INTRODUCTION
Aplastic Anemia (AA), a type of bone marrow failure syndrome, is an uncommon hematological disorder with immune pathophysiology. It is described as diminished or absent hematopoietic precursors of marrow leading to peripheral blood pancytopenia with increased fat spaces in bone marrow in the absence of dysplasia, infiltration and fibrosis.1 The most severely affected patients have neutrophil counts of less than 0.2 x 10^9/L, platelet count less than 20 x 10^9/L and reticulocyte counts of less than 20 x 10^9/L along with marrow cellularity of less than 25%. The disorder may be acquired or genetic. Laboratory and clinical observation have implicated an immunologic pathophysiology particularly for idiopathic variety.2

Causes of acquired AA include toxins (benzene, pesticides and other chemicals), drugs causing marrow aplasia, (as idiosyncratic complication), chemicals and pregnancy.3-7 It is also associated with viruses (seronegative non-A, non-B, non-C and non-G hepatitis and occasionally hepatitis A).8 Five to ten percent of AA follow an episode of seronegative hepatitis in which there is stimulation of immune system leading to T-cell activation, cytokine production and HLA association.8 Acquired AA is an uncommon diagnosis in Western Europe and North America. On the other hand, large number of cases have been reported from Japan, Korea, Thailand, China and Southeast Asia.9-13 It is estimated that AA is at least 4 to 5 fold more common in the East. The peak age of presentation is 15 - 25 years with equal male to female ratio with no difference of distribution in gender.

Recent literature has described an immunologic component resulting in suppression of hematopoiesis hence, implementing a role of immunosuppressive therapy (IST).1 Involvement of the lymphocytes (helper T-cells) has been suggested due to the over expression of class-II histocompatibility antigen HLA DR2 in white patients with aplastic anemia.14 More specific HLA haplotype has been linked to the disorder in Japanese patients. Some HLA antigens may be much more common in subgroup of patients with aplastic anemia e.g. those who respond to Cyclosporine or those who have post-hepatitis marrow failure.15

ORIGINAL ARTICLE

Epidemiologic and HLA Antigen Profile in Patients with Aplastic Anemia
Mehwesh Taj, Tahir Sultan Shamsi, Saqib Hussain Ansari, Tasneem Farzana, Arshi Nazi, Muhammad Nadeem, Rizwan Nabi Qureshi, Kashif Sheikh and Jawwad Hasan Kazmi

ABSTRACT
Objective: To analyze patients suffering from aplastic anemia (AA, peripheral pancytopenia and hypocellular bone marrow in the absence of dysplasia, infiltration and fibrosis) for documenting patient's baseline characteristics and association with various human leucocyte antigens.

Study Design: An observational, cross-sectional study.

Place and Duration of Study: The National Institute of Blood Disease (NIBD), Karachi, from March 2003 to August 2008.

Methodology: All consecutive patients with confirmed diagnosis of AA were evaluated. Data included the baseline characteristics, complete blood counts (CBC), bone marrow biopsy findings, severity of disease, exposure to drugs or chemicals, viral serology and their HLA expression. The data was analyzed on SPSS programme and frequencies were documented.

Results: Among 318 patients, there were 236 (74.21%) males and 82 (25.78%) females. Median age was 16 and 70% belonged to urban population. Drug exposure could be established in 23 (7.23%) of cases, while 4 (1.25%) were HBV surface antigen positive and 7 (2.2%) were HCV antibodies positive. In all, 73 (22.9%) had very severe AA, 195 (61.32%) had severe AA while 50 (15.7%) cases had non-severe AA. HLA B5 (52) showed high expression in 83 patients (26%) in comparison to 5.9% reported in healthy population.

Conclusion: AA was found to affect young adult males living in urban areas. HLA B5 (52) showed higher expression in patients with aplastic anemia.

Key Words: Aplastic anemia. Idiopathic. HLA antigen B5 (52).

