STUDENTS' CORNER
RESEARCH ARTICLE

Hepatitis-associated aplastic anaemia: surveillance of frequency, clinico-haematological features, and demographic distribution at a tertiary care hospital in Pakistan

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Abstract

Objective: We aim to document the frequency of HAAA cases among AA patients presenting at a tertiary care hospital, and to determine the most common agents (viral/drug induced) and Clinico-haematological features among HAAA patients at a tertiary care hospital.

Methods: This study was a retrospective review, conducted at a tertiary care hospital in Karachi, Pakistan.

Results: A total of 21 patients were included in the study. Hepatitis among the HAAA patients was viral in 17 cases, while 4 were idiopathic. All the patients acquired aplastic anaemia within 3-12 months of the Hepatitis episode and most presented with bleeding, bruises and petechiae.

Conclusion: This study indicates and proves that presence and prevalence of this disease in the Pakistani population is quite significant. Unlike the rest of the world, HAAA in Pakistan is not entirely of unknown aetiology, most of the cases can be associated with one of the Hepatitis viruses.

Keywords: Hepatitis, Aplastic Anaemia, Hepatitis Associated Aplastic Anaemia, IST, HSCT.

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Introduction

Hepatitis-associated aplastic anaemia (HAAA) is a well-recognised yet distinct variant of acquired aplastic anaemia (AA) in which an acute episode of hepatitis precedes marrow failure and pancytopenia.[1,2] It was first described in two case histories in 1955[3] and by 1975 more than 200 cases had been reported.[4] HAAA is not considered relative to age, sex, and severity of hepatitis; predominantly it has been noticed in children, adolescent boys and young men who present with severe pancytopenia two to three months after an episode of acute hepatitis.[5] There is no proven correlation with blood transfusions, drugs, or toxins, and most patients have been seronegative for hepatitis A, B, and C.[6] Hepatitis related with aplastic anaemia may be acute and chronic, mild and transient, self-limiting and fulminant and the development of AA is always fatal if not treated in time.[7] The aetiology of HAAA has been linked to various factors including hepatitis as well as non-hepatitis viruses, autoimmune responses, liver transplantation, radiation, and drugs used to curb viral replication.[5] Clinical features relating to aplastic anaemia following hepatitis include pallor, multiple skin bleeding, lymphocytopenia, hypogammaglobulinaemia,[8] low CD4+/CD8 ratio and increased number of cytotoxic cells with neutropenia and fever.[9] The standard therapy employed for the treatment of HAAA is allogenic bone marrow (BM) transplantation from an HLA matched siblings, while immunosuppressive therapy (IST) has also been successful in patients not eligible for transplantation.

Although HAAA is a rare disease, and its prevalence is very low; numerous studies have been conducted across the globe to document the reported cases. HAAA has been documented in 2% to 5%[10] of the AA cases in the West and 4% to 10%[11] in Far East. An epidemiological study was conducted in Europe and 214 cases of HAAA were reported between 1990 to 2007 from 29 countries; these were 5.4% of the total 3,916 cases of aplastic anaemia documented and were evenly distributed over time and geographical areas in Europe. Studies in the Asian region show that despite being a hepatitis prevalent area the prevalence of HAAA in Asia is not so high when compared to Europe.[12] In Pakistan, no such epidemiological studies have been conducted to document the HAAA cases on a provincial or national level. However, cases of HAAA have been documented separately by institutions as case reports. One such case was a Hepatitis G virus-associated case of HAAA from Lahore,[13] and another one with unidentified virus was reported from Military Hospital,

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Case reports of HAAA from different institutes of Pakistan indicate that presence and prevalence of this disease in Pakistani population is quite significant, yet no studies have been conducted to prove this through evidence-based literature. Due to high mortality rate of this disease, it is extremely important that proper studies be conducted in Pakistan. This will not only help in recording the prevalence of HAAA but will find out the agents present in Pakistan among HAAA cases so that proper interventions can be made in time. Hence, the aim of this study, the first of its kind in Pakistan, is to document the frequency of HAAA cases among the AA cases, identify the Clinicohaematological features of the disease, and investigate the role of different types of viruses and drugs among HAAA cases in patients presenting to a tertiary care hospital in Pakistan.

