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Neuron specific enolase as a novel clinical marker of Hyperreactive Malarial Splenomegaly syndrome

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Abstract

Background: Hyperreactive Malarial Splenomegaly Syndrome (HMSS) is characterized by massive splenomegaly in the tropical areas. HMSS is prevalent in malaria endemic areas like Pakistan.

Objective: Serum neuron specific enolase (NSE) in hyperreactive Malarial Splenomegaly Syndrome (HMSS) and its correlation with IgM, IFNγ and IL10 cytokines.

Designs: Case control study

Setting: Department of Medicine, Liaquat University of Medical and Health Sciences Jamshoro/Hyderabad, Sindh, Pakistan, from December 2014 to May 2016.

Materials and Methods: A sample of 37 diagnosed cases of HMSS and 21 normal subjects as controls was selected. Thick and thin films were prepared. Serum NSE, IgM, IFNγ and IL10 were detected by standard methods. Sysmex hematoanalyzer was used for blood analysis. Ethical approval was taken from the Advanced Review Board and Ethics Committee of LUMHS Jamshoro. Study was conducted according to the guideline of the "Declaration of Helsinki" for human studies. Consent form was mandatory for the participation of volunteer's subjects.

Results: HMSS patients revealed showed elevated serum NSE, IgM, IFN-ϒ and IL-10, spleen size and liver span ($P<0.05$). Serum IgM, IFN-ϒ and IL-10 and spleen size showed positive Pearson` correlation with serum NSE ($r = 0.621 – 0.772$, $P = 0.0001$). A multiple regression was run to predict NSE from IgM (mg/mL), IFN-ϒ, IL-10 and spleen size. All 4 variables added statistically significantly to the prediction of NSE ($F= 57.039$, $P= 0001$, $R^2 = 0.761$).

Conclusion: The present study reports the serum neurons specific enolase (NSE) was elevated in hyperreactive malarial splenomegaly syndrome. NSE may be used as alternative to IgM, IFNγ and IL10 for clinical diagnosis.

Keywords: Neuron specific enolase IgM Interferon-γ Interleukin-10

Introduction

According to World health organization an estimated 300 – 500 cases of malaria occur each year with a death toll of 1.5 to 2.7 million people. Malaria prevalence in Pakistan is about 1.5 million cases annually [1, 2]. Hyperreactive Malarial Splenomegaly Syndrome (HMSS) is characterized by massive splenomegaly in the tropical areas. HMSS is prevalent in malaria endemic areas in particular the Southeast Asian and African countries [3, 4]. Natural course of HMSS is not documented and carries a high toll of mortality. A 5-year-mortality rate of 50% has been observed in some countries such as the Uganda and New Guinea [5].

A suggested mechanism of HMS is exaggerated activation of the immune system. Cytotoxic CD8+ T- cells and CD8+ T- suppressor show abnormal physiology. They activate the B cells produce large quantities of IgM. Monocyte-macrophage system hyperplasia results in progressive enlargement of spleen [6]. Activated monocyte- macrophages release many cytokines, enzymes and other mediators. One of such enzyme is the neuron-specific enolase (NSE) [7]. NSE is an enzyme of glycolysis termed as the phosphopyruvate hydratase. Primarily the NSE was isolated from the neurons, but later studies proved it is of Pleiotropic origin [8, 9]. Elevated serum NSE has been reported in bacterial meningitis [10], lung diseases [11], pulmonary tuberculosis [12] and cardiac arrest [13].

Diagnosis of HMSS in early phases is a clinical dilemma in developing countries. There is no any well established serological marker for HMSS diagnosis in clinical practice.

Keeping in mind, the observation of previous study [7] that the macrophages release NSE, the present study was designed to determine the serum NSE as clinical marker of hyperreactive malarial splenomegaly syndrome. The present study determined the serum NSE and its predictive value as clinical marker of hyperreactive malarial splenomegaly syndrome at a tertiary care hospital of Sindh.

Subjects and Methods

The present case control study was conducted at the Department of Medicine, Liaquat University of Medical and Health Sciences Jamshoro/Hyderabad, Sindh, Pakistan, from December 2014 to May 2016.

Inclusion criteria

Diagnosed cases of hyperreactive malarial splenomegaly syndrome of 18 - 60 years of both genders were included.

