RESEARCH ARTICLE

Respiratory viral infections in bone marrow transplant patients: insights from a tertiary care hospital in Rawalpindi, Pakistan

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Abstract

Objective: To determine the incidence of respiratory viral infections in bone marrow transplant patients.

Method: The prospective, descriptive, cross-sectional study was conducted at a tertiary care hospital in Rawalpindi, Pakistan, from September 2019 to August 2020, and comprised respiratory specimens from recipients of haematopoietic stem cell transplant. The specimens were collected in viral transport medium, and were then taken to the Department of Virology. Multiplex polymerase chain reaction was performed on the specimens to ascertain the incidence and prevalence of respiratory viruses. Data was analysed using SPSS 24.

Results: Of the 85 subjects, 53(62.35%) were males and 32(37.65%) were females. The overall median age was 20.0 years (interquartile range: 11.0-32.0 years). Respiratory viral infections were detected in 31(36.4%) specimens. Among them, human rhinovirus was detected in 12(38.7%) cases, respiratory syncytial virus in 5(16.1%), influenza A/H3 in 4(13%), human parainfluenza virus-1 in 3(9.7%), adenovirus in 2(6.4%), human parainfluenza virus-3 in 1(3.2%), human parainfluenza virus-4 in 1(3.2%) and human metapneumovirus in 1(3.2%) case. There were 2(6.4%) cases of co-infection.

Conclusion: More than one-third recipients of haematopoietic stem cell transplant were found to have respiratory viral infections, highlighting the importance of employing multiplex respiratory polymerase chain reaction in early diagnosis and treatment of such infections.

Key Words: Respiratory viral infections, Bone marrow transplantation, Multiplex respiratory PCR, Diagnosis.

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Introduction

Viruses have a vital role in acute respiratory illnesses in infants and children, leading to hospitalisation.[1] Respiratory viral infections (RVIs) in immunocompetent individuals are mostly self-limiting, but these infections in haematopoietic stem cell transplants (HSCT) and solid organ transplant (SOT) recipients are a significant cause of morbidity and mortality.[2] The commonly involved respiratory viruses (RVs) include human rhinovirus (HRV), respiratory syncytial virus (RSV), influenza virus, human parainfluenza virus (HPIV), human metapneumovirus (HMPV) and adenovirus (AdV).[2,3] Typically, RVIs begin as an upper respiratory tract infections (URTI) and half of the patients may progress to complicated lower respiratory tract infections (LRTI), leading to long-term illness with sustained viral shedding, transplant loss or malfunction.[3]

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HSCT is presently being performed for patients suffering from haematological malignancies, like leukaemia, lymphoma and multiple myeloma, non-malignant conditions, like immune deficiency syndromes, aplastic anaemia, autoimmune disorders and myelodysplastic syndrome, and solid tumours, like breast cancer and sarcomas, who have received radiation or chemotherapy that destroyed the cells within the recipient's bone marrow.[4-6] The HSCT procedure consists of harvesting haematopoietic stem cells from a donor and infusing them to the recipient. The HSCT recipients are at a high risk of viral infections because of their immunocompromised state. Strict infection control measures have to be adopted by transplant institutions to control the spread of RVIs in such recipients. A study at the University of Texas on HSCT recipients revealed that 48% of RVIs were related to nosocomial transmission.[7] A study in Brazil on HSCT recipients demonstrated 17% prevalence of RVIs.[8] A comparable study in Switzerland indicated an incidence of 60% for RVIs in HSCT recipients.[9]

Analysis of existing literature indicates that infection control protocols exhibit variations among institution across different countries, and, unfortunately, are not being widely implemented. Enforcing stringent infection control measures, such as maintaining hand hygiene, adhering to standard contact and droplet precautions,

educating staff about the transmission of RVIs, and consistently monitoring healthcare workers' practices, play a crucial role in preventing RVIs among HSCT patients.[10]

Limited data exists regarding the prevalence of various respiratory infections in Pakistan, both before and after the coronavirus disease-2019 (COVID-19) pandemic. A literature review highlighted various viruses, but specific prevalence rates were not provided.[11] A study focussed on the trends of RVIs in the Punjab province of Pakistan during the post-COVID-19 period, and showed that influenza A virus had a prevalence of 26.4%, followed by RSV 10%, and influenza B 6%.[12]

However, to our knowledge, no study has been conducted in Pakistan on HSCT recipients in this regard. The current study was planned to fill the gap in literature by determining the incidence of RVIs in HSCT recipients.