Methods

This was a cross sectional study with retrospective review of patient files, conducted between May 2021 and August 2021 at The Aga Khan University Hospital, a tertiary care hospital in Karachi, Pakistan, over the course of fifteen years from January 1, 2004, to December 31, 2018. The study was approved by the Ethical Review Committee (ERC ID: 2021-5942-17067) of the institute on March 28, 2021. The data was obtained from hospital files, which patients consented to be used for research purposes upon admission to the hospital. It included files of patients of all ages diagnosed with Hepatitis-Associated Aplastic Anaemia (HAAA) and presented at the Department of Haematology and/or the Department of Family Medicine of the study hospital, Karachi, Pakistan, in the past 15 years. Patients with incomplete record files were excluded from the study. Demographic and relevant clinical information (e.g., diagnosis of HAAA, presenting features, haematological (e.g., LFTS, CBC & Bone Marrow tests) and serology tests, management, treatment, and current patient status) was obtained from patients' medical records at Health Management Information System (HIMS) which were then tabulated and compared within patients and between patients.

To determine the most common aetiological agents (viral/drug induced) and Clinicohaematological features of HAAA patients, a minimum sample of 2,290 aplastic anaemia (AA) patients was required which was calculated via OpenEpi software[15] at a 5% significance level. A total of 29 patients were identified as suffering from HAAA out of 3,530 AA patients presenting to the hospital but 8 files had to be excluded due to incomplete data.

Data collected from patient files was imported to SPSS version 26 where a statistical analysis was performed. Continuous data was reported as median while categorical was reported as frequencies and proportions. Since the data was small it was difficult to perform any statistical tests among the variables to see any association between them.

Results

A total of 21 (0.60%) Hepatitis-Associated Aplastic Anaemia (HAAA) patients out of 3,530 AA patients who fulfilled the inclusion criteria of the study were included. The variables recorded during the course of the study were divided into categorical and continuous variables. Table-1 shows the frequency values of demographic variables. The age of patients ranged from 12 years to 63 years while the median presenting age was 37.0 years (IQR:29.5). Predominantly, the patients came from Karachi {n=10 (48%)}, while 7 (33.3%) patients were from other cities of the country, mostly from central and southern Punjab. There was no patient from northern provinces mainly because the hospital is in far south of the country. In addition to this, one patient was referred from Afghanistan.

Important aetiological variables and clinic-haematological features of the presenting patients are given in Table-2. Seventeen (81%) patients had a viral aetiology for Hepatitis and the remaining four (19%) were idiopathic cases. According to the existing literature, most of HAAA cases developed aplastic anaemia (AA) within a couple of months, the same pattern was seen in this study as 10 (48%) patients developed AA within three months of an episode of Hepatitis, 3 (14.3%) patients developed it within six months and 7 (33.3%) within a year. The treatment options offered at the hospital are haematopoietic stem cell transplant (HSCT) or immunosuppressive therapy (IST). The patients are either offered one of these or both depending upon the severity of the case. Among the patients included in this study, 13 (61.9%) patients were treated with IST, 1 (4.7%) with HSCT and 3 (14.3%) were treated with both. Among the patients on IST, 9 (69.2%) are still alive after completing their

Table-1: Frequency table showing distribution of demographic variables.

Characteristics	N (%)
Gender	
Male	14 (66.67)
Female	7 (33.3)
Residence	
Karachi	10 (48.0)
Others	7 (33.3)
Afghanistan	1(4.80)
Missing	3

Table-2: Frequency table showing distribution of aetiological variables and clinicohaematological features variables.

