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DETECTION OF ASYMPTOMATIC CMV IGM POSITIVE BLOOD DONORS IN LOCAL POPULATION BY NEOPTERIN SCREENING

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ABSTRACT

Objective: To estimate serum Neopterin levels in blood donors of local population and to study its association with transfusion transmitted infections (TTIs) including CMV. **Method:** A cross sectional study was carried out in the department of Physiology Liaquat National Hospital and Medical College (LNH&MC) in collaboration with Basic Medical Sciences Institute (BMSI) and Jinnah Post Graduate Medical Centre (JPMC) Blood Bank. Data collection was for a period of six months in 2014. During this period a total of n=174 blood donors were selected through random sampling technique. The selected participants were screened for transfusion transmitted infections through the standard procedures as per WHO recommendations at the Jinnah Post Graduate Medical Centre(JPMC) blood bank. Serum Neopterin was measured using ELISA Kits. Data was analyzed on SPSS version 21. ANOVA and Chi square were applied as test of significance at a confidence level of 95%. **Results:** The Neopterin level in the sera of asymptomatic CMV positive donors was elevated to 17.03 ± 1.83 nmol/l. Neopterin content in the sera of disease negative blood donors was 6.23 ± 2.19 nmol/l as compared to disease positive blood donors who had elevated Neopterin level of 15.10 ± 4.93 nmol/l. (P value 0.001) **Conclusion:** Neopterin screening has the potential to detect transfusion transmitted infections which would not be revealed by routine screening including CMV infection.

Key Words: Blood donors, Transfusion transmitted infections (TTIs), Screening, Neopterin

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INTRODUCTION

Blood transfusion is an essential component of health care which save millions of lives every year. It is however a potential mode of transmission of infections to the recipients[1]. In developed countries, transfusion most commonly supports advanced medical and surgical procedures, including trauma, cardiovascular surgery, neurosurgery and transplantation where

as in the countries with limited diagnostic and treatment options, a much greater proportion of blood is used to treat women with obstetric emergencies and children suffering from severe anemia, often resulting from malaria and malnutrition [2]

The transfusion of blood-borne infections is one of the most important transfusion associated hazard in many parts of the world, particularly in the developing countries. Knowledge of the infectious agents especially those which are endemic in a particular region is essential for reducing the risk of transmission of these agents[3]. There is a long list of viruses, bacteria and parasites which can be transmitted through blood transfusion. Among them the important transfusion transmitted viruses are human immunodeficiency virus (HIV-I/II), hepatitis B virus (HBV), hepatitis C virus (HCV), Parvo virus B-19 and cytomegalovirus (CMV) etc. Recently severe acute respiratory syndrome virus (SARS) and Dengue virus infections have also proved to be transfusion transmissible. Besides these viral infections there are certain parasitic and bacterial infections such as Malaria and Syphilis which are also well known to be transfusion transmissible [4]

The burden of HBV and HCV disease is increasing globally and Pakistan is no exception. As far as the blood donors are concerned, the prevalence of blood transmitted infection is very high in commercial blood donors. Around 20% of them are positive for Hepatitis C and 10% for Hepatitis B infection. Amongst the family replacement/donors prevalence of 5% for hepatitis B infection and 2.5% for Hepatitis C infection was found where as in voluntary blood donors the incidence is of 2% for Hep B and 0.5% for Hep C infections [5-6]

Transfusion transmitted -CMV infection can lead to serious or even fatal clinical manifestations in high-risk transfusion recipients. The patient populations at high risk for CMV disease include CMV-seronegative patients in the following categories: pregnant women and their neonates, preterm low birth weight infants (<1200g), bone marrow and solid organ transplant recipients, AIDS patients and other extremely immunosuppressed individuals, so these group of patients require CMV free blood or components[7]. Study conducted by Mahmood et al in 2014 reported the incidence of 3.4% IgM positivity among blood donors in local population[8]

For screening of donated blood, the World Health Organization has recommended tests for Hepatitis B Surface Antigen, Antibody to Hepatitis C and HIV for subtypes 1 and 2 and serological test for syphilis[9]. However this specific testing alone cannot control for newly emerging or un screened infections. Non-specific screening using immune response marker like Neopterin could reduce this risk[10].

Neopterin is a sensitive indicator of activated cell mediated (T helper cells type1) immune response[11]. Activation of the immune system and subsequent rise in neopterin concentration is a key feature of various pathologies specifically viral infections. Due to its stability in biological fluid, neopterin is used to assess the intensity of cell-mediated immune responses. Numerous studies have established neopterin as an indicator for the onset, progression and outcome of various diseases[12-14]

Raised levels of Serum neopterin levels are found in chronic Hep B positive subjects. In hepatitis B carriers, elevated Neopterin levels may be an indicator of the presence of replication[15].In hep C positive patients elevated neopterin levels are more often associated with a positive PCR[16].