Exclusion criteria

Concomitant systemic disease such as myeloproliferative disorders, Lymphadenopathy, autoimmune disorders, chronic viral hepatitis, liver cirrhosis, portal hypertension, chronic kidney disease and renal failure were excluded.

Clinical history and Physical examination

Inclusion and exclusion criteria were ensured by patient's clinical history. Clinical history findings were noted in a pre structured proforma. Physical examination was performed first by medical officer followed by a Consultant Physician.

Study Groups

A sample of 37 diagnosed cases of HMSS and 21 normal subjects as controls was selected. Hyperreactive malarial splenomegaly syndrome was diagnosed on the basis of standard criteria of Bates et al [14].

All cases with HMS were treated with chloroquine 300mg weekly, in accordance with local treatment protocol, and they were instructed to report to the study team at the hospital for follow-up once a month for 3 months.

Blood sampling

10 ml blood was taken by Venepuncture into a disposable syringe (BD, USA) and processed properly. Blood centrifugation was performed at 4000 rpm for 15

minutes. Sera were stored at temperature of -20^{0}C for the detection of biochemical parameters.

Parasitological Examination

Thick and thin films were prepared from all study subjects, stained with Giemsa's and examined under microscope. Films were considered negative after examination of 300 oil fields without detection of malaria parasites.

Measurement of IgM - IgM total was estimated by MININEPHTM IgM (Human) Kit Binding Site, UK.

Serum Neurons specific enzyme: Serum NSE was measured by "Quantikine Human" solid phase ELISA assay.

IFNγ and IL10 cytokines: IFNγ and IL10 cytokines were measured from sera of cases (HMSS) and controls. Double sandwich ELISA using commercially available kit (R & D system Elisa kits - Germany).

Blood parameters- Haemoglobin, hematocrii (Hct) and white blood cell counts were measured. Sysmex hematoanalyzer was used for blood analysis.

Anti malarial drug administration- All patients with HMS were treated with chloroquine 300mg weekly in accordance with the standard treatment protocol and were instructed to report to the study team at the health centers for follow up once a month for 3 consecutive months.

Ethical approval and consent form

Ethical approval was taken from the Advanced Review Board and Ethics Committee of LUMHS Jamshoro. Study was conducted according to the guideline of the "Declaration of Helsinki" for human studies. Consent form was mandatory for the participation of volunteer's subjects.

Statistical analysis

Data was analyzed on "Statistix 10.0" software (USA). Paired sample t-test and Chi-square test were used for the analysis of continuous (ENO-2, serum cholesterol, hsCRP) and categorical (gender) variables respectively. Pearson`s correlation and Multiple regression analysis was conducted for the ENO-2, hsCRP and serum cholesterol. Statistical significance was at taken 95% confidence interval ($P \leq 0.05$).

Results

The characteristics and laboratory findings of study subjects are shown in the table 1. Of 37 HMSS cases, plasmodia species were isolated in 23 (62.1%). Serum NSE, IgM, IFN-ϒ and IL-10 showed statistically significant differences ($P<0.05$). HMSS showed elevated serum NSE, IgM, IFN-ϒ and IL-10, spleen size and liver span ($P<0.05$) (graph 1-4). Serum IgM, IFN-ϒ and IL-10 and spleen size showed positive Pearson` correlation with serum NSE ($r = 0.621 – 0.772$, $P = 0.0001$) (Table 2, Graph- 5).

Multiple linear regression analysis was performed by Enter method. A multiple regression was run to predict NSE from IgM (mg/mL), IFN-ϒ (pg/ml) and IL-10 (pg/ml). These variables statistically significantly predicted the NSE, $F= 57.039$, $P= 0001$, $R^2 = 0.761$. Results are shown in table 3 for the IgM, IFN-ϒ and IL-10 and spleen size. All 4 variables added statistically significantly to the prediction of NSE ($P= 0.0001$).