Characteristics	N (%)
Family History of any blood disorder	
Yes	0 (0.0)
No	21 (100.0)
Chromosomal breaks	
Yes	18 (86.0)
No	3 (14.0)
Type of hepatitis	
Viral	17(81.0)
Idiopathic	4 (19.0)
Type of virus	
Hepatitis A	2 (12.0)
Hepatitis B and E	3 (17.0)
Hepatitis C	12 (71.0)
Duration between attack of Hepatitis & HAAA	
Within 3 months	10 (48.0)
Within a year	7 (33.3)
Within 6 months	3 (14.3)
Missing	1
Symptoms	
Bleeding	
Yes	13 (62.0)
No	8 (38.0)
Bruises	
Yes	16 (76.0)
No	5 (24.0)
Petechiae	
Yes	15 (71.4)
No	6 (28.6)
RISK FACTORS	
Smoking	
Yes	0 (0.0)
No	21 (100.0)
Alcohol	
Yes	0 (0.00)
No	21 (100.0)
Recreational Drugs	
Yes	0 (0.0)
No	21 (100.0)
Treatment	
Immunosuppressive therapy (IST)	13 (61.9)
Hematopoietic stem cell transplant (HSCT)	1 (4.7)
IST/HSCT or both	3 (14.3)
Missing	4
Patient status	
Alive	12 (57.1)
Expired	9 (42.9)

treatment course, while 4 (30.8%) have expired. Of the patients who received both therapies, only 1(33.3%) is still alive, while 2 (66.6%) have expired. The patient who underwent HSCT only is still alive. Other important features recorded in Table-2 are family history, chromosomal breaks, type of viruses, symptoms and risk factors.

Table-3: Frequency table showing distribution of continuous variables.

Characteristics	Median	Interquartile Range (IQR)	Normal Ranges
Age (in years)	37	29.5	-
Bone Marrow Findings (at time of diagnosis of HAAA)			
Cellularity (%)	15.0	20.0	38.4-50.7
Hb (g/dl)	8.80	2.40	12.3-16.6
TLC	2.35	2.60	4.8-11.3
Plt (x 109/L)	18.0	26.0	154-433
ANC (x 109/L)	0.50	0.95	0.50-1.00
Retic (%)	0.60	2.70	0.5-2.50
LFTs			
ALT (IU/L)	134.0	154.0	7.0-55.0
AST (IU/L)	82.0	101.0	8.0-48.0
GGT (IU/L)	121.0	144.0	8.0-61.0
Total bilirubin (mg/dl)	1.20	1.30	0.1-1.2
Albumin (g/L)	3.40	1.40	3.50-5.0
Transfused products			
Packed RBCs per month transfused	5	5	-
Platelet transfusion per month	33	42	-

Abbreviations: haemoglobin levels (Hb), total leukocyte count (TLC), absolute neutrophil count (ANC), platelets count (Plt), and reticulocyte percentage (Retic). Liver Function Tests (LFTs), Alanine transaminase (ALT), Aspartate transaminase (AST), Gamma-glutamyl transferase (GGT).

Various tests were conducted for all patients for diagnostic and evaluation purposes during the course of their disease. The findings were reported as median along with their interquartile range and are summarised in Table-3. The bone marrow findings for all 21 patients showed a median cellularity of 15.0% (IQR: 20.0). Other variables tested to diagnose aplastic anaemia were haemoglobin levels (Hb), total leukocyte count (TLC), absolute neutrophil count (ANC), platelets count (Plt), and reticulocyte percentage (Retic). All the parameters tested were below their normal values. All the patients were given blood transfusion during their treatment in the hospital. The median transfusion values for the patients were 5 (IQR:5) packed red blood cells and 33 (IQR:42) apheresis platelet concentrates per month.

Liver Function Tests (LFTs) were also conducted to check for hepatitis when the patients were admitted in the hospital. The levels of aminotransferases were high for all patients but were not as high as they should be during hepatitis. Alanine transaminase (ALT) for all 21 patients had a median of 134.0 IU/L(IQR:154) and Aspartate transaminase (AST) had a median of 82.0 IU/L (IQR:101). Other parameters checked among LFTs were Gamma-glutamyl transferase (GGT), Total Bilirubin and Albumin levels (Table-3).

