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Allogeneic Haematopoietic Stem Cell Transplantation : Army Hospital Experience

Velu Nair, Ajay Sharma,*
Satyaranjan Das and Sanjeevan Sharma

ABSTRACT

This article outlines our experiences with **allo**geneic **h**aematopoietic **s**tem **c**ell **t**ransplantation (allo-HSCT) at Army Hospital (Research & Referral) with effect from February 1998 to March 2010. A total of 132 patients underwent 137 transplants, five of them undergoing second transplant due to the failure of first. The initial 18 allogeneic HSCTs were done in single, air-conditioned, side rooms of the general hematology ward. In February 2002, a three-bed, **h**igh **e**fficiency **p**articulate **a**ir (HEPA) filter equipped unit was established, where 119 allo-HSCTs have been carried out. The details of 114 patients who underwent 119 allo-HSCTs in the HEPA filter unit are being discussed in this article. Indications for allo-HSCT included various genetic disorders and haematological malignancies.

One hundred and ninteen transplants were performed in 114 patients for various indications of which 79 were males and 35 were females with a median age of 17 (2-60) years. Peripheral blood stem cells were used in 75 (63%) cases, bone marrow in 43 (36%) and in one patient, bone marrow plus cord blood was used. **G**raft **v**ersus **h**ost **d**isease (GVHD) was noted in forty-two (36%) patients including acute GVHD in seventeen (14%) patients and chronic GVHD in twenty-five (22%) patients. Grade-III/IV acute GVHD was noted in 11 (10%) patients and so was extensive chronic GVHD noted in 11 (10%) patients. Seventy-four (64.92%) patients are surviving on a median follow-up of 34 (3-95) months. The overall mortality was 35.08% (40/114) and the main causes of death were GVHD, infections and regimen related toxicity.

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These results are comparable and in some instances superior to those reported from India and the West.

Keywords : Allogenic haematopoietic stem cell transplantation (allo HSCT), haematological malignancies, graft versns host disease (GVHD)

Introduction

HSCT has revolutionized the treatment of numerous hematological disorders, which were considered incurable in the past (1, 2). The term bone marrow transplantation (BMT) is used synonymously with HSCT. Technically HSCT is the correct nomenclature as stem cells can be sourced from the bone marrow for BMT, from peripheral blood for peripheral blood stem cell transplantation (PBSCT) and umbilical cord blood for cord blood transplant (CBT).

In the developed countries there are adequate numbers of transplant centers, HSCT being an established form of treatment. However, in the developing world there are very few centers, the main limiting factors being, the availability of trained personnel; the prohibitive cost and the high level of multidisciplinary support required for such a center (3).

In India the first HSCT was done in TMH, Mumbai in 1983. This was followed by transplants in CMCH, Vellore; Army Hospital (Research & Referral), Delhi Cantt; AIIMS, New Delhi; Apollo Hospital, Chennai and Sahayadri Hospital, Pune. There are several other centers which have started a transplant program. To date, centers doing more than ten allo-HSCTs yearly are numbered and a total of 1757 allo-HSCTs have been performed till December 2008 in India. Hence, there is a significant supply demand imbalance, necessitating more centers to meet the requirement for a population of over a billion.

Army Hospital (Research & Referral) is the premier referral center for over a hundred Armed Forces hospitals spread all over the country. Presently, we run a successful and effective HSCT program for the Armed Forces of India carrying out approx 35 HSCTs yearly (4).

Healthcare is provided totally free of cost to all entitled patients in the Armed Forces. All soldiers and their dependents (spouse, children, and parents) are entitled for enrolling in the HSCT program and all patients who had a 6/6 HLA antigen match from a related donor were considered for HSCT.

HSCT in Indian setting differs from that in the West in the following aspects.

1. There is no national health program supporting HSCT unlike the NHS (National Health Scheme) in UK. In the public sector, most patients are self supported, whereas, some state and central government agencies and private cooperative enterprises financially support HSCT. Insurance based healthcare has been started but yet is to be set up in a comprehensive manner.
2. There is no functional donor registry for HSCT in India, while this is well established in Europe, North America and Japan. These registries improve the chances of finding a fully matched HLA (unrelated) donor from 60%, to 80%, whereas, it is 25% to 30% when only siblings form the donor pool.
3. Research funding, is substantial in the West. In the past few decades funding for various research activities has significantly increased from agencies such as the Dept of Biotechnology, Dept of Science and Technology, Indian Council of Medical Research etc. In the Armed Forces there is healthy funding for research.
4. There is a shortage of established laboratories to carry out molecular studies for chimerism. This is vital to monitor non-myeloablative HSCT for donor chimerism and decision making regarding donor lymphocyte infusion (DLI)
5. Availability of blood components especially single donor platelets (SDP) harvested by aphaeresis was not freely available in India. However, with awareness and the need, the situation has vastly improved with many blood banks having quality aphaeresis units.
6. All cellular blood products have to be irradiated to prevent transfusion associated GVHD. However, availability of blood irradiators is limited.

Patient and Methods

Patient selection

Once a patient with haematological/ genetic disorder was identified for an allo-HSCT, then a search was done amongst the siblings/parents to find a HLA matched donor, who ideally should have a 6/6 HLA antigen match. Since there is no functioning donor registry in India yet, no unrelated allo-HSCT have been performed at our center. Most transplants have been myeloablative (n=98) wherein, the marrow was ablated using high dose chemotherapy referred

to as 'conditioning'. The age limit for such transplants has been 55 years. Non-myeloablative allo – HSCTs (also called reduced intensity conditioning or 'mini' transplants) have been done in older patients (n=21). In the latter procedure lack of myeloablative conditioning is compensated by the 'graft versus leukemia (GVL) effect' for an effective tumor kill.

HLA matching

Initially, HLA was done by serology and later by molecular typing using sequence specific primers (SSP) which is a low resolution DNA based typing for HLA using PCR technology. HLA loci A, B and D R was done in all patients and of late, we are also doing HLA C, DP and DQ on a case to case basis for doing mismatched HSCTs

Donor screening and pre-HSCT work-up

All donors were thoroughly screened for any underlying disease and they were screened for CMV, hepatitis B and C virus and herpes virus by PCR methods . They were also screened for malaria and VDRL.