Materials and Methods

The prospective, descriptive, cross-sectional study was conducted at a tertiary care hospital in Rawalpindi, Pakistan, from September 2019 to August 2020. After receiving approval from the Institutional Ethical Review Committee of the Armed Forces Institute of Pathology (AFIP) Rawalpindi, Pakistan (Approval No: FC-VIR18-15/READ-IRB/22/1076), and considering an RVI prevalence of 17%[8], with a 95% confidence interval and a margin of error 8%, the estimated sample size was calculated to be 85 individuals. A non-probability consecutive sampling technique was employed, and the sample was collected from both the inpatient and outpatient departments. Those included were patients of either gender having a history of HSCT on medical record and exhibiting symptoms suggestive of RVIs, like cough, fever, runny nose, sore throat, difficult breathing and chest discomfort. Those excluded were pre-transplant patients and transplant recipients who had surpassed 120 days post-transplantation. Data was collected after taking informed written consent. Relevant data was obtained on a predesigned form that had two parts. The first part was demographic characteristics of the recipients, such as age, gender and occupation, while the second part included clinical manifestations suggestive of RVIs.

Various specimens, such as nasopharyngeal / throat swabs, nasopharyngeal aspirates and bronchoalveolar lavage, were collected from the HSCT recipients. The samples were meticulously transported while maintaining a cold chain temperature between 2°C and 8 °C to the Department of Virology where the specimens were subjected to analysis using multiplex respiratory polymerase chain reaction (PCR). The extraction process was performed using a deoxyribonucleic acid (DNA) / ribonucleic acid (RNA) extractor employing magnetic beads technology (Systaaq, United States), RNA amplification was carried out using a respiratory pathogens kit (Fast Track Diagnostic, Luxembourg) on a thermal cycler (CFX96, Bio-Rad, US).

Data was analysed using SPSS24. Median and interquartile range (IQR) were calculated for quantitative variables, while frequencies and percentages were computed for qualitative variables. The association of RVIs was determined with primary disease, human leukocyte antigen (HLA) matching and post-transplant immunosuppression. Stratification was done for qualitative variables to see their effect on RVIs, followed by analysis using chi-square test. $P \leq 0.05$ was considered statistically significant.

Results

Of the 85 subjects, 53(62.35%) were males and 32(37.65%) were females. The overall median age was 20.0 years (IQR: 11.0-32.0 years). RVIs were detected in 31(36.4%) specimens. Among them, HRV was detected in 12(38.7%) cases, RSV in 5(16.1%), influenza A/H3 in 4(13%), HPIV-1 in 3(9.7%), AdV in 2(6.4%), HPIV-3 in 1(3.2%), HPIV-4 in 1(3.2%) and HMPV in 1(3.2%) case. There were 2(6.4%) cases of co-infection (Table 1).

The association of RVIs with age, gender and patients'

Table-1: Patient characteristics, and the respiratory viral infection (RVI) isolates.

Characteristics	Data collected
Age (years)	
Median	20(11.0-32.0)
Gender	
Male	53 (62.4%)
Female	32 (37.6%)
Multiplex Respiratory PCR	
Positive	31 (36.5%)
Negative	54 (63.5%)
Respiratory Viral Infections Isolated Type	
Rhino Virus	12 (38.7%)
RSV(A/B)	5 (16.1%)
Influenza A/H3	4 (12.9%)
HPIV-1	3 (9.6%)
Adenovirus	2 (6.4%)
HPIV-3	1 (3.2%)
HPIV-4	1 (3.2%)
HMPV A/B	1 (3.2%)
RV & HPIV-1	1 (3.2%)
Influenza A/H3 & Adenovirus	1 (3.2%)

PCR: Polymerase chain reaction, RSV: Respiratory syncytial virus, HPIV-1L Human parainfluenza virus-1, HPIV-3: Human parainfluenza virus-3, HPIV-4: Human parainfluenza virus-4, HMPV: Human metapneumovirus.