Discussion

Data on HAAA in Pakistan is almost non-existent and is not enough to calculate the prevalence of the disease. One study has been conducted in a tertiary care hospital about prevalence of aplastic anaemia (AA) in which 34 patients were reported to have developed HAAA over a span of 15 years. Similarly, in our study, which was conducted in one of the largest tertiary care centres of the country, 21 patients were noted to have HAAA over an analogous span of time.[15] The prevalence calculated was 6.0 HAAA patients among every 1,000 AA patients presenting to the hospital. HAAA is not considered relative to age according to the literature and in the current study also, it could not be associated to any specific age group; the age of patients ranged from 12 years to 63 years.[5] Parallel to the existing literature, the results of this study also show a greater prevalence of HAAA among males.[16,17] The development of AA is reported to occur between an interval of 2 to 10 months after a hepatitis attack. Data from the current study indicates the same as all patients included developed AA within 3 to 12 months after a hepatitis episode.[5]

The aetiology of hepatitis in HAAA patients is mostly unknown but isolated studies from different parts of the world associate it with hepatitis viruses, Parvovirus B19, HHV-6, and transfusion-related viruses.[18,19] In the current study, different tests were performed, including PCR and antibody tests, to evaluate the presence of infectious diseases and most of the patients included were infected with hepatitis virus — 2 patients had Hepatitis A infection, 3 had Hepatitis B & E infection, while 12 had Hepatitis C infection. Some studies suggest that the presence of an infectious agent during the course of AA may be due to blood transfusions and not because of the preceding hepatitis.[20] This may or may not be true for this study, as although all the patients presented with a preceding Hepatitis infection, infectious agents were detected in some patients during the course of aplastic anaemia and the study showed a median transfused value of 5 packed red blood cells and 33 platelets per month to all the patients. Symptoms of the patients presenting with HAAA have been reported as bleeding, petechiae and easy bruising. All these symptoms were observed in most of the patients of this study.

Currently, the treatment options available for HAAA all over the world are haematopoietic stem cell transplant (HSCT) if an HLA donor is available, if not then immunosuppressive therapy (IST) is offered. However, new approaches involve the use of glucocorticoids to increase the effect of IST and administration of thrombopoietin agonists to treat aplastic anaemia.[21] HAAA has a better response to HSCT than IST but in Pakistani setting it is difficult to perform HSCT due to financial constraints and non-availability of HSCT in public sector hospitals. Hence, in the present study it was observed that 13 patients received IST treatment augmented with steroid therapy (Table-2), out of which 9 responded to it and are alive till date while the remaining 4 did not respond to it and expired. Three patients were first treated with IST but later they opted for transplant; however, 2 of them expired even after the transplant.

The laboratory investigations for HAAA include bone marrow findings and liver function tests (LFTs) for diagnostic purposes. Current studies show that in order to diagnose hepatitis, LFTs which include ALT, AST, and GGT should on average be 2384.8 U/L, 2309.4U/L, and 204U/L, respectively.[22] However, the current study shows the LFTs level to be higher than the normal values (Table-3) but not as high as given in the existing literature; it was because a tertiary care centre in Pakistan is approached by patients as a last resort and they arrive at the hospital with partially ongoing treatment for hepatitis or have completed their treatment for hepatitis, and that is why when their LFTs were tested they were lower than expected. The findings in this study, which included Haemoglobin, Platelets, Total leukocyte count, etc. for all patients were lower than normal values and were consistent with findings of another study conducted in Pakistan.[15]

Limitations

Since this study was only conducted in one tertiary care centre, a full nationwide demographic representation of the HAAA patients was not possible; hence, no proper prevalence of the disease could be calculated. This study can act as a benchmark for future studies by providing prevalence from the city of Karachi which can help a nationwide calculation of the disease spread.

In addition to this, patients in Pakistan generally present to a tertiary care hospital very late after getting some form of treatment from the primary and secondary care centres, hence they are either partially or completely treated for hepatitis and come to a tertiary care centre for treatment of aplastic anaemia. In addition to that, most of the patients also don't keep the records of their previous treatment, hence it becomes difficult to follow their laboratory values for LFTs and form an association of hepatitis and aplastic anaemia.

Conclusion

This study indicates and proves that the presence and prevalence of this disease in Pakistani population is quite significant. Unlike the rest of the world, HAAA in Pakistan

is not entirely of unknown aetiology; most of the cases can be associated with one of the hepatitis viruses. This may be due to high prevalence of hepatitis in the south Asian region. Better understanding of this disease through studies like these will help in early diagnosis and screening of hepatitis patients for HAAA. However, this data is only from one tertiary care centre of Pakistan. More studies should be conducted on a national level to further understand the disease and this study is the first step towards this long-term goal.

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