Table 1. Characteristics and laboratory findings of study subjects (n=58)

	Study groups	Mean	SD	95% CI		P value
				Low	Upper	
Age (years)	**Controls**	40.81	4.35	7.58	19.25	0.051
	Cases	39.39	12.90			
Body weight (kg)	**Controls**	64.00	8.38	7.58	15.71	0.0001
	Cases	52.35	4.63			
Systolic BP (mmHg)	**Controls**	128.43	10.27	3.91	13.53	0.0001
	Cases	119.70	3.83			
Diastolic BP(mmHg)	**Controls**	78.24	6.91	3.61	10.85	0.0001
	Cases	71.00	5.79			
NSE (ng/dl)	**Controls**	10.54	1.50	-6.58	-3.78	0.0001
	Cases	15.72	3.73			
IgM (mg/mL)	**Controls**	170.48	25.59	-57.18	-25.20	0.0001
	Cases	211.68	34.58			
IFN-ϒ (pg/ml)	**Controls**	60.88	15.67	-27.96	-11.79	0.0001
	Cases	80.76	12.39			
IL-10 (pg/ml)	**Controls**	1.65	.40	-2.54	-1.85	0.0001
	Cases	3.84	.90			
Spleen size (cm)	**Controls**	11.79	.99	-7.25	-5.97	0.0001
	Cases	18.41	1.42			
Liver span (cm)	**Controls**	11.79	.99	-3.09	-1.80	0.0001
	Cases	14.24	1.44			
Hb (g/dl)	**Controls**	14.89	.69	4.08	5.60	0.0001
	Cases	10.04	2.11			
Hct (%)	**Controls**	48.11	1.56	17.03	20.83	0.0001
	Cases	29.18	5.35			
WBC (µL)	**Controls**	7744.14	2770.92	2080.72	4773.50	0.0001

NSE- Neuron specific enolase

IgM- Immunoglobulin M

IFN-ϒ – interferon-gamma

IL-10- Interleukin 10

Hb- Hemoglobin

Hct- Hematocrit

WBC- white blood cells

Table 2. Pearson`s correlation of Neuron specific enolase (n=58)

	Neuron Specific Enolase	
	Correlation co-efficient (r-value)	**P-value**
IgM (mg/mL)	0.772	0.0001
IFN-ϒ (pg/ml)	0.754	0.0001
IL-10 (pg/ml)	0.782	0.0001
Spleen size (cm)	0.621	0.0001

IgM- Immunoglobulin M

IFN-ϒ – interferon-gamma

IL-10- Interleukin 10

Table 3. Multiple Linear Regression Analysis

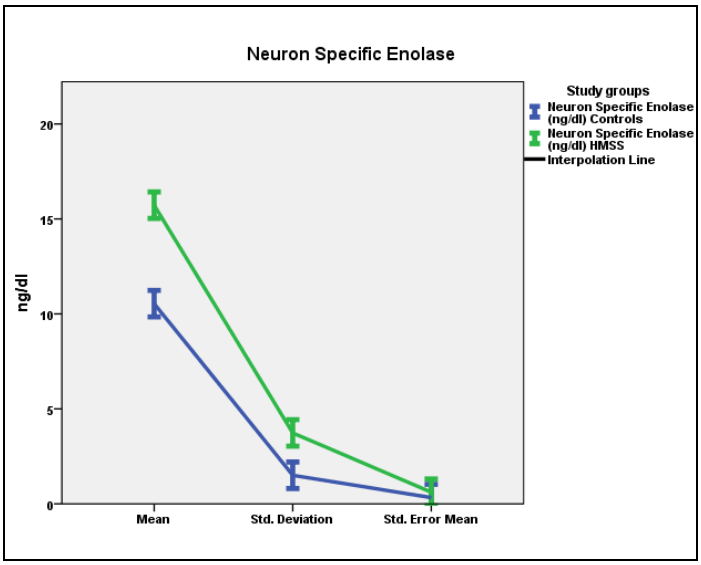
	B	SE B	β	t-value	P-value
(Constant)	-2.522	1.710	-	-1.475	0.146
IgM (mg/mL)	0.044	0.010	0.408	4.546	0.0001
IFN-ϒ (pg/ml)	0.068	0.025	0.282	2.674	0.010
IL-10 (pg/ml)	0.876	0.342	0.298	2.658	0.0001
Spleen size	0.15	0.132	0.013	0.112	0.911

a. Dependent Variable: Neuron Specific Enolase (ng/dl)

