMEGALY SYNDROME

[1]Amar Lal*, [2]Muhammad Junaid Mahboob, [3]Sameeta, [4]Kiran and [5]Sagar

[1]General Practitioner, Golden Health Medical Center, Abu Dhabi.
[2]MBBS, Liaquat University of Medical and Health Sciences.
[3]MBBS, MD, Albert Einstein College of Medicine.
[4]MBBS, MD, Heart and Vascular Institue Detroit.
[5]MBBS, Lecturer of Department of Anatomy, Bibi Aseefa Dental College.

***Corresponding Author: Amar Lal, MBBS**

General Practitioner, Golden Health Medical Center, Abu Dhabi.

Article Received on 14/06/2017 **Article Revised on 03/07/2017** **Article Accepted on 24/07/2017**

ABSTRACT

Objective: The present study investigated the immunological characteristics of IgM, IFNγ and IL10 cytokines in the hyperreactive malarial splenomegaly syndrome (HMSS) at a tertiary care hospital of Sindh. **Study design:** Case control study. **Study setting & Duration:** Department of Medicine- LUMHS Jamshoro, from February 2016 to December 2016. **Subjects & Methods:** A sample of 60 cases of HMSS and 60 normal subjects were selected as controls. The HMSS cases were diagnosed as by Bates et al criteria. Size of spleen and liver was measured by sonography. Serum IgM, IFNγ and IL10 cytokines were measured. Data saved in a pre structured proforma. Confidentiality was maintained. Software "*Statistix 9.0*" (USA) was used for statistical analysis at 95% confidence interval ($P \leq 0.05$). **Results:** Immunological markers the IgM, Interferon-γ (IFNγ) and Interleukin-10 (IL-10) were found elevated in the HMSS cases (P=0.0001). Spleen size was significantly positively associated with the IgM (r= 0.707, P=0.0001), IFNγ (r= 0.70, P=0.0001) and IL-10 (r= 0.826, P=0.0001) and liver span (r= 0.821, P=0.0001). **Conclusion:** The present study noted elevated IgM, IFNγ and IL-10 cytokines levels in the hyperreactive malarial splenomegaly syndrome.

KEYWORDS: IgM, IFNγ, IL-10, Hyperreactive malarial splenomegaly syndrome.

INTRODUCTION

Hyper-reactive malarial splenomegaly syndrome (HMSS) is one of the most common causes of massive splenomegaly in malaria endemic tropical countries like Pakistan.[1,2] Exact cause is not known but abnormal immunological reactions caused by a chronic antigenic stimulation from malaria parasite have been suggested.[3,4] Patients suffering from HMSS show massive enlargement of the spleen in the tropics.[5,6] The condition shows high mortality rate. The natural history of the HMSS pathogenesis is not well documented. A 50% mortality rate at 5 years has been suggested in the countries like the New Guinea and Uganda.[7] Very high mortality rate of 85% has been observed in the hospitalized cases presenting with massive splenomegaly. Whether the HMSS is responsible for the mortality, it is not well established.[6,7] Usual symptoms of HMSS the patients complaint of include; a lump in left side of abdomen, pain and heaviness. Episodic pain is exacerbated by physical activity. Pain may be of severe intensity, becoming persistent and debilitating in chronic cases. Signs of hypersplenism are often present. Anemia results in the shortness of breath. Hemolysis is associated with acute febrile illness. Pregnancy may aggravate the hemolysis process.[8,9] Physical examination shows massive splenomegaly with palpable notch. Spleen is firm to touch, non-tender with regular contours. Hepatomegaly is concurrent to the splenomegaly and parallels the size of the spleen. Cases of HMSS are found only in the malaria endemic countries.[10,11] Clinical diagnosis of HMSS in its early phases is a clinical dilemma, particularly in the developing countries. Currently, the prevalence and incidence of HMSS is not known in the Pakistan.[12] Unusual immune markers may contribute to the understanding of pathogenesis of HMSS[2,10] as this will benefit the community. Keeping this scenario of endemicity of malaria and HMSS in Pakistan, the present study determined the immunological markers of HMSS. The present research studied the immunoglobulin M (IgM), interferon-γ (IFNγ) and interleukin-10 (IL-10) cytokines in addition to the spleen and liver size in diagnosed cases of hyperreactive malarial splenomegaly syndrome at our tertiary care hospital.

