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## Short Communication

# Prevalence of Hepatitis B and C and Assessment of Responsible Risk Factors among the Vulnerable $\beta$ -Thalassemic Patients of Azad Kashmir, Pakistan

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## ABSTRACT

Approximately 350 million patients of hepatitis B and 170 million patients of Hepatitis C are present worldwide according to WHO. Many risk factors are involved in the transmission of these deadly viral infections but blood transfusion in Beta thalassemic patients is working with two faces, one as remedy and the other is key risk factor in the spread of silent killers. Thalassemia patients registered in Combine Military Hospital (CMH) Rawalakot and Sheikh Khalifa Bin Zayed Al-Nahyan Hospital, Muzaffarabad Azad Jammu and Kashmir Pakistan were studied for the viral hepatitis B and C prevalence. A total of 303 (including 164 males and 139 females) individuals, aged between 1 and 12 years were studied. All the understudy participants were interviewed through questionnaire method. After taking written consent from each participant or guardian, 5 ml of blood was collected from each participant and brought to the working laboratory for HBV and HCV screening through ICT kit method. All ICT positive samples were further confirmed through ELISA. Individuals 25(8.2%) were found positive for both hepatitis B surface Antigen (HBsAg) and Anti hepatitis C antibody (Anti-HCV antibody) after initial screening with no coinfection of both diseases. Out of 25 total infected individuals, 05(1.6%) were found HBsAg positive and 20(6.6%) were found anti-HCV positive. All the ICT positive individuals were further confirmed by quantitative Enzyme Linked Immunosorbent Assay (ELISA) and 23(7.6%) individuals were confirmed for both hepatitis B and C including 05(1.6%) HBsAg positive as well as 18(5.9%) anti-HCV antibody positive individuals. We can conclude that 8.2% prevalence of hepatitis B and C among thalassemic patients is an alarming health concern which directly indicates to pay attention for ensuring 100% safe blood transfusion.

## Article Information

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## Authors' Contributions

FS, SA and S. Andleeb designed the study and wrote the manuscript. AR, SAK, FNK, MSH and RAM analysed the samples.

## Key words

Hepatitis B, Hepatitis C,  $\beta$ -thalassemia, Azad Kashmir, Pakistan.

A major risk for the patients of transfusion dependent  $\beta$ -thalassemia is developing blood infections among which, hepatitis B and C are the most common (Jafroodi *et al.*, 2015). It is well-known that hepatitis C virus is a major cause of post transfusion hepatitis infection (Farooq *et al.*, 2018) which may also lead to liver cancer or cirrhosis (Ataei *et al.*, 2012).

The cause of post-transfusion hepatitis as well as chronic liver failure in most of countries is hepatitis C

virus. The factor involved in the increase of survival with hereditary hemolytic anemia is blood transfusion but it is also one of many causes of viral hepatitis transmission among thalassemia patients (Mehta *et al.*, 2017). Thalassemia patients are always at a high risk of diseases transmitted via blood transfusion and post transfusion hepatitis B and C present a major problem round the globe (Ahmadi-Ghezeldasht *et al.*, 2018), hence, in thalassemic patient, treatment of hepatitis C (HCV) is quite hard due to the haemolytic side effects of therapy (Sajjad *et al.*, 2017).

Blood transfusion is the major cause of hepatitis B and C infections in many parts of the world especially in developing countries where the screening system is

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not much established. In Scandinavia, the prevalence of hepatitis in transfusion recipients was 0% (Edgren *et al.*, 2018) and in Rwandait was 1.6% (Twagirumugabe *et al.*, 2017). Patients with  $\beta$ -thalassemia, hemophilia and uremia, who receive multiple blood transfusions, are at an increased risk of transfusion related diseases (Tafesse *et al.*, 2017). 123 (32.45%) out of 379 transfusion dependent thalassemia children were found HCV positive in a descriptive cross sectional study conducted by Ali *et al.* (2016) in Pakistan. In another study, 180 beta thalassemia major children tested for hepatitis C virus out of which 75 (41.7%) children were found positive (Hussain *et al.*, 2008). Another study found hepatitis G virus in Pakistani children with transfusion dependent beta-thalassemia major (Moatter *et al.*, 1999). The rate of hepatitis B and C were found to be very high among thalassemia patients after acquiring some molecular methods (Kadhim *et al.*, 2017). According to the Pakistan Health and Research Council, a national survey showed that the frequency of HCV and HBV infections in Pakistan's general population was 4.8% and 2.5%, respectively. Prevalence of HCV infection alone ranges from 2.2 to 14% in Pakistan, reportedly. In the view of that, about 10 million people in Pakistan are infected with HCV (Ahmed *et al.*, 2016).

The present study was aimed to find out prevalence of hepatitis B and C among thalassemia patients with respected to gender and age as well as to find out associated risk factors other than blood transfusion which are involved in the spread of these silent killers.