Pre-BMT workup of the patient involves a thorough search for any focus of infection, echocardiography for left ventricular ejection fraction, pulmonary function test, ENT and dental check up for any occult focus of infection.

Venous Access

Venous access in all HSCTs was provided by inserting a double lumen Hickman broviac catheter under general anesthesia in children and under local anesthesia in adults. This procedure was carried out by a member of the hematology team in conjunction with the interventional radiologist or anesthesiologist. We have a well trained team of nurses who carry out dressing of these catheters as per laid out SOPs. Each central catheter remains in situ for a period of 1-3 months for blood sampling and transfusion support required post HSCT.

Conditioning

In our center all cases are administered chemotherapy based conditioning as radiation based conditioning is in the process of being established. For acute and chronic leukemias and MDS we used the Busulphan (Bu)-Cyclophosphamide(Cy) regimen proposed by Tutschka (5). Bu in a total dose of 16 mg/kg over a period of 4 days and Cy, 120 mg/kg over two days. For aplastic anemia a combination of fludarabine, Cy and anti-thymocyte globulin (ATG) and in Fanconi's anemia a modified protocol with Cy, ATG ± low dose Bu was used.

Stem cell harvest

PBSC was harvested using a COBE Spectra cell separator. Bone marrow was collected from the donor under general anesthesia from the posterior superior iliac crests using Jamshedi bone marrow aspiration needles. Harvested marrow was collected into a blood bag containing anticoagulant, citrate phosphate dextrose adenine (CPDA) solution without using a filter. The donor is administered 25 units/kg of heparin (UFH) intravenously prior to commencing bone marrow harvest. A minimum nucleated cell dose of 3 x 10^{8}/kg recipient weight is aimed at and harvest volume is accordingly calculated.

Total parentral nutrition

Total Parentral nutrition (TPN) was given to patients when oral intake was compromised because of mucositis. In our center, ready to use commercially available TPN, (kabiven of Fresineus Kabi) was used which contained 19% dextrose, amino acids, electrolytes and 20% lipids. In adults 1026 ml was infused through the central catheter which provided 900 kcal in 24 hours. In children, pediatric formulation of the same was used. No TPN filters were used. Trace elements (chromium, copper, iodine, manganese, selenium, zinc and molybdenum) were administered separately. TPN was discontinued once patient started taking oral fluids. The average period for TPN was 10-14 days.

Blood component therapy

All cellular blood products (RBC, single and random donor platelets and rarely granulocytes) are irradiated (25 Gy per bag) prior to transfusion. RBCs were leucodepleted at collection by the Armed Force Transfusion Center, Delhi Cantt. SDP were harvested using a COBE Spectra (Model: 950000-902) cell separator with leucodepletion using LRS Turbo version 7.0.

The ABO type of blood components transfused after transplantation was defined by the blood groups of the donor and recipient. Patients undergoing major or bi-directional ABO-incompatible transplants received RBCs of blood group O although group A or B recipients of cells from group AB donors could receive donor-type RBCs. This transfusion support was maintained until donor ABO type was demonstrable. Patients undergoing minor ABO-incompatible transplants were also transfused with group O RBCs although group AB recipients of cells from group A or B donors could receive donor-type RBCs (6).

The preferred ABO-type of platelet was that of the donor for major ABO-incompatible transplants and of the

recipient for minor ABO-incompatible recipients. Bidirectional ABO incompatible received plasma-depleted platelet components of donor ABO type. Plasma depleted platelet components of other ABO types were also infused for those patients if a component of the desired type was not available (7).

Prophylaxis for haemorrhagic cystitis

Prevention of hemorrhagic cystitis secondary to use of high dose cyclophosphamide was done by administering continuous infusion of MESNA (2-Mercaptoethanesulfonic acid sodium salt) starting 12 hours prior to and continuing 12 hours following cessation of cyclophospahamide along with hydration. Despite meticulous hydration plus MESNA infusion ,five cases had HC which responded to conservative management. In all these cases a thorough search was made for GVHD and viruses such as BK, CMV and Adeno virus, when hemorrhagic cystitis occurred after engraftment.

GVHD prophylaxis

GVHD prophylaxis in most cases was done using cyclosporine (CSA) with methotrexate (MTX). Intra-venous (IV) CSA was administered beginning Day-3 and continued till mucositis settled and oral fluids were tolerated, following which oral CSA was started (usually double the dose of IV-CSA). CSA levels were done weekly, targeting a level of 250 ng/ml. In cases of CSA intolerance, mycophenolate mofetil/ tacrolimus replaced CSA.

Gluksberg's scale was used to stage acute GVHD. For the treatment of grade-III/IV acute GVHD, intra-venous methyl prednisolone was administered in a dose of 2 mg/kg per day in two divided doses. In steroid non-responders, alternative treatment with ATG (ATGAM-Pfizer), dacluzimab (MoAb against IL-2), or infliximab (MoAb against TNF-á) was resorted to.

Antimicrobials

None of the patients received any gut sterilization with oral quinolones. Oral fluconazole was used as antifungal prophylaxis in the initial transplants. However, it was noticed that colonization by non-albicans candida species occurred in these patients following which fluconazole prophylaxis was discontinued. Intravenous ganciclovir is given as CMV prophylaxis starting on Day-10 continued till Day-2 in a dose of 5 mg/ kg daily. Intravenous acyclovir, 5 mg /kg Q8h was started on Day-1 and continued till patient commenced oral feeds when a switch to oral acyclovir was made. This was continued till immunosuppressives

were used and longer if GVHD occurred (8). A well laid out algorithm to manage febrile neutropenia based on the microbial sensitivity was followed. Usually a third generation cephalosporin with anti-pseudomonal spectrum and an aminoglycoside is the initial therapy followed by addition of anti-staphylococcal (vanomycin/ teicoplanin) and antifungal agents if fever persists. A high index of suspicion for fungal infections was kept and antifungal therapy was instituted early with voriconazole / amphotericin.