Table-2: Association of respiratory viral infections (RVIs) with age, gender, occupation and education..

Frequency of RVIs by Age				
Age Groups	**Respiratory viral Infections**		**Total**	**p-Value**
	Positive	**Negative**		
01-16 years	10 (33.3%)	20(66.7%)	30	0.851
17-50 Years	20 (37.7%)	33 (62.3%)	53	
51-65 Years	1 (50%)	1 (50%)	2	
Frequency of RVIs by Gender				
Gender				
Male	16 (30.2%)	37 (69.8%)	53	0.095
Female	15 (46.9%)	17 (53.1%)	32	
Frequency of RVIs by Occupation				
Occupation				
Student	18 (37.5%)	30 (62.5%)	48	0.897
Public Servant	5 (38.5%)	8 (61.5%)	13	
Business man	3 (30%)	7 (70%)	10	
House Wife	5 (35.7%)	9 (64.3%)	14	
Frequency of RVIs by Education				
Education				
Below Matric	17 (37.8%)	28 (62.2%)	45	0.016
Matric &above	11 (57.9%)	8 (42.1%)	19	
Bachelor & above	3 (14.3%)	18 (85.7%)	21	

RVI: Respiratory viral infection

Table-3: Association of RVIs with primary disease, HLA matching, post-transplant immunosuppression, post-transplant CMV viraemia and GvHD.

Primary Disease				
	Multiplex Respiratory PCR			
	Positive	**Negative**	**Total**	**p-Value**
Acute myeloid leukaemia	7 (46.6%)	8 (53.4%)	15	0.551
Beta thalassemia major	5 (29.4%)	12 (70.6%)	17	
Aplastic Anaemia	2 (15.4%)	11 (84.6%)	13	
Myelodysplastic Syndrome	8 (57.1%)	6 (42.9%)	14	
Fanconi Anaemia	2 (40%)	3 (60%)	5	
Hodgkin Lymphoma	3 (37.5%)	5 (62.5%)	8	
Chronic myeloid leukemia	0	1 (100%)	1	
Glanzmann's Thrombocytopenia	1(25%)	3 (75%)	4	
HLA Matching				
Full Matched	27 (45%)	33 (55%)	60	0.034
Half Matched	4 (18.1%)	18 (81.9)	22	
No Matched	0	3 (100%)	3	
Post-transplant Immuno-suppression				
Cyclosporine	28 (34.2)	54 (65.8%)	82	0.144
Others	3 (100%)	0	3	

AML: Acute myeloid leukaemia, BTM: Beta thalassemia major, MDS: Myelodysplastic syndrome, CML: Chronic myeloid leukemia.
HLA: Human Leukocyte Antigen

occupation was not significant (p>0.05), while education was significantly associated (Table2).

There was no significant association of RVIs with primary disease (p=0.551) or post-transplant immunosuppression (p=0.144). However, a notable association was identified between RVIs and allogeneic transplant recipients (p=0.034) (Table 3).

Discussion

RVs represent a significant source of infection among immunocompromised patients, often linked to prolonged viral shedding, nosocomial acquisition and heightened mortality rates in HSCT recipients.[13] Over the past decade, extensive research has been conducted in developed nations concerning RVIs complicating stem cell transplants. However, there is dearth of data on RVIs among HSCT recipients originating from developing countries. While RVIs in immunocompetent individuals caused by RSV, AdV, HPIVs, HMPV and HRV are typically mild and self-limiting, in immunocompromised patients, these viruses can result in severe illness and complications, often leading to increased morbidity and mortality.[13-15] The frequency of these viral infections typically mirrors the prevailing epidemiological patterns within the community. However, their clinical course tends to be more severe, especially during the early post-transplant period.[14,15] In the current study, RVIs were detected in 31(36.4%) HSCT recipients. The frequency of RVIs observed was higher than the reported range (3.5% to 29%) documented in a comprehensive review encompassing studies conducted in multiple countries.[16] This significant contrast likely reflects a combination of factors related to the study population, diagnostic methods, seasonality, geographical location, and infection control practices. A study in the Netherlands, involving a cohort of 110 patients, reported that 55(50%) recipients experienced a post-transplant RVI. Among those affected, the majority exhibited solely URTI symptoms, some required oxygen therapy, and 1recipient had to be shifted to ventilator support due to severe bacterial infection. The rest had an uneventful clinical recovery within 7-14 days.[17]