SUBJECTS AND METHODS

The present case control study was carried out in the Department of Medicine, Liaquat University of Medical

and Health Sciences (LUMHS), Jamshoro. HMSS cases and controls were selected from February 2016 to December 2016. The LUMHS hospital is a referral tertiary care center which caters patients from whole Sindh. Patient's clinical history and physical examination was performed by a medical officer followed by a Consultant Physician and final diagnosis of HMSS was made as suggested by Bates et al criteria.[13] A sample of 60 cases of HMSS and 60 normal subjects were selected as controls. The controls were age and sex matched. Diagnosed cases of hyperreactive malarial splenomegaly syndrome of ≥12 years of both genders were included in the study. Patients suffering from chronic viral hepatitis, liver cirrhosis, portal hypertension, chronic kidney disease (CKD) and miliary tuberculosis were excluded. HMSS cases with severe diseases were also excluded. Willing patients and their heirs were informed for the purpose of study. The HMSS cases were treated with 300 mg chloroquine once a week. Patients were informed that the reduction of spleen size will take time. Patients and attendants were taken into confidence that the enlarged spleen occurred because of chronic malaria, and regular follow ups are essential for its therapy once a month for 3 months. Volunteer's cases and control were asked for the blood sampling. Sonography (Model No; SSA- 590 A) detected the size of spleen and liver span. Venepuncture was performed after area was sterilized with alcohol swab.10 ml blood was taken into a disposable syringe (BD, USA). 5ml was taken into EDTA vacutainers for thick and thin blood films for the parasitological examination. 5ml into gel tubes, centrifuged at 4000 rpm for 15 minutes to separate the serum. The sera were stored at -20^{0}C temperature and used for biochemical assays. Haemoglobin, hematocrit (Hct) and white blood cell counts were measured on Sysmex hematoanalyzer. Peripheral blood smear was examined for exclusion of leukemia, myeloproliferative disorders. EDTA vacutainers blood was processed, thick and thin films were prepared. They were stained Giemsa's stain for the microscopic examination of parasites. Negative film was defined as no malaria parasite detected at the 300 oil fields. Serum IgM levels were measured by MININEPHTM IgM (Human) Kit Binding Site, UK. While the IFNγ and IL-10 cytokines were measured by double sandwich ELISA assay kit (R & D system Elisa kits - Germany). Permission was taken from ethical committee of the institute. Research protocol was handled as per "Declaration of Helsinki". Patients or legal heirs were asked to sign the consent form. All patients data was entered and saved in a pre structured proforma. Confidentiality was maintained. Software "*Statistix 9.0*" (USA) was used for statistical analysis. Independent samples t-test, Chi-square test and Pearson`s correlation were used for the analysis of continuous, categorical variables and correlation respectively. Microsoft excel was used for the scatter graphs. Level of significance was defined at 95% confidence interval ($P \leq 0.05$).