HSCT Program in Non HEPA Settings

The first 18 allo-HSCTs were done in single air-conditioned side rooms of the general Hematology Ward (May 1998 – Dec 2001) Indications included 10 CML; 04 AML; 02 ALL; 01 CLL and 01 thalassemia major. The median age was 26.5 (4.5-39) years and median cell dose administered was 6.1 x 10^8 MNC/ kg (1.7-15). Acute GVHD was noted in 9 (50%) and chronic GVHD in 3 (16%) patients. Out of these eighteen transplants, eight (44%) are disease free on a median follow-up of 11 (9-11) years. Ten (56%) patients died, of which three died of grade-III/IV acute GVHD, three of extensive chronic GVHD, three of sepsis and one of VOD and pneumonia. The high mortality was attributed to the non-HEPA environment, patient selection and initial learning curve (9)**.** The HLA typing in these patients was done by serological typing.

HSCT Program in HEPA Settings

The next 119 Transplants between February 2002-March 2010 were done in a positive pressure HEPA filtered units, each having all intensive care backup facilities. The air-quality was monitored regularly for bacterial and fungal spore contamination using appropriate settle plates (9).

We allow one attendant, who is usually the mother/father in case of pediatric patients and spouse / relative in case of adults. Foldable couches in addition to the patient bed are provided in all rooms for use by the attendant. In our experience the presence of a family member as an attendant has been a huge success and provides great moral support through this labour intensive procedure, which keeps the patient in isolation for a period of 3-4 weeks. We also have an attached common kitchen in the transplant unit, wherein, the attendants are allowed to cook. All food is pressure cooked and no raw / uncooked food is allowed for consumption. Only filtered water is used, which is also boiled for drinking purposes. We also have a dedicated air conditioned, non-HEPA filtered, 4-bed step-down unit, where patients are shifted once neutrophil

Table 1: Baseline Data and Outcomes in 114 allo-HSCT patients

BMTS	AML (n=30/33)	ALL (n=13/13)	CML(n=19/20)	AA(n=15)	Thal Maj (n=27	Miscellaneous(n=10/11)
M/F	23/7	11/2	8/11	9/6	18/9	7/3
Median Age (years)	24(5 - 54)	19 (9 - 32)	27.5(7 - 46)	22(12 - 46)	6 (2.5 - 13)	16(2 - 60)
Median Follow-up (months)	58.5 (7- 84)	.50.77 (76 - 92)	59(2 – 95)	16 (2- 56)	35 (2-92)	42 (2-58)
Stem Cell Source BM+PB: 1	BM:5; PB:28	BM:2, PB:11	BM:5; PB:14 BM+PB:1	PB: 15	BM:26, BM+CB:1	BM:4; PB: 5
Median Cell Dose (MNC x 108/kg)	7(2.33 - 9.2)	6.4(1.9 - 7.5)	5 (2.5 - 8.2)	6.75(3.1 - 7.7)	5.3 (2.2 - 9.7)	5.2 (2.9 - 8.6)
Median Neutrophil Engraftment (days)	11(8 -16)	12 (9 - 14)	11(9-14)	10 (8 - 12)	13 (9 - 14)	11(7 - 20)
Acute GvHD	3(10%) Gr III/IV:3	4(30.77%) Grade I:1 grade II: 1 Grade III:2	4(21.05%) Gr III/IV-04	1(6.67%) Gr II:1	3(11%) Gr I:1, Gr III/IV :2	2(18.18%) Gr II:1 Gr IV:1
Chronic GvHD	5(16.67 %) Ext: 3 Ltd: 2	.4(30.77%) Ltd: 2, Ext: 2	8(42.11 %) Ext: 3 Ltd : 5	3 (20.00%) Ltd: 1 Extd: 2	2(7.5%) Ltd: 2	2(20%) Ext: 1 Ltd: 1
VOD	4(13.33%)	3(23.08%)	3(15.79%)	Nil	2(7.41%)	3(30%)
Infections	5(16.67%)	3 (23.08%)	4(21.05%)	3(20.00%)	5(18.52%)	1(10%)
Mortality & causes of death	53.33% (16/30) Relapse: 6; Pneumonia+ Sepsis:4 VOD:1 Ac GVHD IV: 2 Others: 3	46.15% (6/13) Relapse: 2, Acute GVHD:2, Sepsis: 1 Ch GVHD with CMV:1	31.58%(6/19) Relapse: 1 Septicemia:1 VOD 1 Ac GVHD :2 Ch GVHD:1	20% (3/15) Infections : 2 pericardia effusion: 1	22.22% (6/27) Severe VOD: 2 Acute GVHD: 2 Sepsis:1 Ch GVHD with CMV :1 infection	30% (3/10) Relapse: 1; Ch GVHD +(BOOP): 1 Ac GVHD-IV):1
DFS/EFS*	46.77%(14/30)	53.85% (7/13)	68.42%(14/19)	80% (12/15) *	77.78% (21/27)*	70% (7/10)*

engraftment has occurred. A year-wise distribution of allo-HSCTs carried out at this centre between March 1998 to February 2010 is shown in Fig. 1 and the distribution of various indications in Fig. 2.

Results

The base line data and outcomes are outline in Table 1.

