Original Article

Neopterin Screening Significantly Improves Safety of Blood Donations

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ABSTRACT

Introduction: The transfusion of blood borne infections is one of the most important transfusion associated hazards in many parts of the world specially in developing countries. Potentially hazardous agents include certain viruses, bacteria and parasites such as HIV, HCV, HBV, Syphilis, Malaria and other pathogens. These may remain undetected either because they are known and not screened or they may also be unknown by usual screening methods. Moreover it is also possible that blood is donated during the diagnostic window period. Neopterin is a sensitive indicator of activated cell mediated (T helper cells type1) immune response. Its significant role in blood screening was acknowledged after many years of trials therefore we hypothesized that additional non-specific screening using highly sensitive immune marker like neopterin could significantly improve the safety of blood donations

Study Design: A cross-sectional prospective study

Place and Duration of Study: This study was carried out in the department of Physiology, BMSI in collaboration with JPMC blood bank from May 2011 to Oct. 2011.

Patients and methods: A total of 174 blood donors were included in the study who were screened for HIV, HBV, HCV, Malaria and Syphilis by routine screening (ELISA, RPR and slide method). Serum neopterin level was measured using ELISA.

Results: 174 subjects participating in this study, 21 showed increased serum neopterin levels beyond the acceptable cut-off level of 10nmol/l. Out of them 7 were positive for HBV, 6 for HCV and one of the blood donors was found to have HIV and HCV co-infection. Thus increased neopterin levels were found to be highly significant with a p value of 0.001 in donors having viral infections. Further testing of the remaining 7 samples with increased neopterin level that were seronegative by routine screening, led to the detection of acute CMV infection in three of these donors. These subjects were asymptomatic at the time of donating blood. Three subjects tested positive for syphilis by RPR but their neopterin level was found to be within normal range.

Conclusion: Screening of blood donations for serum neopterin levels significantly increases the safety of blood donations regarding various viral pathogens in a non-specific way.

Key Words: Neopterin, Blood donations.

INTRODUCTION

Blood transfusion is an essential component of health care which saves millions of lives each year.[1] Currently known dangerous infectious agents associated with transfusion include HIV, Hepatitis B and C , Malaria, Syphillis, Acute CMV, EBV, Parvo virus, West Nile virus and agents responsible for Filiriasis Leishmaniasis, Toxoplasmosis and Chagas disease.[2,3] The W.H.O. recommends that blood donations worldwide should at least be screened for Hepatitis B Surface Antigen ,Antibody to Hepatitis C ,Antibody to HIV, usually subtypes 1 & 2 and Serologic test for Syphilis.[4]In 2006 the W.H.O report on blood safety revealed that in the area of blood testing, 56 out of 124 countries did not screen all of their donated blood for HIV, hepatitis B & C and syphilis. Reasons given for this include scarcity or unaffordability of test kits, lack of infrastructure and shortage of trained staff. [5] In Pakistan there is high prevalence of Transfusion transmitted infections, with the paid donors showing 25% HCV and 10% HBV infection. In the family donors HCV infection was found to be 2.5% and HBV was 5%, where as the voluntary donors showed 2% prevalence for Hepatitis B and 0.5% for HCV infection.[6]

Neopterin is a sensitive indicator of activated cell mediated (T helper cells type 1) immune response.[7] Human monocytes/macrophages produce and release large amounts of neopterin following activation by T-lymphocyte-derived gamma-interferon.[8,9] It is determined by RIA, ELISA and HPLC in body fluids. It concentrations measure 5.3 ± 2.7 nmol/l in the serum of healthy adults.[10] Activation of the immune system and subsequent rise in neopterin concentration is a key feature of various pathologies like: viral infections, autoimmune diseases , malignant tumours, allograft transplantation and cardiovascular disease. Neopterin screening of blood donations allows to detect and exclude viral infections during the acute phase and allows to further shorten the diagnostic window in addition to specific serologic screening methods.[11] For

blood donation screening serum neopterin level is acceptable below 10nmol/l .[12]There are a number of studies have proved the capability of Neopterin screening to improve safety of blood donations regarding the transmission of viral infections such as HIV, Hep B, Hep C, EBV, Parvo Virus B19 and acute CMV infection.[13]So additional non-specific screening using immune response marker like Neopterin could reduce this risk.[14] This study was under taken to study neopterin relationship with transfusion transmitted infections and assess the potential role of Neopterin screening as a safety marker in blood donations.