INTRODUCTION
Aplastic Anemia (AA), a type of bone marrow failure syndromes, is an uncommon hematological disorder with immune pathophysiology. It is described as diminished or absent hematopoietic precursors of marrow leading to peripheral blood pancytopenia with increased fat spaces in bone marrow in the absence of bone marrow fibrosis, dysplasia or infiltration.1 The most severely affected patients have neutrophil counts of less than 0.2 x 10^9/L, platelet count less than 20 x 10^9/L and reticulocyte counts of less than 20 x 10^9/L along with marrow cellularity of less than 25%. The disorder may be acquired or genetic. Laboratory and clinical observation have implicated an immunologic pathophysiology particularly for idiopathic variety.2

Causes of acquired AA include toxins (benzene, pesticides and other chemicals), drugs causing marrow aplasia, (as idiosyncratic complication), chemicals and pregnancy.3-7 It is also associated with viruses (seronegative non-A, non-B, non-C and non-G hepatitis and occasionally hepatitis A).8 Five to ten percent of AA follow an episode of seronegative hepatitis in which there is stimulation of immune system leading to T-cell activation, cytokine production and HLA association.8

Acquired AA is an uncommon diagnosis in Western Europe and North America. On the other hand, large number of cases have been reported from Japan, Korea, Thailand, China and Southeast Asia.9-13 It is estimated that AA is at least 4 to 5 fold more common in the East. The peak age of presentation is 15 - 25 years with equal male to female ratio with no difference of distribution in gender.

Recent literature has described an immunologic component resulting in suppression of hematopoiesis hence, implementing a role of immunosuppressive therapy (IST).1 Involvement of the lymphocytes (helper T-cells) has been suggested due to the over expression of class-II histocompatibility antigen HLA DR2 in white patients with aplastic anemia.14 More specific HLA haplotype has been linked to the disorder in Japanese patients. Some HLA antigens may be much more common in subgroup of patients with aplastic anemia e.g. those who respond to Cyclosporine or those who have post-hepatitis marrow failure.15
Previously, a couple of case series were reported from Pakistan. Here the epidemiological report and HLA profile of aplastic anemia at a specialized hematology centre in Pakistan reflects the disease pattern and severity in the last 7 years.

The aim of this study was to analyze patients suffering from aplastic anemia (AA, peripheral pancytopenia and hypocellular bone marrow in the absence of dysplasia, infiltration and fibrosis) for documenting patients' baseline characteristics and association with various human leucocyte antigens.

**METHODOLOGY**

It was an observational cross-sectional study to document the clinical variables and hematological parameters of AA. Data included all confirmed cases of AA who had peripheral pancytopenia with hypocellular marrow, presented from March 2003 to August 2008. Data was collected from NIBD and BMT, Karachi, Pakistan, which is the pioneer of Bone Marrow Transplantation in Pakistan and is a tertiary care centre for the treatment of hematological disorders. Patients who received chemotherapy or radiation and confirmed cases of Fanconi’s anemia or paroxysmal nocturnal hemoglobinuria were excluded from the study. Fanconi’s anemia was excluded by cytogenetic analysis for chromosomal breaks and CD55 and CD59 evaluation by ID-PNH from untransfused samples during their disease work up.

The pre-determined questionnaire included a detailed personal biodata, date of diagnosis, and type of treatment offered. All patients were evaluated clinically including general physical examination and systemic examination. Personal biodata included the age, at the time of diagnosis, gender, ethnicity, and the area of residence. The proforma also included history of prolonged use of any type of drugs for at least 6 months prior to developing pancytopenia. The hepatitis virology status (hepatitis B virus surface antigen and hepatitis C virus serology) as well as Human Immunodeficiency Virus (HIV) status was also recorded. HIV status was established to determine the possible cause of hypoplastic bone marrow. The description of HLA antigen expression of patients as a part of work up for bone marrow transplantation was also recorded from the same clinical records.

The reported data was gathered with the Institutional Review Board (IRB) approval from Bioethics Committee of NIBD, in compliance with the principles of the Declaration of Helsinki and then anonymized. Statistical analysis (rates, proportions and median) were carried out using Statistical Package for Social Sciences (SPSS) version 17. Descriptive statistical analysis was performed by calculating frequency and percentages for qualitative variables. Quantitative variables were expressed as the mean ± standard deviation.