Acute myeloid leukemia

Total of thirty-three allo-HSCTs

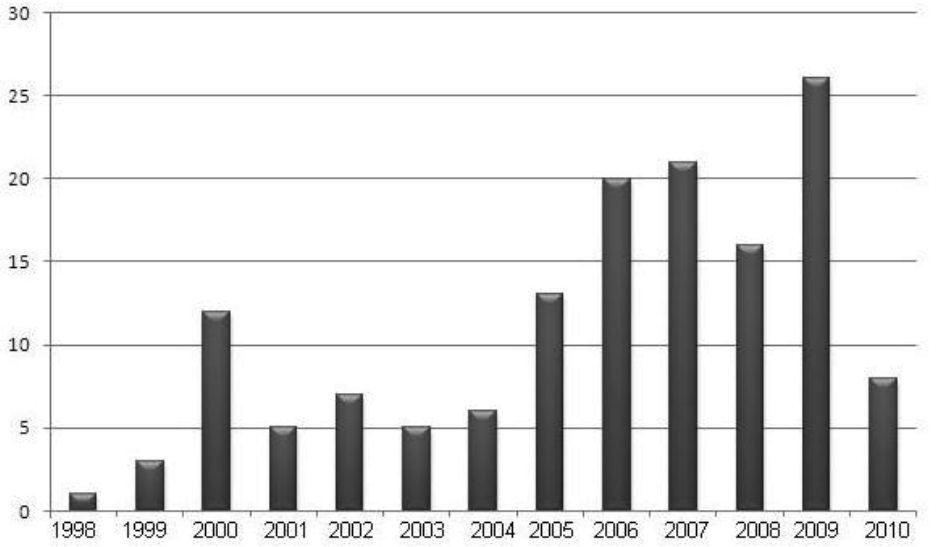

Fig. 1: Allo-HSCTs at AHRR between March 1998–Feb 2010

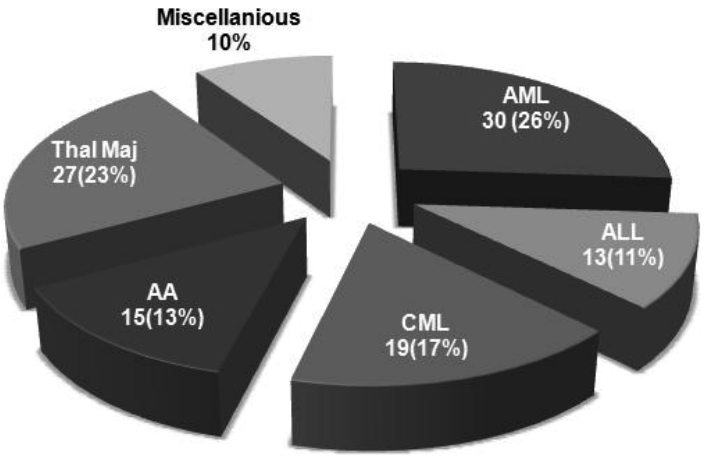

Fig. 2: Indications for allo-HSCT at AHRR between Jan 2002-Feb 2010

were done in thirty patients of AML, where twenty-eight were PBSCTs and five were BMTs. Three patients who underwent a second transplant were all PBSCTs. Out of 30 patients, 25 were in complete remission (CR)1, four in CR2

and one patient who underwent a second transplant was in CR3. Median age was 24 (5-54) years and male-female ratio was 23/7. Grade-III/IV acute GVHD was noted in three (10%) patients and chronic GVHD in five (16%) patients, three of whom had extensive chronic GVHD (2 skin & liver; 1 lung). Fourteen (47%) patients are alive and disease free on a median follow-up of 25.5 (2-59) months. Sixteen (53%) patients died including, six of relapse (4 of CR1; 1 of CR2 and 1 of CR 3); six of pneumonia with sepsis; two with VOD; one of grade-III/IV acute GVHD and one due to chronic lung GVHD.

Acute lymphoblastic leukemia

Thirteen patients underwent HSCT of which eight were in CR1 with high risk disease including three Ph+; and five were in CR2. There were eleven males and two females with a median age of 19(9-32) years. Seven (53.85%) patients are alive and disease free on a median follow-up of 50 (3-99) months. Grade-III/IV acute GVHD was seen in two patients and chronic GVHD in four (31%) patients (two limited; two extensive). Out of six (46.15%) deaths, two patients died due to grade-III/IV acute GVHD; one due to extensive chronic GVHD; three due to relapse(2 with CR2 and one with Ph+ disease).

Chronic myeloid leukemia

CML was one of the most frequent indications for allo-HSCT in the pre imatinib mesylate era. However, after the introduction of imatinib mesylate the number of transplants done has been negligible due to the excellent response to imatinib mesylate. Nineteen patients in the first chronic phase (CML-CP-1) underwent 20 allo-HSCTs out of which 14 were PBSCT and 6 were BMT. The median age was 27.5(7-46) years with a male-female ratio of 8:11. Grade-III/IV acute GVHD was seen in four (20%) patients and chronic GVHD in 8 (40%) patients, of which three had extensive chronic GVHD (one skin & liver, two lung). Thirteen (68%) patients are alive and disease free on a median follow-up of 59 (2-95) months while 32.58%(6/19) mortality was observed. One patient transplanted using PBSC developed grade-II acute skin GVHD which was successfully treated with CSA and a short course of steroids. One-year post-HSCT, this patient developed significant weight loss, extensive oral ulcers and severe cholestatic jaundice. Skin and mucosal biopsy confirmed chronic GVHD (Fig. 3). The liver biopsy revealed portal fibrosis with lymphocytic infiltration. There was loss of bile duct with cholestasis consistent

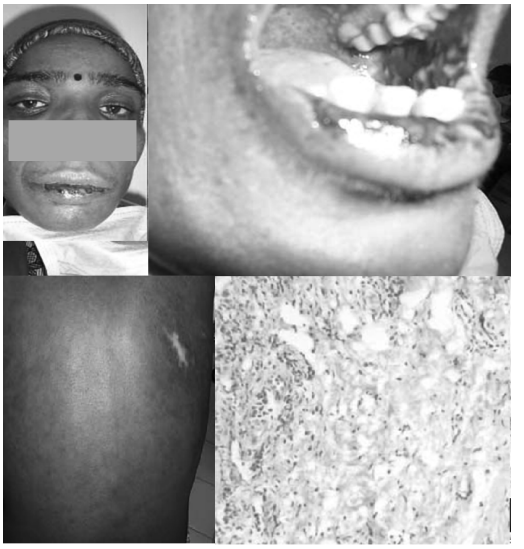

Fig. 3: Extensive chronic GVHD in patients underwent allo-HSCT (vanishing bile duct syndrome)

with a diagnosis of 'Vanishing bile duct syndrome'. This patient died due to severe extensive chronic GVHD (10). The other causes of death included VOD in one; grade-III/IV acute GVHD in two; septicaemia in one and relapse in one.