HRV stands as the predominant RVI in humans and has emerged as the most commonly isolated virus within the spectrum of RVIs among HSCT recipients.[18] A prospective surveillance study in the US documented a cumulative incidence of HRV infections amongst HSCT recipients at 22.3%.[19] However, the current findings regarding HRV infection indicated a comparatively higher prevalence, with HRV detected in 12 out of 31 positive cases (38.7%). The significant differences observed are likely attributed to the higher occurrence of rhinovirus infections within the general population, weakened immune system and the use of immunosuppressive medications, which increases the likelihood of HSCT recipients to HRV. HPIVs have gained recognition as a common contributor to

morbidity and mortality among cancer patients, particularly in those who having undergone HSCT or having haematological malignancies due to their compromised immune status. In HSCT recipients, HPIV infection affects around 3-7% of HSCT recipients, and roughly 3-5% of lung transplant recipients. Up to half of HSCT patients with HPIV infection may experience the development of LRTI, accompanied by a mortality rate ranging 12-57%.[20] However, other studies have highlighted that HPIV can lead to complications, such as LRTI, in 20-40% of HSCT recipients, often linked to a mortality rate reaching upto 27%.[20,21] In the current study, HPIV was detected in 5(16.1%) cases. Notably, no mortality was observed among those with HPIV infection in the current study. Regarding RSV, it demonstrates a consistent prevalence year-round, with a higher incidence typically observed between the months of September and April. RSV infection poses a commonly transmissible complication, documented to affect up to 2-17% of HSCT recipients and among SOT recipients. RSV demonstrates its most significant impact during the initial year following transplantation, except for lung transplant recipients, among whom detection rates remain consistent over time following the transplant.[22] The incidence of RSV infection noted in the current study was 16.1%. AdVinfection typically manifests during the first 100 days following transplantation, with occurrence rates ranging 2.5-14% among autologous HSCT recipients, and 5-47% among allogeneic transplant recipients.[23] In the current study, the frequency of AdV infection among HSCT recipients stood at 6.4%. The reported frequency of influenza virus infection by various studies amongst transplant recipients ranged 4-5% among HSCT recipients and 0-13% among SOT recipients.[18] In the current study, influenza A/H3 virus was detected in 13% cases. HMPV is being increasingly identified as a leading cause of RVIs in transplant patients. It usually starts as a URTI, but can escalate to being an LRTI. Alarmingly, HMPV infections have been linked to mortality rates as high as 50%. The majority of studies assessing the prevalence of HMPV infection in HSCT recipients reported estimated frequencies ranging 5-9%.[24] In the current study, HMPV frequency was 3.2%. The notable variation in the reported frequencies of RVIs among HSCT recipients are likely influenced by various factors, including the degree of immunosuppression, the efficacy of monitoring practices, diagnostic methodologies, and the prevalence of RVIs in distinct populations. Furthermore, a trend was observed indicating a possible link between gender and RVIs, with significance levels ranging from p=0.05 to p=0.1. The data suggest that females may be more susceptible to RVIs compared to males, suggesting a gender-based disparity in post-HSCT respiratory infections, which warrants further investigation.

Although the current study documented the incidence of RVIs in HSCT recipients, the results cannot be applied to renal or liver transplant recipients. Additionally, to know the long-term effects of RVIs on HSCT recipients' outcomes is challenging due to long-term follow-up. Addressing these limitations requires a multi-centred approach with larger sample sizes that will validate the current findings.

Despite the limitations, however, the current study was the first of its kind in Pakistan, and was able to set the stage for further research, paving the way for comprehensive approaches aimed at enhancing the care and outcomes of HSCT recipients vulnerable to RVIs in the country.

Conclusion

More than one-third HSCT recipients were found to have RVIs, highlighting the importance of employing multiplex respiratory PCR in the early diagnosis and treatment of such infections.

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Authors' Contribution:
NS: Concept, drafting, data collection and analysis.
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EG: Design, revision and final approval.
RI: Drafting and revision.
AR: Study design, data interpretation, drafting, critical review and final approval.
MN: Drafting, SPSS statistical analysis and revision.