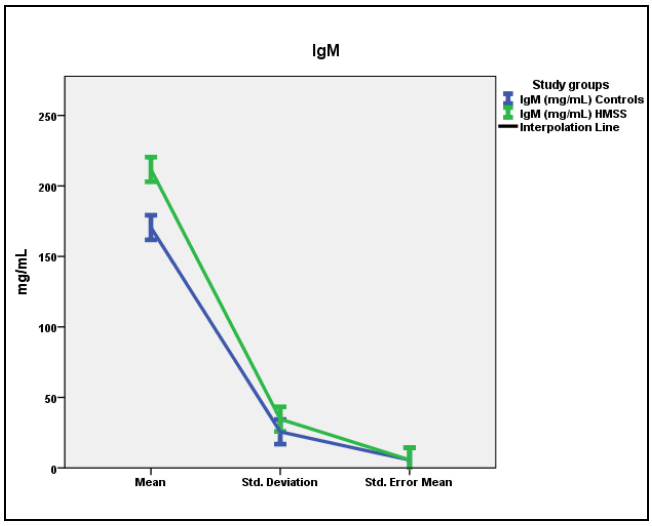
IgM- Immunoglobulin M

IFN-ϒ – interferon-gamma

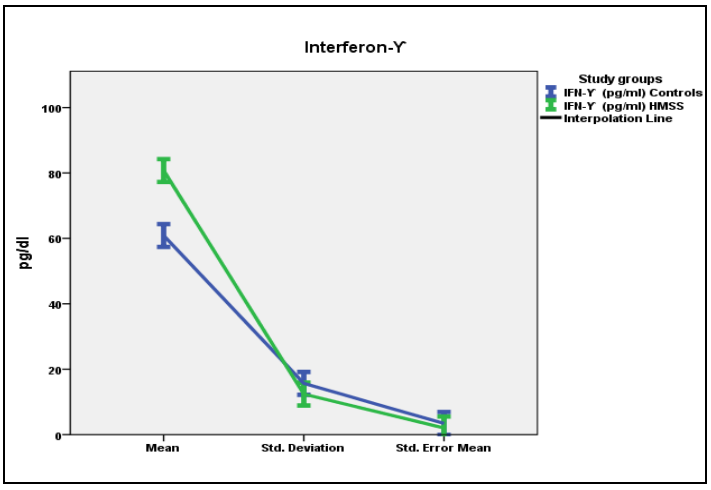
IL-10- Interleukin 10



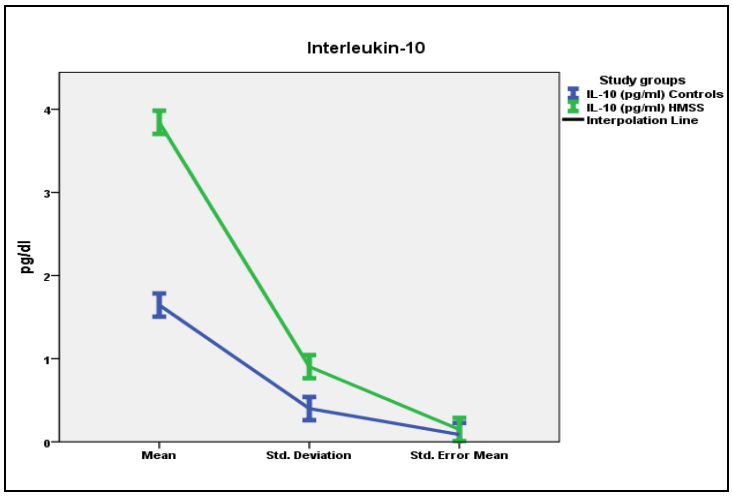
Graph 1. Serum Neuron specific enolase of controls and cases



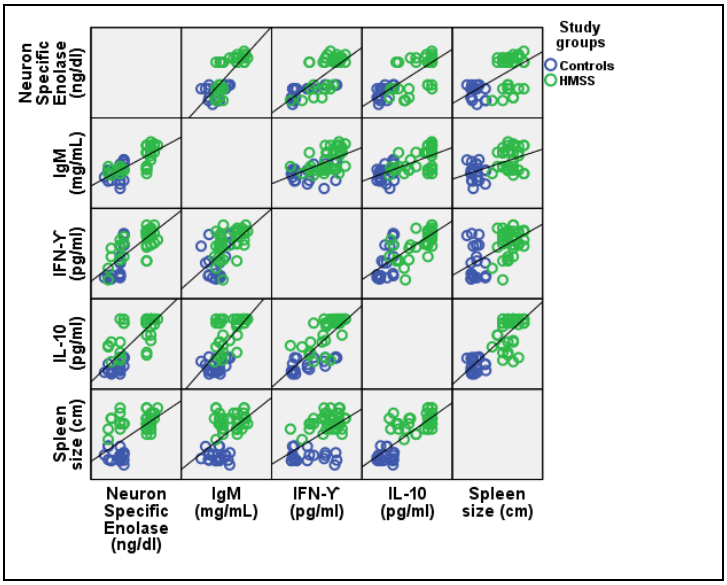
Graph 2. Serum Immunoglobulin M (IgM) of controls and cases