Thalassemia major

Total of twenty seven thalassemia major patients underwent transplant using CB and BM in one and BM in 26 patients. The median age was 6 (2.5-13) years with a male-female ratio of 2:1. All patients were risk stratified using Lucarelli's criteria, which proposed three classes based on, evidence of good iron chelation, hepatomegaly and presence of portal fibrosis on liver biopsy. Class-I were patients with good iron chelation, no hepatomegaly or portal fibrosis. The presence of one or two factors was classified as class-II and presence of all three factors as class-III. In our series 4 patients belonged to class-I, 15 to class-II and 8 to class-III (Table 2). The only patient who underwent CBT rejected the graft but was successfully transplanted a second time, using BM from the same sibling donor. Three out of four class-I patients died, two due to infection and one due to severe VOD; one in class- II died due to grade-III/IV acute GVHD, and sepsis. Two patients died in class-

Table 2: Results as per Lucarelli's classification

Outcome variables	**Class I (%)**	**Class II (%)**	**Class III (%)**
No. of patients	4(14.81%)	15(56%)	8(30%)
Severe VOD	1(25%)	3(20%)	2(25%)
Acute GVHD (III/IV)	Nil	1(6.7%)	2(25%)
Chronic GVHD	Nil	1(6.7%)	1(13%)
Infections	2(50%)	2(13%)	2(25%)
Mortality	3(75%)	1(6.7%)	2(25%)
EFS	1(25%)	14(93%)	6(75%)

III; one due to grade-III/IV acute GVHD and another due severe VOD. Event free survival (EFS) was noted in 77.7% (21/27) patients on a median follow-up of 35 (2-92) months.

Acquired severe aplastic anaemia

Fifteen patients of severe aplastic anemia underwent allo-HSCT and all were PBSCT (11). The median age was 22 (12-46) years with a male-female ratio of 3:2. Twelve (80%) patients are alive, free of disease on a median follow-up of 16 (2-56) months. Only four (26.5%) patients had GVHD including one acute GVHD (grade II) and three chronic GVHD (one limited and two extensive) without any fatal outcome. Three (20%) patients died, two due to sepsis and one due to massive pericardial effusion with cardiac arrhythmia.

Miscellaneous

In this group, there were three patients of Fanconi's constitutional anaemia who underwent four transplants. Two were males and one female who underwent a second transplant due to graft rejection. The median age of this cohort was 14 (8-16) years. Regimen related toxicity was seen in one patient in form of VOD and one also had extensive chronic skin GVHD. All three patients in this group are surviving and are disease free on a median follow-up period of 42 months. There were 2 patients of JMML, both infants, where a combination of Bu/Cy as conditioning regimen and CSA+MTX as GVHD prophylaxis was given. Out of these two patients, one patient relapsed and died 5- month post-BMT and the second

child is surviving, disease free, on a follow-up period of 42 months. There were two patients of MDS, one male and one female, where combination of Flu, Bu and ATG was used as conditioning regimen and CSA+MTX was given for GVHD prophylaxis. Out of these two patients, one patient developed acute GVHD and second patient developed chronic GVHD of lung with septicaemia and succumbed to these complications, 11 months post-BMT. There was one patient of PNH who developed grade-III/IV acute GVHD and died 5-months post-BMT. Two patients with congenital PRCA were transplanted of which one patient also had Duchenne muscular dystrophy (DMD) (2). Though, HSCT was done only for transfusion dependent PRCA, outcomes are suggestive of transplant being of some benefit in DMD also. The child is transfusion free and interestingly has not shown any deterioration in his muscular power 39 months post HSCT. His CPK levels have reduced from 20,000 U/L pre BMT to 350 U/L. The muscle biopsy (biceps) done two years post-HSCT has shown 8% cells of donor origin. Sequential chimerism studies, using whole blood, established trilineage engraftment with 100% donor chimerism. However, immunostains for dystrophin I were markedly reduced, while dystrophin II and III were absent. The other patient with PRCA, developed mild-VOD and hemorrhagic cystitis which responded to conservative treatment and is disease free four years post-transplant.

Infections

There were 130 documented infections in 114 transplants. This included 61% bacterial, 24% viral, 14% fungal and one parasitic infection. There was no difference between patients transplanted for malignant and non-malignant indications.

Bacterial infections

In 76 patients there were 92 documented bacterial infections, 53 % of which occurred in the first 30 days, post–HSCT. Gram negative bacteria (GNB) were isolated in 77% and gram positive in 23% of cultures. The common GNB isolated included, E.coli (48%), P. aeroginosa (13%), non-fermentative Gram negative bacteria (12%), Enterobacter (2%) and K. pneumonia (2%). The gram positive organisms were, S.aureus (13%) and coagulase negative Staphylococcus (8%).

Positive bacteriological cultures were obtained from blood (65%) followed by urine (12%), sputum (8%) and catheter related infections (5%).

Viral infections: In all, 26 of the

transplant patients had 34 documented viral infections. The common pathogens were CMV, transfusion related hepatitis viruses and herpes group of viruses. CMV was detected in 13 patients (CMV-DNA by PCR) and Hepatitis B infection in 3 patients ,all three detected post BMT. Hepatitis C virus infection was seen in one patient.

Fungal infections: fungal infections were documented in 20 transplants and the common fungi identified were, Candida spp (57%), Aspergjllus, spp (33%) and Zygomycetes (10%). For infections due to Aspergillus, all patients fulfilled the CDC criteria of either proven or possible fungal infection. Majority of the infections were seen in the first 100 days post transplant and the most common site was the lung (52%). The other sites included the CNS, para-nasal sinuses, gastrointestinal tract, skin, catheter related, isolation from blood (Candida) and disseminated forms.

Parasitic infections: Only one transplant patient showed seropositivity post transplant for toxoplasma. There were no infections with tuberculosis or malaria.

