References

Case Report
Transfusion associated graft versus host disease
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Abstract
This case report describes an immunocompetent lady who developed transfusion associated graft versus host disease following transfusion from a close relative. The diagnosis was established by HLA typing and STR analysis of the patient and her family.

Introduction
Transfusion associated graft versus host disease (TAGvHD) is a dreadful and one of the most feared complications of blood transfusion. It can occur following transfusion of red cells, platelets and granulocytes. Like GvHD occurring after bone marrow transplantation, TAGvHD is also characterized by fever, skin rash, diarrhoea and hepatitis. However, it is remarkably different from bone marrow transplant associated GvHD.

The diagnosis of TA-GvHD is often delayed due to lack of awareness and seemingly non-specific manifestations. The rarity of this syndrome prompted us to share our experience of the diagnosis of a lady with this dreadful albeit rare complication, precipitated by an unfortunate combination of preventable local circumstances.

Case Report
A 25 years old lady was admitted in Armed Forces Bone Marrow Transplant Centre (AFBMTCT), Rawalpindi, Pakistan, with a two month history of recurrent fever with jaundice and skin lesions for one month. She gave birth to a baby girl on 29th March 2007, following which she was transfused one unit of whole blood, the donor being her real sister, in Sargodha. Fifteen days after delivery she developed high grade fever and her blood counts dropped (Table). In the meantime, she also developed jaundice. She was referred to a hospital in Lahore where her bone marrow aspiration and trephine biopsies were reported as “Not Diagnostic” and AFB and blood culture were reported as negative. After about two weeks of initial transfusion, she had developed generalized exfoliative skin rash all over her body. After a month of treatment, she was taken home to Faisalabad by her relatives. During her 10 days stay at home, her blood counts were done regularly. On 5th of June, her platelets suddenly dropped to 5000/ul. She was referred to AFBMTCT. At the time of admission in AFBMTCT, she had very low blood counts; bilirubin was 4.62 mg/dl and ALP 1050 U/l. She was admitted to be investigated for pancytopenia and was given adequate treatment. Her initial investigations revealed Aspergillus flavus and MRSA in nasal swabs. USG and CT scan abdomen showed space occupying lesion in spleen. Red blood cells were not deficient for CD59, Coomb's test and G6PD screening were negative and FDP were <250 mg/ml. Keeping in view her fever and rash, she was referred to Armed Forces Institute of Pathology (AFIP) for investigations regarding autoimmune disease, where after a review, TAGvHD was suggested to be a possible cause of her pancytopenia and deranged LFTs. Specimens were collected to carry out tissue typing and short tandem repeat (STR) analysis of the patient, her sister and her brother. Buccal swab was obtained as the source of pretransfusion DNA sample of the patient. Later laboratory investigations for suspected autoimmune disease showed ANA, anti dsDNA and anti ENA antibodies to be negative.

Table. Serial Blood Counts of the Patient.

<table>
<thead>
<tr>
<th></th>
<th>15th April*</th>
<th>1st June</th>
<th>5th June</th>
<th>7th June**</th>
<th>22nd June</th>
<th>26th June</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>8.0</td>
<td>_</td>
<td>_</td>
<td>7.9</td>
<td>9.2</td>
<td>9.5</td>
</tr>
<tr>
<td>WBCs (cells/ul)</td>
<td>1,000</td>
<td>1,500</td>
<td>1,300</td>
<td>2,400</td>
<td>2,200</td>
<td>3,000</td>
</tr>
<tr>
<td>Platelets (cells/ul)</td>
<td>15,000</td>
<td>20,000</td>
<td>5,000</td>
<td>15,000</td>
<td>21,000</td>
<td>28,000</td>
</tr>
</tbody>
</table>

* 1 unit whole blood transfused
** 2 units RCC and 6 units platelets transfused
but complement levels were reduced (C3 0.7 g/l and C4 0.1 g/l). Skin biopsy was consistent with grade III GvHD.

On the 16th day of admission, her condition worsened. Despite full supportive care, she continued to have swinging pyrexia, worsening hepatitis and bone marrow failure, and she expired on 20th day of admission.

**HLA typing and chimerism**

During the course of her admission, HLA typing of the patient, her brother and her sister was performed, to investigate the donor homozygosity and to evaluate her other siblings for a possible bone marrow transplant. Blood samples were taken from the patient, her brother and sister. For pre-transfusion DNA sample of patient, buccal mucosa cells were used for DNA extraction. Tissue typing tests were performed by SSOP using INNO-LiPA, Innogenetics, Belgium, which showed that donor was homozygous for A*26, B*08 and DRB1*03 while patient was homozygous for B*08 and DRB1*03 only, and her HLA-A typing was A*26, A*33. These results revealed that homozygosity for A*26 in her sister's results was the most probable cause of TAGvHD.

STR analysis of patient, her sister and her brother was carried out using STR markers, D21S11, D3S1358, D5S818, D8S1179, FGA and ThO1. The results confirmed a mixed chimerism of 90% recipient and 10% donor origin (Figure).