RESULTS

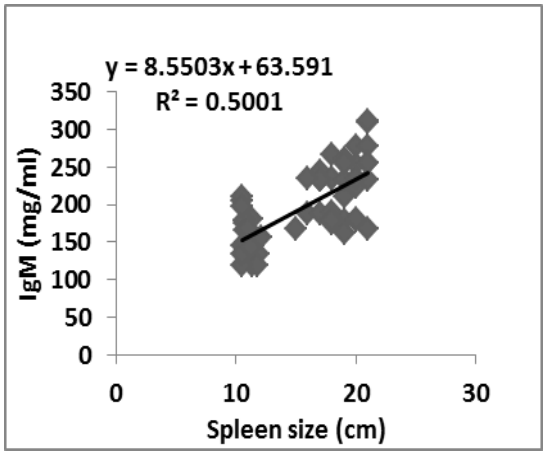
The present case control study was conducted to analyze the immunological markers in hyperreactive malarial splenomegaly syndrome. Mean ± SD age in controls and cases was noted as 42.81± 4.35 and 42.25±7.37 years respectively (P=0.071). Age, body weight, systolic BP and diastolic BP showed non-significant differences between 2 groups. While the hemoglobin, hematocrit, and White blood cells (P=0.0001), (P=0.0371) and (P=0.010) are shown in table 1. Of 120 control and cases, 87 (72.5%) were male and 33 (22.2%) were female (P=0.0001). Male to female ratio was 2.6:1. Immunological markers the IgM, IFNγ and IL-10 showed highly significant differences between control and cases (P=0.0001). Spleen size and liver span showed significant differences (P=0.0001) (table 1). Pearson`s correlation showed spleen size was significantly positively associated with the Ig M (r= 0.707, P=0.0001), IFNγ (r= 0.70, P=0.0001) and IL-10 (r= 0.826, P=0.0001) and liver span (r= 0.821, P=0.0001) (table 2, Graphs 1- 4).

Table 1: Characteristics and laboratory findings of study subjects (n=120)

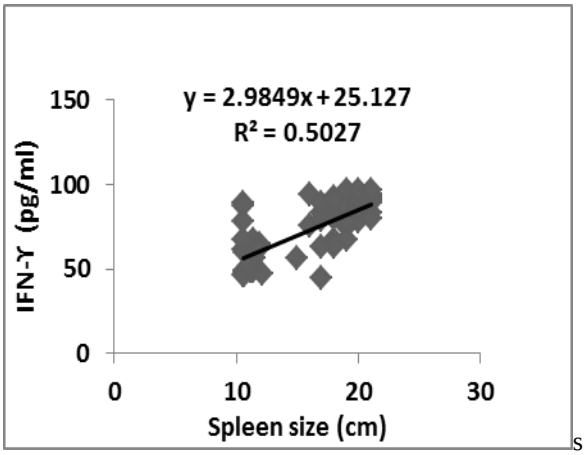
	Control		Cases		P- value
	Mean	SD	Mean	SD	
Age (years)	42.81	4.35	42.25	7.37	0.071
Body weight (kg)	51.29	9.13	52.14	4.67	0.087
Systolic BP (mmHg)	123.38	8.18	120.22	4.75	0.067
Diastolic BP (mmHg)	75.57	6.17	70.95	5.84	0.068
Hemoglobin (g/dl)	13.87	1.61	9.76	1.97	0.0001
Hematocrit (%)	45.45	6.21	26.09	6.20	0.0001
White blood cells (µL)	7988.7	1084.8	4076.9	680.63	0.0001
IgM (mg/mL)	157.14	27.65	224.57	40.48	0.0001
Interferon-ϒ (pg/ml)	58.12	13.30	81.14	12.65	0.0001
Interleukin-10 (pg/ml)	1.69	0.37	4.06	1.01	0.0001
Spleen size (cm)	10.97	0.52	18.81	1.63	0.0001
Liver span (cm)	11.45	0.75	14.69	1.33	0.0001

Table: 2. Pearson`s correlation of Spleen size (n=120)

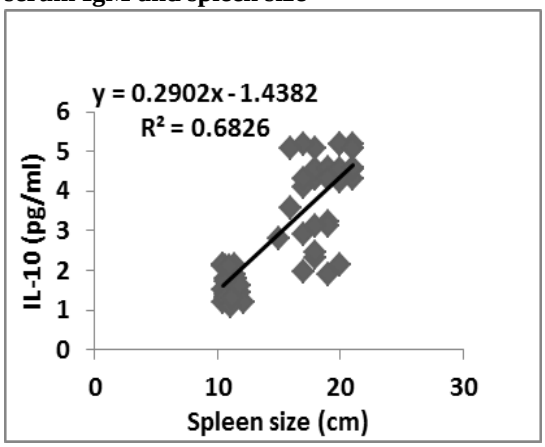
	Correlation co-efficient	P-value
IgM (mg/mL)	0.707	0.0001
Interferon-ϒ (pg/ml)	0.709	0.0001
Interleukin -10 (pg/ml)	0.826	0.0001
Liver span (cm)	0.821	0.0001