Discussion

The Hematology unit of AHRRR was set up over a 2 year period starting in February 1994. The minimum infrastructure to carry out a HSCT was in place by 1997 except for a blood irradiator. In 2002, a Gamma Cell 1000 elite (NORDION), blood irradiator with a cesium 137 source was procured by the Armed Forces Transfusion Center which supplies our blood components. Till 2002, irradiation of all cellular products was carried out in the Radiotherapy department of our hospital. The more difficult part was to train a team of nurses to carry out the following tasks,

1. Identify venous access
2. Care of venous catheters, specially central catheters
3. Administer chemotherapeutic agents and blood components
4. HES sedimentation for major mismatched transplants
5. Stem cell harvest, both BM and PBSC
6. Familiarize with various transplant protocols and supportive care

In addition the blood bank and laboratory assistants had to be trained in,

1. Cryopreservation of stem cells and thawing protocols
2. Cell viability studies and
3. Flowcytometry

HLA typing was initially outsourced and then set up in-house using serological methods. In 2002 molecular typing

by SSP, was established and in 2005, chimerism studies were also set up.

From a single consultant to start with, we have come a long way, and now have four consultants with a fully trained nursing team. Now we have a good, effective HSCT program which offers transplant for all hematological disorders including genetic diseases, catering for all Armed Forces personnel and their dependants.

In the non-HEPA filter setting, a overall survival (OS) of 44.4% (8/18) was noted, whereas, OS of 65.8% (75/114) was noted in the HEPA filter setting. Though this is not statistically significant (p=0.139) as the numbers were very small in the non-HEPA group (n=18) compared to the HEPA group (n=114), the trend is clear, with an advantage in the HEPA group (Fig. 4). Taking into account the environmental

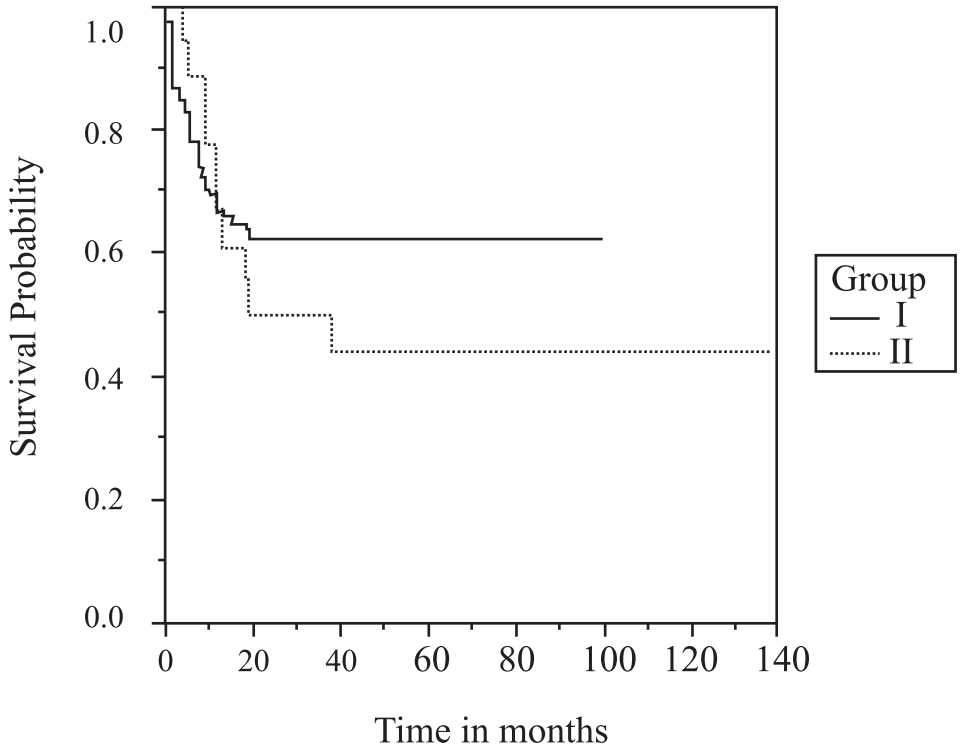

Fig. 4: Kaplan –Meier probability of survival for allo-HSCTs in Non-HEPA (1998-2001) versus HEPA (2002-2010) setting

conditions with a high incidence of antimicrobial resistance we believe that it is prudent to perform HSCTs in a HEPA filtered unit despite the feasibility of doing the same in clean side rooms, albeit, with inferior results (9).

For all our thalassemia major transplants, BM was used as the source of stem cells, except in one case, where BM+CB were used. In most cases of hematological malignancies and bone marrow failure syndromes, PBSC was used. In all pediatric donors only BM was harvested as PBSC harvest was not feasible in this age group. The donor was educated about the merits and demerits of BM versus PBSC, and allowed to make a choice as and when possible.

There was no significant difference in the incidence of acute GVHD between the BM and PBSC groups. Acute GVHD was seen in three (6.98 %) out of 43 BMTs and in 14(18.42%) out of 76 PBSCTs (p=0.142). However, the incidence of chronic GVHD was significantly higher in patients transplanted with PBSC. Chronic GVHD was noted in 6.98% in the BMT group where as it was noted in 30.70% in the PBSCT group (p=0.0058). This is as observed by other workers.

Twenty-six patients with thalassemia major underwent BMT and one was given BM plus CB. A EFS of 77.7 % was noted on a median follow-up of 35 months (Fig. 5). A 93% EFS was observed in 15 patients of thalassemia major classified to Lucarell's class-II. These results are comparable with that of Pessaro, Italy and with CMC, Vellore (12, 13). Both these centres have amongst the largest series for thalassemia major transplants in the west and in India, respectively. Three of our four Class I patients died, two of sepsis and one of severe VOD which emphasizes the unpredictability of sepsis and regimen related toxicity in HSCT (13).

In 15 patients with severe aplastic anemia with a median age of 22 years a EFS of 80% was noted on a median follow-up of 16 months (Fig.5). Though the number of transplants are small, results are superior to those published from India (13) and the west. This is because most of our patients were diagnosed early and minimally transfused given the network of more than 100 armed forces hospitals spread all over India. A delay in diagnosis and multiple transfusions, pre-transplant, negatively impacts transplant outcomes in this group of patients.