Serum neopterin levels are found to be raised in HIV infected patients not only during the acute phase but also in the window period and correlate with the extent and severity of the disease. The robust performance of Neopterin, as an inexpensive and reliably measured serum marker supports its potential suitability for HIV patient monitoring particularly in limited resource setting[17-19]. Studies have demonstrated higher serum Neopterin concentrations in Dengue Fever which has the potential of transfusion transmission [20-22].Neopterin levels are also found to be elevated in malaria[23].

After many years of trials nation wide screening for elevated Neopterin was introduced in Austria in 1994 when its significant role in blood screening was acknowledged. Since then a number of studies have proved the capability of Neopterin screening to improve safety of blood donations regarding the transmission of virus infections such as HIV, Hep B, Hep C, EBV, Parvo Virus B19 and Acute CMV[10]. We conducted a similar study in our population to estimate serum Neopterin levels in blood donors and to study its association with transfusion transmitted infections.

MATERIALS AND METHODS

This cross sectional study was carried out in the Department of Physiology Liaquat National Hospital

TABLE 1:
NEOPTERIN LEVELS IN DONORS WITH POSITIVE AND NEGATIVE SCREENING TESTS (N=174)

Screening test	No. (%)	No. of TTI positive with elevated Neopterin level (>10 nmol/L)	Neopterin level (nmol/L)	
			Mean ± S.D	P-value
Negative	154 (88.51)	4 (2.6%)	6.23 ± 2.19	0.001*
Positive	20 (11.49)	17 (85.0%)	15.10 ± 4.93	

TABLE 2:
NEOPTERIN LEVELS IN TRANSFUSION TRANSMITTED INFECTIONS (N=174)

Transmitted disease	No. (%)	No. of TTI positive with elevated Neopterin level (>10 nmol/L)	Neopterin levels nmol/l	*P value*
Hepatitis "B"	7 (4.02)	7 (100%)	16.39 ± 3.21	
Hepatitis "C"	6 (3.45)	6 (100%)	15.59 ± 1.63	0.001*
HIV + Hepatitis "C"	1 (0.57)	1 (100%)	25.01 ± 0.00	
CMV	3 (1.72)	3 (100%)	17.03 ± 1.83	
Syphilis	3 (1.72)	0	5.86 ± 0.78	

TABLE 3:
VARIATION OF NEOPTERIN LEVELS IN TRANSFUSION TRANSMITTED INFECTIONS (N=174)

Neopterin range (nmol/l)	No. of subject	Transfusion transmitted disease				
		Hep "B"	Hep "C"	Hep "C" & HIV	CMV	Syphilis
3.1 – 6.0	70	0	0	0	0	1
6.1 – 10.0	83	0	0	0	0	2
10.1 – 14.0	4	2	1	0	0	0
14.1 – 18.0	11	3	4	0	2	0
18.1 – 22.0	5	2	1	0	1	0
22.1 – 26.0	1	0	0	1	0	0
Total	174	7	6	1	3	3

and Medical College (LNH&MC) in collaboration with Basic Medical Sciences Institute (BMSI) and Jinnah Post Medical College Blood Bank. Data collection was for a period of six months in 2015. During this period a total of n=174 blood donors were selected through random sampling technique. All participants fulfilling the inclusion criteria and consenting to participate were selected on odd dates of the month excluding the official holidays.

The selected participants were screened for transfusion transmitted infections through the standard procedures as per WHO recommendations at the JPMC blood bank. Serum Neopterin was measured with commercial ELISA Kits. Blood samples were obtained in 4-5 ml evacuated sterile gel tubes. After centrifugation plasma samples were stored at -20ºC until neopterin assay was performed. Since Neopterin is photosensitive, all samples were kept in dark during all procedures. The cut off for serum neopterin concentration for healthy donors was taken as < 10nmol/l[11].

The data feeding and analysis was on computer package SPSS (Statistical Packages of Social Sciences) version 21.0. Clinical characteristics were summarized in terms of frequencies and percentages for qualitative/ categorical variables (age groups, blood groups, screening results of blood transfusion transmitted diseases, etc.) mean ±S.D. for quantitative variables (neopterin level nmol/l). Chi square test was used to find the association of qualitative/categorical variables and Student t-test/ANOVA was used for quantitative data. In all statistical analysis only p-value <0.05 were considered significant. The study was approved from the ethical review committee of respective institutions.