Graph 3. Serum interferon-ϒ (pg/ml) of controls and cases



Graph 4 Serum Interleukin-10 (pg/ml) of controls and cases



Graph 5. Scatter plot of NSE, IgM, IFN-Ɣ, IL-10 and spleen size

Discussion

Pakistan is reported as the hyper endemic country for the malaria. The malaria exists in the community throughout the year. Chronic inflammatory reaction against malaria parasite casues splenomegaly, increases antibody production IgM, and release of different cytokines such serum NSE, IFNγ and IL10. The present study is the first research on the hyperreactive malarial splenomegaly syndrome (HMSS) conducted at a tertiary care hospital which evaluated diagnostic and predictive value of serum NSE for the first time. Malaria is a significant cause of tropical splenomegaly [2, 15]. The production of IgM, IFNγ and IL10 cytokines in response to malaria antigens has been shown to be important in induction and maintenance of immunity to malaria in naturally exposed population. IFN-γ plays an important role in host defense against many infectious diseases [16]. Several studies [15, 16] have suggested that IFN-γ plays an important role in the regulation of immune responses during the course of infection by the activation of macrophages involved in both the intracellular and extracellular destruction of the parasite.

A number of studies have shown that IFN-γ is associated with the pathogenesis, as seen in malaria-infected mice, and that the pathogenic effect of IFN-γ is counterbalanced by the anti-inflammatory cytokine, IL10 [17]. The IL-10 is a pleiotropic immunomodulatory cytokine regulating not onlyTh1 but also Th2-type reactions in many instances [18, 19]. High levels of IL-10 observed during malaria episodes may be beneficial in reducing the inflammatory response, but it may also be detrimental by decreasing antiparasitic cellular immune responses [18]. We found that the plasma levels of NSE, IgM, IFN-γ and IL10 cytokines were significantly elevated in HMS patients. This is the first research showing the diagnostic utility of serum NSE and its predictive value from the IgM, IFN-γ and IL10 in HMSS patients, suggesting that serum NSE might have an important role in the protection and/or pathogenesis of HMSS which may be exploited for the earlier diagnosis of HMSS in malaria endemic countries.

A reliable biological marker of HMSS is major problem for clinicians in developing countries, which often result in treatment failures, massive splenomegaly, Splenic rupture and mortality. Serum NSE has been reported as a disease marker for small cell carcinoma lungs, neruoblastoma, retinoblastoma [8, 9] and tuberculous meningitis [20, 21]. At present sufficient evidence is present to use serum NSE as disease marker in malignant and non-malignant disorders where there is involvement of macrophages, such as the HMSS [8, 9, 20, 21]. However, role of serum NSE in HMSS prediction needs further exploration. Serum NSE was raised in HMSS 15.72 ±3.73 (pg/ml) compared to controls as 10.54 ±1.50 (pg/ml). The findings of serum NSE correlated with serum IgM, IFNγ and IL10 and spleen size. Serum NSE revealed positive correlation with IgM (r = 0.772, p = 0.0001), IFNγ (r = 0.754, p = 0.0001), IL10 (r = 0.782, p = 0.0001) and Spleen size (r = 0.621, p = 0.0001) (Scatter plot 5).

The evidence based findings of present study show the serum NSE may prove useful clinical laboratory marker for HMSS diagnosis and prediction. Further research is needed to set cut off values of NSE for the HMSS.

As the malaria is endemic in Pakistan [22, 23] the clinical diagnosis is highly suspicious and clinicians are always in search of some specific serological tests for diagnosis. If serum NSE proves validated it will be a great success in clinical diagnosis. The origin of serum NSE is certainly from macrophages which secrete it. The present study reports 2 important findings, first- the serum NSE levels were found elevated in HMSS similar IgM, IFNγ and IL10 levels. Second – serum NSE levels were positively correlated with IgM, IFNγ, IL10 and spleen size.