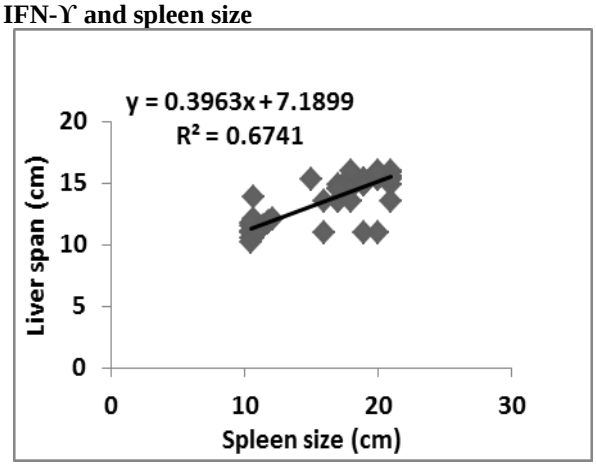
Graph 1. Scatter plot showing correlation of serum IgM and spleen size



Graph 2. Scatter plot showing correlation of serum IFN-ϒ and spleen size



Graph 3. Scatter plot showing correlation of serum IL-10 and spleen size



Graph 4. Scatter plot showing correlation of serum Liver span and spleen size

DISCUSSION

WHO estimates show 300–500 million cases of malaria occur annually, and counts for a mortality toll of 1.5- 2.7 million people. In Pakistan, estimates show 1.5 million cases per year.[1,2] In chronic cases of malaria, the spleen becomes enlarged massively called the hyperreactive malarial splenomegaly syndrome (HMSS). HMSS is endemic in the malaria endemic countries, particular in the Southeast Asian and African countries.[3,4] The present case control study was conducted to analyze the immunological markers in hyperreactive malarial splenomegaly syndrome. Mean ± SD age in controls and cases was noted as 42.81± 4.35 and 42.25± 7.37 years respectively (P=0.071). Hemoglobin, hematocrit, and White blood cells (P=0.0001), (P=0.0371) and (P=0.010). Of 120 control and cases, 87 (72.5%) were male and 33 (22.2%) were female (P=0.0001). Male to female ratio was 2.6:1. The findings are in keeping with previous studies.[1,2] In present study, the immunological markers the IgM, IFNγ and IL-10 were elevated significantly in the HMSS cases (P=0.0001), the findings are supported by previous studies.[8-10] Pathophysiology of HMSS is not well known. Earlier hypothesis of Fakunle and Ziegler that the HMSS results from IgM overproduction by the B-cell stimulation induced by the malaria antigen has been suggested.[8,9] Immune reactions show abnormalities of cytotoxic CD8+ T- cells and CD8+ T- suppressor cells. These stimulate the B lymphocytes; differentiate them into plasma cells producing large quantities of IgM. Hyperplasia of monocyte-macrophage system is responsible for the progressive enlargement of spleen. Genetic factors may play role in these immune reactions due to occurrence of HMSS in tribal and family clusters, resulting in hyper Ig M production.[10,11] Spleen size and liver span showed significant differences (P=0.0001). The finding corroborates with previous studies[14-16] which were reported form malaria endemic countries with stable