RESULTS

A total of 174 blood donors were recruited in the study. All the donors were screened for routine basic screening tests (HIV 1 and 2, HBs Ag, HCV, MP and RPR for syphilis). After screening out of 21 donors with elevated Neopterin, 14 were positive for infections screened routinely which included HBs Ag n=7 (4%), HCV n=6 (3.4%) and HCV + HIV co-infection n=1 (0.6%). The remaining 7 donors with elevated Neopterin were further screened with an advanced panel of tests including CMV IgM and Dengue IgM antibodies. Three of these asymptomatic donors with elevated Neopterin showed presence of CMV IgM antibodies. The Neopterin level in the sera of these CMV positive donors was 17.03 ± 1.83 nmol/l

In our study out of 174 subjects, 153 were negative for the screening of transfusion transmitted infections. The Neopterin content in the sera of these blood donors was 6.23 ± 2.19 nmol/l where as in the blood donors who tested positive for TTIs either by routine screening or through our advanced panel of screening markers, the neopterin level was elevated to 15.10 ± 4.93 nmol/ l. When compared statistically the difference between these values was found to be highly significant with p value of 0.001. (Table 1).

When neopterin levels were assessed in blood donors suffering from transfusion transmissible infections, significant increase in its levels was observed as compared to healthy donors *p* value=0.001(Table 2).

Hep B seropositive blood donors showed elevated neopterin level between 10.1-26.0 nmol/l. Hep C seropositive donors also showed neopterin level between 10.1-22.0 nmol/l. A donor who was positive for HIV+HCV co-infection showed raised neopterin level of 25.01nmol/l. CMV positive donors also had elevated neopterin level between 14.1-22.0 nmol/l. However Syphilis positive donors showed normal range of neopterin level 3.1-10.0 nmol/l (Table 3).

DISCUSSION

Although the transfusion of blood and blood products is a life saving measure and helps innumerable people worldwide, at the same time however blood transfusion is an important mode of transmission of infections to the recipients. In developing countries the prevalence of transfusion transmitted infections is much higher and quite far from attaining a zero risk level at the present moment[1]

In our study out of 174 donors n=21 (12.06%) showed elevated Neopterin level which is in accordance with the study done by Banu et al. (2011) who showed n=58 (19.09%) donors with elevated neopterin level[24]. In another study conducted by Honlinger et al. (1989) only n=12 (1.6%) blood donors showed increased Neopterin level where as in the study of Fisenk et al. (2005) n=57 (5%) donors showed elevated Neopterin levels[25-26].

Transfusion transfused cytomegalovirus infection is associated with considerable morbidity and mortality in at risk population, which include CMV seronegative neonates, immune-compromised patients and transplant recipients[27] among blood donors CMV IgM was positive in 3 donors (1.724%). When compared to studies conducted by Honlinger et al. (1989) and Mahmood

et al. (2014) it was almost half which showed 3.7% and 3.4% positivity respectively[25-8]. The neopterin level in the sera of these CMV positive donors was 17.03 ± 1.83 nmol/l which is in agreement with the studies of Schennach et al (2002) and Parrak et al. (2006) who found similarly elevated neopterin levels in CMV positive asymptomatic blood donors[28-29]. Schennach et al. (2002) reported a 20 fold increased incidence of Acute CMV infection in blood donors with elevated neopterin level, where increased neopterin level was found even before CMV IgM seroconversion[28]. Cytomegalovirus infection is usually asymptomatic or self-limiting, but the risk of acquiring the infection through blood transfusion from asymptomatic donors remains a major problem for immunocompromised CMV seronegative patients[30]

In this study out of 174 donors, n=7 (4.02%) were positive for Hbs Ag. The Neopterin content in the sera of viral Hepatitis B positive donors was 16.39 ± 3.21 nmol/l. The study done by Banu et al. (2011) also found similar results of neopterin elevation in Viral Hepatitis B positive blood donors[24].

In our study one HIV positive donor showed elevated neopterin level of 25.01 nmol/l. Study conducted by and Mildvan et al. (2005) also showed increased neopterin level in 100% of HIV positive subjects[17]

Hepatitis C virus infection also correlated with elevated Neopterin levels. The n=6 (3.4%) HCV positive samples had neopterin values in the range of 15.59 ± 1.63 nmol/l. similar results were obtained by Schennach et al. (1998) and Banu et al. (2011) in their studies[15, 23].

A limitation of our study was the sample size which was restricted because of constraint in funds. However the strengths of the study from the novelty of topic to the importance of neopterin levels as a screening tool cannot be overlooked. The random sampling technique supported the small sample size by decreasing the bias in the study.

On the basis of our findings, we suggest and recommend that further extensive research be undertaken on a larger scale to explore the beneficial role of Neopterin screening in blood donation in our local population, so that we may implement it as a routine assay in our blood banks.

CONCLUSION

Serum Neopterin levels were found elevated in asymptomatic CMV IgM positive blood donors (15.10 ± 4.93 nmol/l). Thus it is concluded that Neopterin screening has the potential to detect transfusion transmissible CMV infection which would not be revealed by routine screening

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Authors contribution:
* Concept and design, data collection** Critical review of the paper *** Collection and assembly of data **** Data analysis

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