PATIENTS AND METHODS

This cross-sectional prospective study was carried out in the department of physiology BMSI, in collaboration with JPMC blood bank. The study comprised of a single group which included 174 blood donors. All healthy blood donors of both gender age between 18-60 years were included in the study. Patients with history of Cancers, history of heart disease, history of active tuberculosis, history of known infectious diseases e.g. HIV, Hepatitis B and C and history of STD / High risk sexual behavior were excluded from this study. These were screened for HIV, HBV, HCV by ELISA, Malaria by slide method and Syphilis by RPR. Serum neopterin level was measured using ELISA.

RESULTS

This study was conducted on blood donors at the JPMC blood bank. A total of one hundred and seventy four blood donors were selected, who were all male and the age group in our study was in the distribution of 18-50 yrs. 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 includes HBs Ag (7) HCV (6) and HCV + HIV co-infection (1). The remaining 7 donors with elevated neopterin were screened for CMV IgM and Dengue IgM antibodies. Three of these asymptomatic donors showed presence of CMV IgM antibodies.

Table No.1: Age distribution of blood donors with Neopterin level

Age group (in years)	Number	Percent	Neopterin level (nmol/L)	
			Mean ± S.D.	Elevated neopterin level
<20	5	2.9	9.24 ± 5.01	1 (20.0%)
20-24	58	33.3	7.36 ± 3.58	8 (13.8%)
25-29	60	34.5	7.15 ± 4.06	8 (13.3%)
30-34	37	21.3	7.03 ± 4.09	3 (8.1%)
35-39	10	5.7	6.51 ± 1.42	-
≥ 40	4	2.3	8.57 ± 6.35	1 (25.0%)
Total	174	100.0	7.25 ± 3.87	21 (12.1%)

Average neopterin level (Mean ± S.D) and Elevated neopterin level according to age were not significant difference p>0.05

Table No.2: Neopterin levels in donors with positive and negative screening tests

Screening test	No. (%)	No. of 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 No.3: Neopterin levels in transfusion transmitted infections

Transmitted disease	No. (%)	No. of 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	0.001*
Hepatitis "C"	6 (3.45)	6 (100%)	15.59 ± 1.63	
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	1.86 0.78	

Table No.4: Distribution of neopterin levels in various transfusion transmitted disease

Neopterin range (nmol/L)	No. of subject	Transfusion transmitted disease				
		Hep "B"	Hep "C"	Hep "C" & HIV	CMV	VDRL
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

DISCUSSION

The specific testing cannot control newly emerging and/or unrecognized infections. Additional non-specific screening using immune response marker like Neopterin could reduce this risk. In 1994 nationwide screening for elevated Neopterin was introduced in whole Austria when its significant role in blood screening was acknowledged after years of trials. One of the major goals of this non-specific screening

strategy was to build a kind of umbrella against virus infections which are not routinely screened for.[15] Recent data further supports the concept that neopterin screening should be able to detect a wide range of acute viral infections, and thus also newly emerging viral infections should be detectable by elevated neopterin concentrations e.g highly elevated neopterin levels were observed in patients suffering from acute dengue virus infections. [16]

Considering this background we designed this study to estimate serum Neopterin levels in blood donors of local population and to study its relationship with transfusion transmitted infections. We also intended to assess the potential role of Neopterin screening as a safety marker in blood donations in our local population as it will be a pilot study for the south Asian population with regards to blood donation safety and role of neopterin.

In our study out of 174 donors 21(12.06%) showed elevated neopterin level which is in accordance with the study done by Banu et al[17], who showed 19.09% (58)donors with elevated neopterin level. In another study conducted by Honlinger et al[18] only 1.6% (12) blood donors showed increased neopterin level.