Fourteen (46%) out of 30 patients with AML are disease free on a median follow up of 25.5 months. Out of 13 patients with ALL, seven (54%) are alive

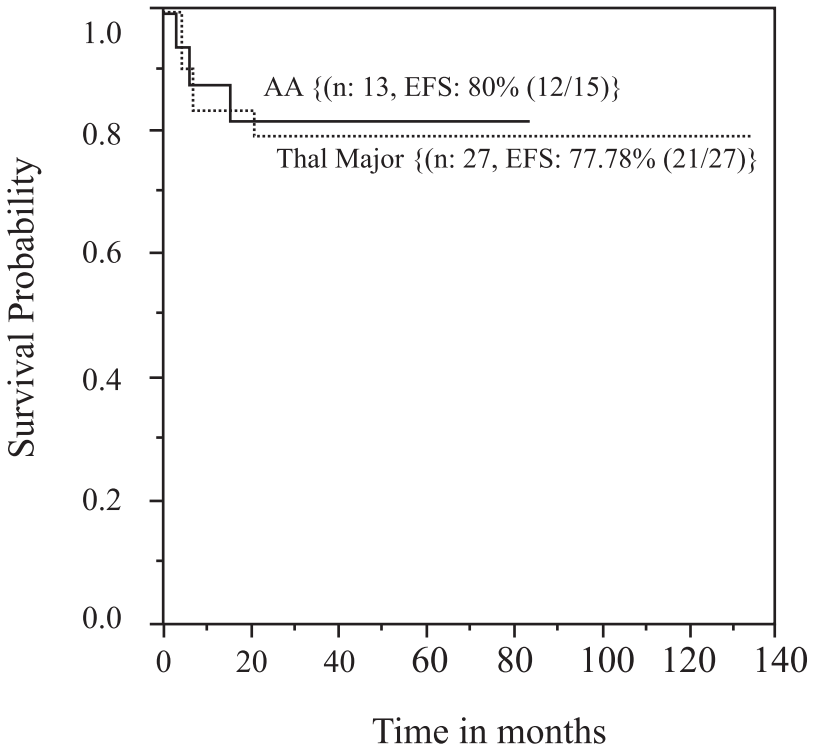

Fig. 5 : Kaplan-Meier probability of survival for patients with Thalassemia Major and Aplastic Anaemia

on a median follow up of 50 months. Results for both AML and ALL are comparable with those published from India and the west.

CML is the commonest leukemia in India, and was the most frequent indication for allo-HSCT at our center, till the advent of Imatinib mesylate. Imatinib mesylate has revolutionized the treatment of CML, with long term molecular responses noted, as long as the patient is on the drug. However, HSCT remains the only curative treatment and may be a cost effective option, if done early within the first year of diagnosis, in younger patients (<30years), especially in developing countries. This is more so because, Imatinib mesylate has to be continued long term and maybe life long, which adds to the cost. In our series a DFS of 68% was noted in 19 patients in CML-CP1, over a median follow up of 59 months (Fig.6).Our results are superior to those published from India and the west. The American Society of Hematology panel reported 50%

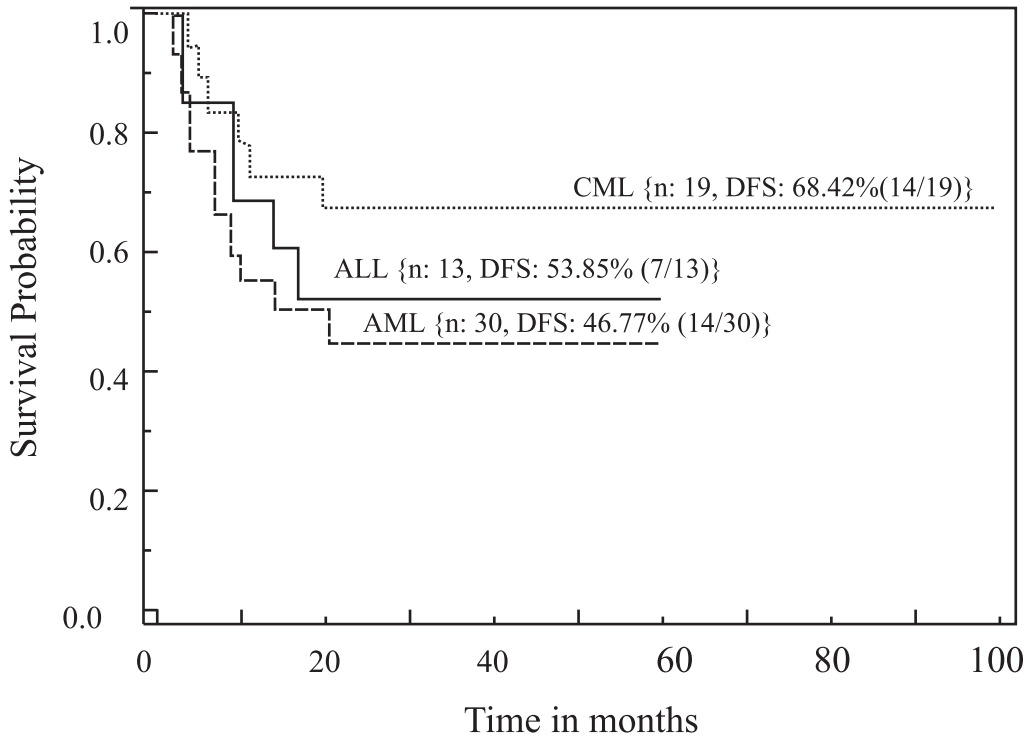

Fig. 6 : Kaplan-Meier probability of survival for ALL, AML and CML.

leukemia free survival after 5 years in CML-CP following a matched related allo-HSCT. The cumulative incidence of relapse at 18 years was 25% in these patients (14). Hence a long term close follow up is warranted in these patients with BCR-ABL monitoring for relapse and early intervention with donor lymphocyte infusion or Imatinib mesylate. The European bone marrow transplant registry reported a 10 year survival of 65 to 70% in children (15). Early diagnosis and transplant within one year of diagnosis in our patients with CML-CP1 with a median age of 27.5 years have largely contributed to our excellent results (15).