malaria transmission. Pearson`s correlation showed spleen size was significantly positively associated with the Ig M (r= 0.707, P=0.0001), IFNγ (r= 0.70, P=0.0001) and IL-10 (r= 0.826, P=0.0001) and liver span (r= 0.821, P=0.0001).These findings are in agreement with previous studies.[3,17-19] Production of IgM, IFNγ and IL-10 in response to chronic persistent antigen stimulation is important against the malaria as it is naturally produced. IgM is important in primary immune response during active malaria parasite replication. While the IFN-γ plays pivotal role against infectious disease for a lasting response.[20] Several studies[3,21] have suggested the role of IFN-γ in the immune response regulation. It plays central role for the activation of macrophages for intracellular and extracellular parasite killing. The IFN-γ is a pro inflammatory cytokine which is counter acted by anti inflammatory cytokine the IL-10.[21] As observed in the present study; both cytokines were raised in the HMSS. The IL-10 is an immunomodulatory cytokine of pleiotropic origin. It regulates both type 1 and 2 helper T cells (Th_1 & Th_2).[22,23] Raised IL-10 levels are in inflammatory reactions is suggested to exert beneficial anti- inflammatory effects. IL-10 reduces the immune reactions, but in large quantities may slow down the intracellular parasite destruction.[22] In present study, the plasma levels of IFNγ and IL-10 cytokines were significantly elevated in HMSS patients ($P<0.05$). The present study is the first research on the raised serum levels of IgM, IFNγ and IL-10 from a malaria endemic area. The raised plasma levels of pro-inflammatory cytokine- the IFNγ and anti-inflammatory cytokine- the IL-10 needs further research as this may be exploited to halt the pathogenesis of HMSS. This will help in reducing the hyperreactive malarial splenomegaly syndrome related morbidity and mortality. The present study has certain limitations like a small sample size, particular geographical area, specific ethnicity, genetic factors, etc, however, prospective study designs with inclusion and exclusion criteria of cases and control increases the strength of the present study.

CONCLUSION

The present study shows a significant proportion of patients with massive splenomegaly are having hyperreactive malarial splenomegaly syndrome (HMSS). Immunological characteristics of elevated plasma levels of IgM, IFNγ and IL-10 cytokines were found in these patients. Raised plasma levels of pro-inflammatory cytokine- the IFNγ and anti-inflammatory cytokine- the IL10 needs further research as this may be exploited to halt the pathogenesis of hyperreactive malarial splenomegaly syndrome (HMSS).

