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Molecular epidemiology and genotyping of SEN Virus in thalassemia patients in Pakistan

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Dear Editor,

Pakistan is among the countries where rate of blood transfusion is quite high with approximately 1.5 million bags transfused annually. According to WHO, Pakistan has a high disease burden of thalassemia, approximately 5000 children are born with, and 70,000 patients are registered with the disease annually (WHO, 2016). Most of the health care facilities to these patients are provided by private blood transfusion centers, run by non-governmental organizations (Luby et al., 2000). WHO recommend the local authorities to screen all blood samples for hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV), at least in the major cities if not nationwide. Despite HBV, HCV and HIV screening, the rate of transfusion-transmitted infections (TTI) is increasing (Afzal, 2016; Waheed et al., 2012). Among these infections, the major proportions are of hepatic infections. Approximately 20% of hepatic infections are not associated with hepatitis viruses (A–E) and might be due to the other viruses (Hosseini and Bouzari, 2016a).

To explore other possible causative contagion agents, this study was designed to estimate the preponderance of SEN Virus (SENV, SEN for the initials of the patients in whom the virus was isolated for the first time) in Pakistan. The reason for selecting SENV was that the infection with SENV can lead to hepatitis and its prevalence was positively correlated with transfusion (Karimi et al., 2013). Although epidemiology of SENV varies by geographic region, but it has been found that the sero-prevalence of the virus was 10 times more in transfusion recipients compared to patients who did not receive transfusions (Akiba et al., 2005). It is non enveloped, single stranded circular DNA virus. It has nine genotypes (A–I) where genotypes D and H are more often consorted with hepatitis of unknown origin compared to other genotypes (Mohamed et al., 2011). To check the safe blood transfusion efficacy HBV and HCV infections were also analyzed in multi transfused thalassemia patients as a part of the study.

To estimate the prevalence of TTI induced SENV in Pakistan, a study was designed. For this purpose initially the sample size was calculated for both patient and control groups.

$$n = \left(\frac{r+1}{r}\right)\frac{(\overline{p})(1-\overline{p})(Z_{\beta}+Z_{\alpha/2})^2}{(p_1-p_2)^2}$$

Power was set at 80%, it was expected to detect the odd ratio of ≥2.0. The ratio of control to patients was set at 1:1 therefore the r value was equal to 1. We assumed that the proportion exposed in the control group was 20%. For 80% of power Z_{β} is equal to 0.84. For 0.05 significance level, $Z_{\alpha} = 1.96$, $r = 1$ (equal number of cases and controls). The proportion exposed in the control group is 20%. To get proportion of cases exposed:

$$p_{case\,\exp} = \frac{ORp_{controls\,\exp}}{p_{control\,\exp}(OR-1)+1} \quad p_{case\,\exp} = \frac{2.0(0.20)}{(0.20)(2.0-1)+1} = \frac{0.40}{1.20} = 0.33$$

$$\text{Average proportion exposed} = (0.33 + 0.20)/2 = 0.265.$$

$$n = 2\frac{(0.265)(1-0.265)(0.84+1.96)^2}{(0.33-0.20)^2} = 181$$

Therefore, $n = 362$ (181 cases, 181 controls).

So 181 thalassemia patients (aged ranged 1–15 years) and for healthy control group same numbers of volunteers were enrolled. Blood samples were taken after written consent from each participant. The samples were collected from different areas of Rawalpindi (Punjab), Islamabad (Federal Capital) and Sawat (Khyber Pakhtunkhwa). The samples were collected from different areas in order to get preliminary information about SENV infection in different localities of the country and to avoid any biased or false finding. Out of total 181 samples 46 patient samples were collected from Rawalpindi, 45 were taken with written informed consents from Islamabad and 90 from Swat. The same number and age matched healthy participants were also enrolled from each area. As Rawalpindi and Islamabad are twin cities with advanced health facilities so half study participants were from these cities and other half were enrolled from Swat (Khyber Pakhtunkhwa) which is comparatively remote area. The study was approved by departmental ethical committee and was carried out in Molecular virology lab at COMSATS institute of Information Technology, Islamabad. Among the study participants, 53% (96) patients were male while 47% (85) were females and in control group 62% (112) were male and 38% (69) were females. Blood DNA was isolated with extraction kit (Pure Link™ Genomic DNA kits) according to manufacturer's protocol. The quality of DNA sample was checked by amplification of a housekeeping gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH). All study participants were subjected to SENV presence by using specific primers (Tangkijvanich et al., 2003) on Verity 96 well thermal cycler (Applied Bio system, USA). The virus positive individuals were further characterized for the viral genotype (SENV-D and SENV-H) by using specific primers (Tangkijvanich et al., 2003).