### RESULTS

A total of 318 patients were identified as a case of AA. Mean age of patients at diagnosis was 20.54 ± 14.731 years ranging from 2 - 81 years, while the median was 16 years. Out of all, 237 (74.52%) were male while 81 (25.5%) were female. Considering 15 years as the age limit, 133 patients (41.82%) were in pediatric age group. Two hundred and twenty three patients (70.12%) were urban residents and rest of 95 patients belonged to rural areas as shown in Table I. There were patients belonging to different ethnic groups and 165 were Urdu speaking (51.8%), 38 were Sindhi (11.9%), being two largest ethnic groups residing in Sindh province. Among remainders, 24 were Punjabi (7.5%), 33 were Pathan (10.3%) while 58 patients (18.2%) belonged to other ethnic groups. This segregation is a rough reflection of ethnic groups affected by AA, sent for tertiary care hospital referral (Table I).

Fifty cases (15.7%) were diagnosed as non-severe AA, 195 patients (61.3%) were labelled as severe AA while 73 patients (22.9%) had very severe AA. Although cases were diagnosed throughout the year, there was a slightly increased number of cases in the month of September and October.

Idiopathic aplastic anemia was diagnosed in 294 patients (92.4%) as no absolute cause could be determined from their detailed medical history, systemic examination or relevant investigations. All these patients had normal bone marrow cytogenetics. Total 23 patients (7.23%) had exposure to medications: Indomethacin (n=6), anti-epileptics (n=4), Sulphonamides (n=5), Ticlopidine (n=3) and 5 patients had exposure to insecticides and other benzene based solvents, 4 patients (1.25%) were found to be hepatitis B surface antigen carriers.

<table>
<thead>
<tr>
<th>Ethnic group</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urdu speaking</td>
<td>165 (51.88%)</td>
</tr>
<tr>
<td>Sindhi</td>
<td>38 (11.9%)</td>
</tr>
<tr>
<td>Punjabi</td>
<td>24 (7.54%)</td>
</tr>
<tr>
<td>Pathan</td>
<td>33 (10.3%)</td>
</tr>
<tr>
<td>Others</td>
<td>58 (18.2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Drug exposure</th>
<th>Number of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs</td>
<td>23 (7.23%)</td>
</tr>
<tr>
<td>Viral hepatitis</td>
<td></td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>4 (1.25%)</td>
</tr>
<tr>
<td>Hepatitis C</td>
<td>7 (2.2%)</td>
</tr>
<tr>
<td>Idiopathic</td>
<td>294 (92.4%)</td>
</tr>
</tbody>
</table>

Table I: Detailed characteristics of the 318 patients with aplastic anemia.
antigen positive and 7 patients (2.2%) were anti HCV positive but the exact time of seropositivity was not known. Hence, it was merely an exposure and direct effect of hepatitis on pancytopenia could not be established.

Data of HLA typing (class-I and II HLA antigens was available for 82 cases was also analyzed (Figure 1). The highest antigen frequency of HLA noted in AA patients was that of HLA B5 (B52), which was seen in 27 out of 82 cases (32.92%) while second highest antigen seen was HLA B7, reported in 51 cases (16%, Figure 1).

**DISCUSSION**

This is a large case series of aplastic anemia patients from Karachi, Pakistan. Several studies supported a higher rate of AA in the Eastern hemisphere than in the West. The reported incidence in the Western countries including Canada, Australia, Spain, France and elsewhere in Europe is 1 - 2 per million populations per year, hence regarded as a rare disorder. The annual incidence in the three cities of Thailand which were rigorously studied and showed incidence of aplastic anemia more than 3 per million populations, considering the environmental factors and poverty as the most common incidents. The incidence was 3.9 cases per million persons in Bangkok, 3.0 per million in Songkla, and 5.0 per million in Khonkan. In Turkey, 73 confirmed cases were included in a 3 years survey. The median age was 30 years and females were younger at diagnosis. Severe disease was confirmed in 89% of patients. The occurrence was found to be 1.96/million population of children/year in the four northern districts of West Bengal in India. Malaysian data reported 39 proven cases of AA between January 1999 to March 1996. The median age was 23 years and the reported incidence was 4.8 per million per year.