The finding serum NSE may be interpreted as of clinical importance for the diagnosis of HMSS as a single laboratory investigation, however this needs further validation with large sample size conducted at the national level. The main limitation of present study is the small sample size, but the finding of raised serum NSE and its predictive value for HMSS are worth findings to report.

Conclusion

The serum NSE was found raised in hyperreactive malarial splenomegaly syndrome. Serum NSE showed positive correlation with IgM, IFNγ, IL10 and spleen size. The present study reports the clinically diagnostic and predictive value of serum NSE; hence it may be used as novel clinical marker for the hyperreactive malarial splenomegaly syndrome.

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Conflict of Interest: None

Contribution:

Dr Suhail Ahmed Almani - concept of study design, review of available literature, final manuscript check and plagiarism checking

Dr Bilal Razaque Memon - proforma designing, data collection, compiling of data

Dr Omesh Kumar - Data collection, compiling of data

Dr Anum Atique- Data collection, compiling of data

Burhan Memon- Data collection, compiling of data

Rahul Roy - Proforma designing, data collection, compiling of data

Dr Haji Khan Khoharo - Concept of study design, review of available literature, Statistical analysis, final manuscript check and plagiarism checking

Reference

1. 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
2. Dabadghao VS, Diwan AG, Raskar AM. A Clinicohaematological Profile of Splenomegaly. Raskar Bombay Hosp J 2012; 54(1):10-18.
3. 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.
4. 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.
5. 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; 93:573–6.
6. 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.
7. Honda K, Ohga S, Takada H, et al. Neuron-specific enolase in hemophagocytic lymphohistiocytosis: a potential indicator for macrophage activation? Int J Hematol 2000; 72:55-60.
8. Kuralay F, Tokgoz Z, Comlekci A. Diagnostic usefulness of tumour marker levels in pleural effusions of malignant and benign origin. Clin Chim Acta 2000; 300:43-55.
9. Li CS, Cheng BC, Ge W, Gao JF. Clinical value of CYFRA21-1, NSE, CA15-3, CA19-9 and CA125 assay in the elderly patients with pleural effusions. Int J Clin Pract 2007; 61:444-448.
10. Inoue S, Takahashi H, Kaneko K. The fluctuations of neuron-specific enolase (NSE) levels of cerebrospinal fluid during bacterial meningitis: the relationship between the fluctuations of NSE levels and neurological complications or outcome. Acta Paediatr Jpn 1994; 36:485-488.
11. Collazos J, Esteban C, Fernandez A, Genolla J. Measurement of the serum tumor marker neuron-specific enolase in patients with benign pulmonary diseases. Am J Respir Crit Care Med 1994;150:143-145.
12. Nam SJ, Jeong JY, Jang TW, Jung MH, Chun BK, Cha JH, et al. Neuron-specific enolase as a novel biomarker reflecting tuberculosis activity and treatment response. Korean J Intern Med 2016; 31: 694-702. http://dx.doi.org/ 10.3904/ kjim. 2015.407.
13. Stammet P, Collignon O, Hassager C, et al. Neuron-specific enolase as a predictor of death or poor neurological outcome after out-of-hospital cardiac

arrest and targeted temperature management at 33°C and 36°C. J Am Coll Cardiol 2015; 65:2104-2114.

14. Bates I, Bedu-Addo G. Review of diagnostic criteria of hyper-reactive malarial splenomegaly. *Lancet* 1997; 349(9059):1178.
15. 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.
16. Wallace S, Bedu-Addo G, Rutherford TR, Bates I. Serological similarities between hyperreactive malarial splenomegaly and splenic lymphoma in West Africa. Transactions Royal Soc Trop Med Hyg 1998; 92:463–467.
17. Chelimo K, Sumba PO, 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 (37): 1-7.
18. Li C, Sanni A, 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. Infect Immun 2003; 71 (9): 4850–4856.
19. Villegas EN, Wille U, Craig L. Blockade of costimulation prevents infection-induced immunopathology in interleukin-10-deficient mice. Inf Immun 2000; 68 (5): 2837–2844.
20. Kang YJ, Jo JO, Ock MS, et al. Over-expression of thymosin beta4 in granulomatous lung tissue with active pulmonary tuberculosis. Tuberculosis (Edinb) 2014; 94:323-331.
21. Heitmann L, Abad Dar M, Schreiber T. The IL-13/IL-4Rα axis is involved in tuberculosis-associated pathology. J Pathol 2014; 234:338-350.
22. 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.
23. 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.