The result of the study showed that epidemiology of SENV is significantly associated with transfusions in Pakistan (Table 1). While the genotyping data of SENV depicts that out of total 30 SENV

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Table 1
Epidemiology of SEV-V, HBV and HCV in thalassemia patients in Pakistan.

Study groups	SEN-V			HBV[†]		HCV[†]	
	Positive %	Negative %	*P* value[*]	Positive %	Negative %	Positive %	Negative %
Patients ($N = 181$)	13	87	0.0398	2	98	12	88
Control ($N = 181$)	4	96		–	–	–	–

[†] HBV and HCV were only screened in patient group.
[*] *P* value was calculated by Fischer's exact test. *P* values smaller than 0.05 were considered significant.

positive samples (both patient and controls) in our data set 12 belong to SENV genotype D group while 8 belong to SENV genotype H, while remaining sample were some unknown genotypes as they were not positive for both primer sets. Recently Karimi et al. (2013) reviewed that SENV is endemic throughout the world but its prevalence is varied (1–23%) in different geographical regions. Our results showed that prevalence of SENV is significantly high in experimental group and it could be a potential threat for future (Table 1). The prevalence of SENV in Pakistan is comparatively lower both in patient and control groups as compared with previous reports from different geographical regions of the world (Hosseini and Bouzari, 2016b; Mu et al., 2004; Schréter et al., 2006; Schröter et al., 2003; Shibata et al., 2001). Other reports from different parts of the globe showed lower prevalence of SENV when compared with the results of current study (Bowden, 2001; Mushahwar, 2000; Serin et al., 2005) which confirms the variability in the sero-prevalence of SENV across the world. A recent report from Iran reported that SENV was detected in 90% of healthy individuals even higher than high risk group (Hosseini and Bouzari, 2016b) while other report from different province of Iran (Ghasemi Dehkordi and Doosti, 2011) showed contrary results. The possible reason behind this variability of SENV prevalence in different geographical regions of the world might be the life style, socio-economic status, standard of health care facilities, knowledge, skills and training of health care providers, number of surgical procedures, number of transfusions, duration of hemodialysis, intravenous drug use, unsafe sexual practices, homosexuality and professional exposure. Current study just focused on considering the presence of SENV in thalassemia patients who receive multiple transfusions and no statistical analysis was applied to find out the association between numbers of transfusions, ages of considered groups and other possible risk factors. The future studies should focus on the correlation of SENV prevalence with number of transfusions received, age of the patient and other potential risk factors.

Our study also showed that HBV and HCV are still prevalent (Table 1) in spite of strict WHO guidelines. The overlap between HCV, HBV and SENV infection has been observed in several studies (Bowden, 2001; Ghasemi Dehkordi and Doosti, 2011; Hosseini and Bouzari, 2016b; Mu et al., 2004; Mushahwar, 2000; Schréter et al., 2006; Schröter et al., 2003; Serin et al., 2005; Shibata et al., 2001); while the current study reported a minor trend of SENV co-infection with HCV (2%) but none of the SENV positive patient was co infected with HBV. High HCV prevalence showed that blood transfusion is not safe even in metropolitan cities. On the basis of these findings, proper following of the WHO guidelines for TTI screening is highly recommended for future transfusions. Pakistan is endemic for hepatitis infection and has a high disease burden (Afzal et al., 2014a; Afzal et al., 2014b), if effective strategies will not be devised for future, these infectious diseases will be a main threat for the community and health sector. Although SENV screening is not a part of the current guidelines for TTI screening programme but further studies on its epidemiology are warranted. Another progressive suggestion is to establish a surveillance system for patients with co-infection of SENV/HBV and SENV/HCV; it will help in analyzing hepatic disease progression and management for further studies in Pakistan. It is the need of hour to include SENV screening in HBV/HCV infected individuals and in healthy blood donors.

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Conflict of interest

There is no conflict of interest.