A report from Rawalpindi (Pakistan) followed 31 confirmed cases of AA in the year 1997. There is another case series of 144 patients reported from Karachi in 2001. They reported 144 cases in 7.5 years with median age of 17 years. Idiopathic AA was labelled in 74.3% of cases.

This data was collected from a tertiary care centre for hematology and bone marrow transplant centre of Pakistan. It is the largest series from a hospital-based data, with AA ever reported till now in literature but still the study might not determine the true incidence of AA in this region. Some inference could be made from the fact that 318 patients presented in 6 years at a single centre being referred from different areas of the country. These figures might reflect a comparatively higher incidence of AA in this part of world.

In this study, aplastic anemia was found to affect predominantly young adult males living in urban population. Previous studies from Pakistan reported a similar finding, while data from Thailand exhibited slight female preponderance in few areas. The higher number of male patients in this study might indicate their social dominance and easy accessibility to health facilities. While on the other hand, culturally they may be more exposed to various environmental factors.

Patients included in the study were between 2 - 81 years of age. Majority of patients were teenagers and belonged to 7 - 22 years age group. One peak was noted around the age of 12 years and the second at 22 years of age, nearly same note in other studies (Figure 1). Median age was 16 years in our series. A local case series reported from Karachi in 2001, evaluated 144 cases in 7.5 years with median age of 17 years. Aplastic anemia reported from other parts of the world observed different median age e.g. 26 years noted from Bangkok (43 from Khonkean and 57 Songkla), 21 from Spain and from Japan and 23 years from Malaysia. The data also shows that mostly patients belonged to urban population and majority of them were Urdu speaking while next common populations were Sindhi and Pathan. This demography basically reflects the population structure of the Metropolitan city of Karachi. Most cases in data were of idiopathic variety well supported from a Japanese study while as well as from a regional study of Pakistan. Small number of patients developed drug induced or post-hepatitis AA, 10% reported in Western studies, contrary to the eastern countries.

The data of HLA typing of 82 patients was available among 318 patients (Figure 1). The study revealed that some HLA antigens were more frequently found in patients with AA as compared to normal healthy individuals. The frequency of HLA B5 (B52) antigen was found to be 27% in patients with AA; however, it is reported as only 5.9% in the normal healthy Pakistani population. Similarly, frequency of HLA B7 antigen was noted to be 16% patients of AA while it is reported 7.9% in normal healthy Pakistani population. A local study...
mentioned the frequency of class-I and class-II allele in genetic susceptibility of AA. DRB1*15 emerged as a susceptible allele and DRB1*03 as a protective allele in Pakistani AA patients and control samples. No significant difference was found in allele frequencies of other HLA class I and HLA class-II alleles for both patients and controls.25 Japanese data debated upon the significance of HLA class-I alleles in bone marrow failure syndromes and has reported over expression of HLA DR-2 antigen in AA.14,15 Studies have shown that HLA-DRB1*1501 allele and DRB1*1501-DQA1*0102-DQB1*0602 haplotype had a better response to CsA treatment. However, DRB1*04 alleles had a worse response to CsA and a tendency is associated with poor prognosis.25

Further studies are needed to confirm these observations and its association with the disease severity and finally response to treatment. This data is insufficient to document the epidemiology as it was not a population based study. There are a number of associations with the etiology of this life threatening disorder but due to vast number of idiopathic cases we still need to know the other environmental factors, drug exposure and genetic susceptibility if present. We need to explore the reason why it is more common in the East. The difference of HLA antigens in these patients might have made them susceptible to the seasonal or viral insults or there might be some unexplored genetic reasons contributing in our part of world for such a frequent presentation of a rare disorder.

CONCLUSION
AA was found to affect young adult males living in urban areas. HLA B5 (52) showed higher expression in patients with aplastic anemia.

REFERENCES