#### Materials and Methods

The study was approved by the Directorate of Advance Studies and Research (DASR), which have authority to approve research and deal ethical issues in University of Azad Jammu and Kashmir, Muzaffarabad.

Thalassemia patients registered in Combine Military Hospital (CMH) Rawalakot and Sheikh Khalifa Bin Zayed Al-Nahyan Hospital, Muzaffarabad, Azad Jammu and Kashmir Pakistan were studied for the viral hepatitis B and C prevalence. A total of 303 (including 164 males and 139 females) individuals, aged between 1 and 12 years were studied. All the understudy participants were interviewed through questionnaire method. A filled and signed consent was collected from each participant or guardian. The consent form based upon collection of general information (age, sex and locality) and for risk factor assessment.

Blood samples (5ml) were collected from each participant and injected in the gel tube (Biotube Gel Clot Activator). Serum was separated by centrifugation. Each serum sample was labeled and stored at  $-20^{\circ}\text{C}$ . The hepatitis B surface antigen (HBsAg) and hepatitis C virus antibody (anti-HCV) were detected by using ICT kit

method through ICT devices (SD BIOLINE HBsAg and Anti-HCV kits). Moreover, all the initially HBsAg and anti-HCV positive individuals were further quantified for viral antigens and antibodies respectively through quantitative ELISA with the cut-off value (Non-Reactive  $< 0.10$ , Equivocal  $0.10 - 0.50$  and Reactive  $> 0.50$ ) provided with diagnostic kit for HBsAg and anti-HCV by the Linear Chemicals S.L., Spain.

Data was analyzed through GraphPad Prism (Version 5.0) to calculate the mean age (with 95% Confidence Interval), standard deviation and Chi-square test was used to detect the significance level of different risk factors involved in the spread of hepatitis B and C in thalassemia patients.

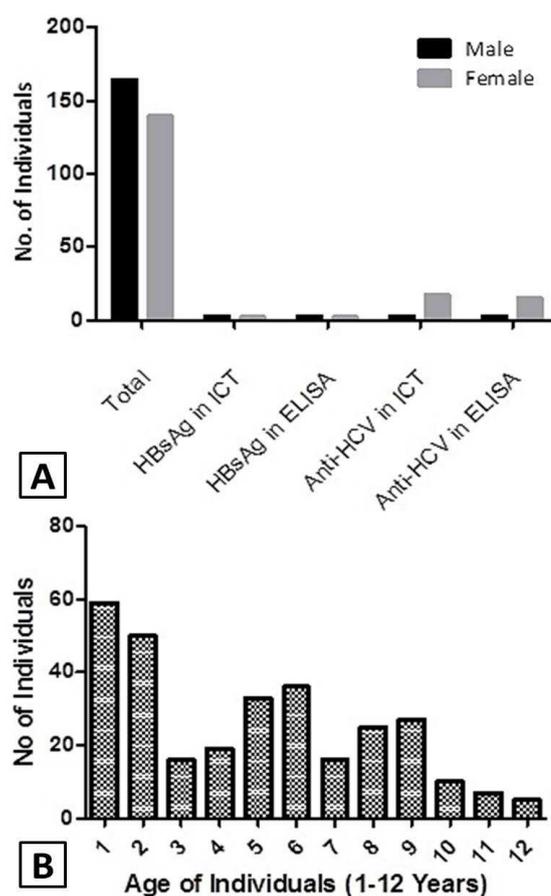


Fig. 1. Comprehensive prevalence (A) and age wise frequency distribution (B) of hepatitis B and C in thalassemia patients of Azad Kashmir, Pakistan.

#### Results

For the present study total, 303  $\beta$ -thalassemia patients were screened through immune-chromatography devices (ICT) including 164 (52.1%) male and 139 (45.9%) female individuals. 25 (8.2%) individuals were found

positive for both hepatitis B surface antigen (HBsAg) and Anti hepatitis C antibody (anti-HCV antibody) after initial screening with no coinfection of both diseases. Out of 25 total infected individuals, 05(1.6%) were found HBsAg positive (3 male and 2 female) and 20(6.6%) were found anti-HCV positive (3 male and 17 female).

All the ICT positive individuals were further confirmed by quantitative enzyme linked immunosorbent assay (ELISA) and we found 23(7.6%) individuals confirmed for both hepatitis B and C including 05 (1.6%) HBsAg positive (3 male and 3 female) as well as 18 (5.9%) anti-HCV antibody positive (3 male and 15 female) individuals.

The male population was found with 1% HBsAg prevalence as compare to 0.6% prevalence of HBsAg in female population. In case of Anti-HCV prevalence, the females were found more prevalent with 5.6% prevalence as compare to 1% of HBsAg in male population (Fig. 1A).