REFERENCES

1. Waqar T, Khushdil A, Haque K. Efficacy of Chloroquine as a first line agent in the treatment of uncomplicated malaria due to Plasmodium vivax in children and treatment practices in Pakistan: A Pilot study. J Pak Med Assoc, 2016; 66: 30-33.
2. Humaira M, Ahmed A, Memon A, Siddique SA, Hayee PA, Ghani MH, Ghouri R. Etiological Pattern of Splenomegaly in Adults at Tertiary Care Hospital Jamshoro Sindh. J Liaquat Uni Med Health Sci., 2016; 15(01): 40-5.
3. Alkadarou T, Musa A, Alkadarou A, Mahfouz MS, Troy-Blomberg M, Elhassan AM, Elhassan IM. Immunological Characteristics of Hyperreactive Malarial Splenomegaly Syndrome in Sudanese Patients. Journal of Tropical Medicine, 2013. Article ID 961051:1-5.
4. Dabadghao VS, Diwan AG, Raskar AM. A Clinicohaematological Profile of Splenomegaly. Raskar Bombay Hosp J., 2012; 54(1): 10-18.
5. Bisoffi Z, Leoni S, Angheben A, Beltrame A, Eseme FE, Gobbi F, et al. Chronic malaria and hyper‑reactive malarial splenomegaly: a retrospective study on the largest series observed in a non‑endemic country. Malar J., 2016; 15(230): 1-8.
6. Leoni S, Buonfrate D, Angheben A, Gobbi F, Bisoffi Z. The hyper-reactive malarial splenomegaly: a systematic review of the literature. Malar J., 2015; 14: 185.
7. Bedu-Addo G, Bates I. Causes of massive tropical splenomegaly in Ghana. The Lancet, 2002; 360(9331): 449– 454.
8. Jaroensuk J, Stoesser N, Leimanis ML, Jittamala P, White NJ, Nosten FH, et al. Treatment of suspected hyper-reactive malarial splenomegaly (HMS) in pregnancy with mefloquine. Am J Trop Med Hyg, 2014; 90: 609–11.
9. Camara B, Kantambadouno JB, Martin-Blondel G, Berry A, Alvarez M, Benoit-Vical F, et al. Hyperreactive malarial splenomegaly: Three clinical cases and literature review. Med Mal Infect, 2009; 39: 29–35.
10. McGregor A, Doherty T, Lowe P, Chiodini P, Newsholme W. Hyperreactive Malarial Splenomegaly Syndrome—Can the Diagnostic Criteria Be Improved? Am J Trop Med Hyg, 2015; 93(3): 573–576.
11. Allam MM, Alkadarou TAMY Ahmed BG. Hyperreactive Malarial Splenomegaly (HMS) in malaria endemic area in Eastern Sudan. Acta Tropica, 2008; 105(2): 196–199.
12. Khattak AA, Venkatesan M, Khatoon L, Quattara A, Kenefic LJ, Nadeem MF, et al. Prevalence and patterns of antifolate and chloroquine drug resistance markers in Plasmodium vivax across Pakistan. Malaria J., 2013; 12: 310.
13. Bates I, Bedu-Addo G. Review of diagnostic criteria of hyper-reactive malarial splenomegaly. Lancet, 1997; 349(9059): 1178.
14. Tobena Rue M, Coll Usandizaga F, Moraga Llop FA, Toran Fuentes N, Garcia Pena MP. [Hyper-reactive malarial splenomegaly in a 9 year-old Equatorial Guinean child] (in Spanish). An Pediatr (Barc), 2011; 75: 80–1.
15. De Iaco G, Saleri N, Perandin F, Gulletta M, Ravizzola G, Manca N, et al. Hyper-reactive

malarial splenomegaly in a patient with human immunodeficiency virus. Am J Trop Med Hyg, 2008; 78: 239–40.
16. Jaroensuk J, Stoesser N, Leimanis ML, Jittamala P, White NJ, Nosten FH, et al. Treatment of suspected hyper-reactive malarial splenomegaly (HMS) in pregnancy with mefloquine. Am J Trop Med Hyg, 2014; 90: 609-11.
17. Eltayib EE. Hyper-Reactive Malarial Splenomegaly Syndrome (HMSS). Cureus, 2012; 4(11): e72. DOI 10.7759/cureus.72.
18. Verma S, Aggarwal A: Hyper-reactive malarial splenomegaly: rare cause of pyrexia of unknown origin. Indian J Pediatr, 2007; 74: 409-11.
19. Mitjà O, Hays R, Malken J, Ipai A, Kangapu S, Robson J: HMS-related hemolysis after acute attacks of Plasmodium vivax malaria. Am J Trop Med Hyg, 2011; 85: 616-8.
20. Chelimo K, Sumba PO, J. W. Kazura JW, Ofula AV, John CC. Interferon-gamma responses to Plasmodium falciparum liver-stage antigen-1 and merozoite-surface protein-1 increase with age in children in a malaria holoendemic area of western Kenya. Malaria J., 2003; 2: 1-37.
21. Li C, Sanni LA, Omer F, Riley E, Langhorne J. Pathology of Plasmodium chabaudi chabaudi infection and mortality in interleukin-10-deficient mice are ameliorated by anti-tumor necrosis factor alpha and exacerbated by anti-transforming growth factor β antibodies. Infec Immun, 2003; 71(9): 4850–4856.
22. de Waal Malefyt R, Yssel H, Roncarolo MG, Spits H, De Vries JE. Interleukin-10. Current Opin Immunol, 1992; 4(3): 314–320.
23. Villegas EN, Wille U, Craig L. Blockade of costimulation prevents infection-induced immunopathology in interleukin-10-deficient mice. Infec Immun, 2000; 68(5): 2837–2844.