In our study the age distribution of blood donors was between 18-50 yrs. Most of the blood donors were in the age group of 25-29 years i.e 34.5% (60) donors and 20-24years i.e 33.3% (58) donors. This was followed by 21.3% (30) donors in the age group of 30-34 years , 5.7% (10) donors in the age group of 35-39 years, 2.9% (5) donors below the age of 20 years and 2.3% (4) donors over the age of 40 years.

The results of our study showed that the average Neopterin level (Mean ± S.D) and elevated Neopterin level according to age were not significant statistically.(difference P>0.05) This is not in agreement with the study of Spencer et al[19] who found a positive correlation between neopterin level , age and gender This could be due to the fact that all of our study subjects (blood donors) were male in the age distribution of 18-50 yrs in contrast to the age distribution of 20-80 yrs in their study who belonged to either gender. Furthermore in that study, they suggested that the relative contribution of age and gender to modulating neopterin levels in normal physiological events may reflect the biology of underlying aging, late-age onset diseases, and perhaps gender differences in morbidity and mortality.

In our study out of 174 subjects, 154 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 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.

Among blood donors CMV IgM was positive in 3 donors (1.724%). When compared to study conducted by Honlinger et al[18] it was almost half which showed 3.7% positivity (12) donors. The neopterin level in the sera of these CMV positive donors was 17.03 ± 1.83 nmol/l which is in agreement with the study of Schennach et al [20] who found similarly elevated neopterin levels in CMV positive asymptomatic blood donors. who 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. Another study conducted by Schennach et al[21] Who also found that in donors with increased neopterin levels the occurrence of an acute Epstein-Barr virus or Parvo virus infection was 4 to 6 times more likely than in donors with neopterin level within the normal range.

In conducted study out of 174 donors 7 (4.02%) were positive for Hbs Ag . The neopterin content in the the sera of viral Hepatitis B positive donors was 16.39 ± 3.21nmol/l. The data was obtained for an evident increase in neopterin levels associated with viral B hepatitis, this was well supported by another study done by Samsonov et al[22] (1992) who found elevated neopterin level of 19.9±5.7 nmol/l in the serum of Hepatitis B positive patients. Kalkan et al[23]in their study also found elevated neopterin levels of 15.6±5.1 nmol/l in chronic HBV positive patients which is in well agreement with our study. The study done by Banu et al [17] also found similar results of neopterin elevation in Viral Hepatitis B positive blood donors.

In our result one HIV positive donor showed elevated neopterin level of 25.01nmol/l. The Study conducted by Fuchs et al also showed increased neopterin level in 100% HIV positive donors. They found that in HIV infection, neopterin levels increase with progressive disease and inversely correlated with CD4+/CD8+ T cell subset ratios and are of predictive significance [24]. Recently in the study of Nubling et al [25] concluded that the diagnostic sensitivity of neopterin screening during the HIV window phase is similar to p24 antigen test therefore neopterin screening of blood donors may identify window phase of HIV infection.

Hepatitis C virus infection also correlated with elevated neopterin levels. The 6 (3.4%) HCV samples had neopterin values in the range of 15.59 ± 1.63nmol/l.Similar results were obtained by Schennach et al[26] and Banu et al[17] in their studies.

Three of the donors in the study were positive for syphilis .The neopterin concentration in their serum was 5.86±0.78 nmol/l which was below the cutoff level of 10nmol/l. Similar findings were observed by N'Gom et al [27] who did not find any neopterin elevation with syphilis. This is probably due to the fact that in contrast to the viral infections which trigger the cytotoxic immune response involving TH1 cells, the systemic bacterial infections invoke the humoral immunity thus involving TH2 instead of TH1, and therefore no change in neopterin levels was observed.[12]

CONCLUSION

Screening of blood donations for serum neopterin levels significantly increases the safety of blood donations regarding various viral pathogens in a non specific way.

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