For determining prevalence of hepatitis B and C among  $\beta$ -thalassemia patients, the age-wise data was analyzed by using GraphPad Prism (Version 5.0) software and the population was divided in to two age groups, 1-6 years and 7-12 years, respectively. Standard deviation for the total population was found 3.13 and mean age with 95% Confidence Interval was found  $4.80 \pm 0.35$  for the total population. Moreover, the age group 7-12 years was found with higher prevalence of both hepatitis B (4.4%) and hepatitis C (14.4%) as compare to age groups of 1-6 years. This is due to the increase rate of multi transfusion with the increase of age in the case of thalassemia because the blood transfusion is the key risk factor involve in the spread of hepatitis B and C world-wide (Table I; Fig. 1B).

For the present study risk factors were assessed through a questions based proforma from each participant or from the guardian on the behalf of participants. Data was analyzed through two sided chi-square test (at  $p < 0.05$ ) by using GraphPad Prism (version 5.0). We found significant relationship of hepatitis B and C spread with family hepatitis history and dental treatment. Treatment from a local Practician was found not significant with  $p$  value of 0.0625 regarding to spread of hepatitis B and C in thalassemia patients. Table II shows all the details related

to risk factors assessment in the thalassemia patients other than blood transfusion.

**Table I.- Age-wise prevalence of hepatitis B and C among  $\beta$ -thalassemia patients.**

Age	No. of Ind.	SD	Mean age with 95% CI	HBsAg +ve	Anti-HCV +ve
01-06	213	1.89	$3.11 \pm 0.25$	01 (0.5%)	07 (3.3%)
07-12	90	1.37	$8.80 \pm 0.29$	04 (4.4%)	13 (14.4%)
Total Ind.	303	3.13	$4.80 \pm 0.35$	05 (1.6%)	20 (6.6%)

Ind., individuals, SD, standard deviation.

### Discussion

In the present study, the sero-prevalence of HBV markers (HBsAg and anti-HCV) was 22.8% in beta-thalassemia patients living in Azad Kashmir which indicated a high exposure rate. While it was observed that the prevalence of HBsAg was 1.6%. No anti-HBc detection procedure was used to study the selected population.

In a similar study conducted by Jang *et al.* (2017) 140 patients were tested, out of which only one patient was found positive for HBsAg that accounts for a prevalence of 6.4% and anti-HCV was found to be 45.7%. While in the present study 303  $\beta$ -thalassemia patients were screened in total through immune-chromatography devices (ICT), in which 164 (52.1%) were male and 139 (45.9%) were female individuals. Among 303 beta thalassemia patients 25(8.2%) individuals were found to be positive for both hepatitis B surface antigen (HBsAg) and anti-hepatitis C antibody (anti-HCV antibody) that were believed to be uninfected after initial screening. Out of 25 infected individuals, 05 (1.6%) were confirmed as HBsAg positive (3 males and 2 females) and 20 (6.6%) were found anti-HCV positive (3 males and 17 females). While the average age of patient was 1 to 12 years. A higher prevalence of both hepatitis B (4.4%) and hepatitis C (14.4%) infections was observed in the age group of 7-12 years. Another study was conducted in Pakistan in which total 95 patients observed were found  $\beta$ -thalassemia major (96%) and  $\beta$ -thalassemia intermedia (4%). Among these, 47 (49%) were found positive for anti-HCV antibodies and three for hepatitis B surface antigen (Din *et al.*, 2014).

**Table II.- Risk factors assessment regarding to spread of hepatitis B and C.**

Risk factors	Response options	Overall responses	Responses of diseased	Responses of non-diseased	Chi-square value P-value (two sided)	Statistical significance (at P-0.05)
Hepatitis history in the family?	Yes	2	1	1	0.0313	Yes
	No	301	24	277		
Dental treatment?	Yes	1	1	0	0.0008	Yes
	No	302	24	278		
Treatment from a local practician?	Yes	106	13	93	0.0625	No
	No	197	12	185		

It is revealed from other studies that the chances of infection significantly rise with the increasing age as the demand of blood transfusions increases with the passage of time (Manisha *et al.*, 2015). Similar findings we observed in our study where a higher prevalence of HVC (14.4%) and HBV (4.4%) infections were observed in 7-12-year age group due to the increase rate of multiple transfusions to meet the demand in case of thalassemia.

According to a study, 206 individuals were tested for anti-HCV through ELISA. 28.15% of the 206 patients were found anti-HCV positive (Boroujerdnia *et al.*, 2009). While in the present study the total population was 303, the standard deviation for the total population was found 3.13 and mean age with 95% Confidence Interval was found 4.80±0.35. Moreover, after ELISA, 5.9% individuals were confirmed for hepatitis C infection which is too low as compared to 28.1% prevalence of hepatitis C found in the study of Boroujerdnia *et al.* (2009).

#### Conclusions and recommendations

The high (8.2%) prevalence of hepatitis B and C in a vulnerable population such as thalassemic patients is an alarming health concern that directly indicates that attention should be paid to ensure pre-screened blood transfusion.

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#### Statement of conflict of interest

The authors declare no conflict of interest.

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