References

Afzal, M.S., 2016 Jun. Are efforts up to the mark? A cirrhotic state and knowledge about HCV prevalence in general population of Pakistan. Asian Pac. J. Trop. Med. 9 (6), 616–618. http://dx.doi.org/10.1016/j.apjtm.2016.04.013.
Afzal, M.S., Anjum, S., Zaidi, N.U., 2014 Jan 6a. Changing of HCV clade pattern in iran; the possible means for something good. Hepat. Mon. 14 (1), e11879. http://dx.doi.org/10.5812/hepatmon.11879.
Afzal, M.S., Ahmed, T., Zaidi, N.U., 2014 Jan 12b. Comparison of HCV prevalence in pakistan and iran; an insight into future. Hepat. Mon. 14 (1), e11466. http://dx.doi.org/10.5812/hepatmon.11466.
Akiba, J., Umemura, T., Alter, H.J., Kojiro, M., Tabor, E., 2005 Jul. SEN virus: epidemiology and characteristics of a transfusion-transmitted virus. Transfusion 45 (7), 1084–1088.
Bowden, S., 2001. New hepatitis viruses: contenders and pretenders. J. Gastroenterol. Hepatol. 16, 124–131.
Ghasemi Dehkordi, P., Doosti, A., 2011. The prevalence of SEN virus infection in blood donors and chronic hepatitis B and C patients in Chaharmahal VA Bakhtiari province. J. Cell Anim. Biol. 5 (8), 182–186.
Hosseini, S.A., Bouzari, M., 2016 Mar 8a. Detection of SENV virus in healthy, hepatitis B- and hepatitis C-infected individuals in Yazd Province, Iran. Iran. Biomed. J. (Pii-IBJ-A-10-1571-1).
Hosseini, S.A., Bouzari, M., 2016 Julb. Detection of SENV virus in healthy, hepatitis B- and hepatitis C-infected individuals in Yazd Province, Iran. Iran. Biomed. J. 20 (3), 168–174.
Karimi, G., Gharehbaghian, A., Tafti, M.F., Vafaiyan, V., 2013 Jun. Emerging infectious threats to the blood supply: seroepidemiological studies in iran - a review. Transfus. Med. Hemother. 40 (3), 210–217. http://dx.doi.org/10.1159/000351540 (Epub 2013 May 17).
Luby, S., Khanani, R., Zia, M., Vellani, Z., Ali, M., Qureshi, A.H., Khan, A.J., Abdul Mujeeb, S., Shah, S.A., Fisher-Hoch, S., 2000 Jun. Evaluation of blood bank practices in Karachi, Pakistan, and the government's response. Health Policy Plan. 15 (2), 217–222.
Mohamed, S., Thabit, A.G., Abd-El Rahman, S.A., Abdelmohsen, E.E., SeifEldin, S.S., Ghandour, A.M.A., 2011. Prevalence of SEN virus infection in multi transfused patients in Assiut University Hospitals, Egypt. J. Am. Sci. 7 (1), 687–696.
Mu, S.J., Du, J., Zhan, L.S., Wang, H.P., Chen, R., Wang, Q.L., Zhao, W.M., 2004 Aug 15. Prevalence of a newly identified SEN virus in China. World J. Gastroenterol. 10 (16), 2402–2405.
Mushahwar, I.K., 2000. Recently discovered blood-borne viruses: are they hepatitis viruses or merely endosymbionts? J. Med. Virol. 62, 399–404.
Schréter, I., Kristian, P., Jarcuska, P., Porubcin, S., Siegfried, L., Birosová, E., Rajnic, A., Gocalová, A., 2006. Detection of SEN virus in the general population and different risk groups in Slovakia. Folia Microbiol. (Praha) 51 (3), 223–228.
Schröter, M., Laufs, R., Zöllner, B., Knödler, B., Schäfer, P., Feucht, H.H., 2003. A novel DNA virus (SEN) among patients on maintenancehemodialysis: prevalence and clinical importance. J. Clin. Virol. 27, 69–73.
Serin, M.S., Koksal, F., Oksuz, M., Abayli, B., Aslan, G., Tezcan, S., Yildiz, C., Kayar, B., Emekdas, G., 2005 Dec. SEN virus prevalence among non-B and non-C hepatitis patients with high liver function tests in the south of Turkey. Jpn. J. Infect. Dis. 58 (6), 349–352.
Shibata, M., Wang, R.Y., Yoshiba, M., Shih, J.W., Alter, H.J., Mitamura, K., 2001 Aug 15. The presence of a newly identified infectious agent (SEN virus) in patients with liver diseases and in blood donors in Japan. J. Infect. Dis. 184 (4), 400–404.
Tangkijvanich P, Theamboonlers A, Sriponthong M, Thong-Ngam D, Kullavanijaya P, Poovorawan Y.·SEN virus infection in patients with chronic liver disease and hepatocellular carcinoma in Thailand. J. Gastroenterol. 2003; 38(2): 142-148.
Waheed, U., Khan, H., Satti, H.S., Ansar, M.A., Malik, M.A., Zaheer, H.A., 2012. Prevalence of transfusion transmitted infections among blood donors of a teaching hospital in Islamabad. Ann. Pak. Inst. Med. Sci. 8 (4), 236–239.
WHO, 2016. http://www.emro.who.int/pak/programmes/blood-safety.html (access on 19-03-2016).

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