**Discussion**

Barnes and Loutit first described GvHD, in mice that were given allogenic spleen cells after irradiation developed fatal symptoms. TAGvHD, in humans, is a fatal immunological transfusion complication, caused by engraftment of immunocompetent donor lymphocytes in a susceptible host. Mature donor T cells recognize alloantigens in recipients and become activated, leading to cytokine production and lymphocyte proliferation. Normally, recipient's lymphocytes are able to reject foreign HLA and prevent the development of donor anti host immune response. Two factors may allow such a response to develop. Firstly, sharing of HLA haplotypes between donor and the recipient. The incidence and severity of GVHD increase with increasing disparity in the HLA type between donor and recipient. This is likely when blood products are obtained from close relatives or in a population with restricted pool of HLA haplotypes, as observed in Japanese population. Second factor is defective recipient cell-mediated immunity, inherited or acquired. GVHD has not been reported following infusions with FFP and cryoprecipitate or coagulation factor concentrates, as they are devoid of viable lymphocytes. The total number of TA-GVHD cases reported in the world's literature was fewer than 200. TA-GvHD typically occurs 4-30 days after transfusion. Fever is the first symptom to appear, followed by erythematous maculopapular skin rash spreading all over the body. Hepatic abnormalities and bloody diarrhoea follow. Pancytopenia is typically seen 2-3 weeks after transfusion. The disease is rapidly fatal despite aggressive therapy. Fatality rate is around 90%. Severe systemic infections occurring 3-4 weeks after transfusion are the most common cause of death. Diagnosis is often difficult requiring differentiation from viral infections and drug eruptions. In an appropriate clinical background, constellation of skin, gastrointestinal tract, liver and bone marrow symptoms should arouse suspicion. A lower threshold for performing skin biopsy aids in supporting the diagnosis, the findings are however supportive and not diagnostic. Definite diagnosis depends upon the demonstration of lymphocytes of donor origin in patient's blood/tissue. However, it must be emphasized that donor leucocytes may remain in otherwise healthy individuals for a few days to many years; hence skin biopsy specimen from the patient is preferable. Donor lymphocytes in patient can be demonstrated by HLA typing of pre and post transfusion patient's DNA and donor's DNA. Presence of donor alleles in post transfusion patient's sample, not found in pre-transfusion sample, strongly support the presence of chimerism. Pre-transfusion DNA can be obtained from hair follicles, nail clippings and buccal mucosa. Alternatively, analysis of variable number tandem repeats (VNTR) and short tandem repeat (STR) profile analysis can be done. As the disease is invariably fatal in most of the patients, prevention is of utmost importance. Unfortunately, in our patient, the disease was triggered by a sinister combination of factors, of which the most important was the lack of awareness on safe blood transfusion practices in the medical
community, thus allowing transfusion of blood donated by a close relative.

**Conclusion**

Keeping in mind the local culture of marriages in close relatives, a relatively restricted gene pool is expected in the local population. These facts combined with prevalent myths about blood transfusion are likely to give rise to a relatively large number of similar mishaps.

**References**


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**Opinion and Debate**

**Safe medical practice in Pakistan: are we prepared to adopt a path?**

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While there are many discussions about improvements in medical education in Pakistan, reports, personal communications and print media write ups are not concurring with these anecdotal hearsays. Ironically, medical education in public sector medical colleges with large hospitals attached, are not fulfilling the training and teaching needs of the prospective medical graduates. There are a number of factors for this including a lack of motivation among teachers, bad regulatory control and subtle politics. With the upsurge of a number of private sector medical colleges, it was assumed that this situation would improve in view of effective quality control, incentives for teachers and some sort of accountability. The problem with private institutions is the inadequate number of patient turnovers in ill equipped hospitals. There is more teaching with less patient exposure and hands-on training. Medical students in the public sector made their personal efforts in learning through their patients under the umbrella of larger hospitals with huge patient turn out. At the end of the day, it transpired that a number of medical graduates from the private sector did well in their Professional and Linguistic Assessment Board (PLAB) and United States Medical Licensure Examination (USMLE) exam, some scoring up to 99% of marks in USMLE exams. So which is better? We cannot compare one over the other, as both have inherent disadvantages. However, it is important to look at the end product: which is the 'Medical Doctor.' A large number of medical graduates commence and complete their postgraduate training (specialization) in Pakistan and start their career. Some after completing their internship would immediately go into general practice. The dilemma remains about safe practice of a doctor. In the current era, there are no formal assessments, observations, or periodic reviews of practice or revalidation of certification. There is a big change in how the western world is making medical practice safer and, once it is in full bloom, there will be an expectation from medical practitioners aspiring to work in the western world to have undergone this exercise, on a periodic basis. There are some reports where bad practices in medicine have reflected dangerously on a number of patients. It is therefore pertinent to initiate such a system in Pakistan. For this country, designing and implementing new policy has always been a nightmare. However, it is necessary to look at some models designed by the western world and find a way where a suitable system can be developed in Pakistan. The Irish Medical Council initiated discussions on Competence Assurance Structures (CAS) in 1997 and intends to fully implement the policy for performance assessment in practice.1 The salient features of this plan include participation of all doctors in a Continuing Quality Assurance (CQA) programme for 50 hours per year. These doctors must also align themselves with a postgraduate training body where there is a requirement to submit evidence of participation, involvement in clinical audit and peer review activities as part of ongoing education. The structured peer review assessment is carried