Three patients with Fanconi's constitutional anemia and two with congenital PRCA are disease free on a median follow up of 42 and 48 months respectively. Interestingly, one of the PRCA patients also suffered from DMD, which is a fatal form of muscular dystrophy. Though the transplant was

successfully performed, primarily for transfusion dependent PRCA, it seems to have also benefited DMD, as evidenced by a static clinical course over the last 39 months. Muscle biopsy has also shown 8 % donor cells on DNA typing with 100 % donor chimerism in the blood in this patient (2).

The cost of an allo-HSCT in private hospitals is approximately 10 to 12 lakhs Indian rupees (US $ 20-24,000).The cost in our hospital is half of this while the same in the West can cost between 150 to 400,000 US $. This vast difference in cost opens up a huge scope for medical tourism.

Hence, it has been possible to establish an excellent HSCT centre and program over the last 12 years. A wide range of indications for HSCTs has been transplanted successfully with outcomes which have been comparable/ superior to published data from India and the west**.** In the armed forces, health care is a priority and all efforts are made to ensure delivery of the recommended 'standard of care' in our transplant program. This coupled with an excellent tracking system ensures a near 100% follow up, which has helped us in achieving good outcomes. Our future plans include starting HLA matched unrelated donor transplants and setting up a haploidentical transplant program.

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The Effect of Genetic Loading and Gender on Cognitive Functions of Unaffected Full Biological Siblings of Schizophrenia Patients

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ABSTRACT

Recent studies are giving consideration to the cognitive functions of schizophrenia which include deficits in abstraction, verbal memory, vigilance, language and executive functions. Only a small number of studies have addressed the effects of genetic loading and gender on cognitive performance in schizophrenia. In this article we shall be focusing on the following two aspects, firstly compare the performance of male and female full biological siblings of schizophrenic patients on cognitive tests and secondly, to find the influence of the genetic load on the performance of these tests.

This is a single point non-invasive study of non affected full biological siblings of patients with schizophrenia, involving administration of a battery of neuropsychological tests to assess the cognitive function in the siblings of schizophrenia patients.

On Wisconsin card sorting test (WCST), there was no significant difference between the male and the female siblings, except on percentage non-perseverative errors ($p=0.026$). On comparison on spatial working memory test (SWMT) and continuous performance test (CPT), there was no significant difference between males and females of sibling group.

The simplex group performed significantly better on all three tests as compared to the multiplex group.

In our study female sibling group performed significantly better than males on one parameter of WCST and one parameter of SWMT. Also, the siblings from multiplex families performed poorer as compared to siblings from simplex families on various

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parameters of WCST, CPT and SWMT.

Keywords : Male, female, simplex, multiplex, schizophrenia, cognition, Wisconsin card sorting test, continuous performance test, spatial working memory test, unaffected siblings

Introduction

Schizophrenia is arguably the most severe and disabling psychiatric disorder adversely affecting all the major domains of the patient's life-living independently and caring for themselves, working or attending school, fulfilling parental or other role obligations, and enjoying close relationships and rewarding leisure activities (1). In addition to the classically described 'positive' and 'negative' symptoms of Schizophrenia, recent studies are giving consideration to the cognitive functions of schizophrenia which include deficits in abstraction, verbal memory, vigilance, language and executive functions (2, 3, 4).

Although cognitive deficits have not yet been included in the diagnostic criteria of schizophrenia, they are one of the important core manifestations of this disorder and provide important diagnostic information, both in the phenotypic and prodromal phases. It has been proposed that abnormal cognitive functioning is a possible causal risk factor for psychosis, representing a third group of symptoms (5).

Owing to the large number of genetic and environmental variables which might lead to the development of Schizophrenia, researchers have found it difficult to identify those people who are currently asymptomatic and are at risk of developing schizophrenia at a later date. A possible way to find susceptibility genes for complex disorders may be using traits like variables associated with the disorder as phenotypes in genetic studies (6,7,8). For this reason, traits that can be observed both in patients and their relatives have come into focus. Because some relatives of patients carry genes for the illness although the illness is not expressing in them, the abnormal brain functioning that is often observed in them can be attributed to the effect that the illness genes have on the brain even in the absence of the full-blown illness (9). This effort to identify intermediate phenotypes, or endophenotypes, is driven by the idea that they involve the same biological pathways as the disorder but are closer to the relevant gene action than the categorical diagnoses,

thus adding power to genetic studies. Studies so far suggest that cognitive and psychophysiological aspects of brain function are more sensitive and reliable factors as endophenotypes for schizophrenia as compared to neruoanatomy or neurochemical findings (10). In several studies, cognitive deficits in relatives of schizophrenia patients have been found to parallel those observed in the patients, although to a milder degree. (11, 12).

Although it is known that female patients of schizophrenia tend to have better functioning and prognosis than males, previous studies have been unsatisfactory in eliciting a significant difference in the neuropsychological performance of the two sexes (13, 14).

Only a small number of studies have addressed the effects of genetic loading on cognitive performance. The majority suggest the expected negative relationship between genetic loading and aspects of neuropsychological abilities such as spatial delayed response task (15), delayed story recall, semantic verbal fluency, and inhibition response errors on a word completion test (16). Therefore the studies that examined associations with genetic loading indicated that both visual and verbal memory deficits hold particular promise as endophenotypes for the disorder.

This article is a part of a study conducted in the Department of Psychiatry, CSMMU, Lucknow, UP, India in which the performance of full biological siblings of schizophrenia patients was compared with that of unaffected controls (17). In this article we shall be focusing on the following two aspects, firstly comparing the performance of male and female full biological siblings of schizophrenic patients on cognitive tests and secondly, finding the influence of the genetic load on the performance of these tests.

Methods

This is a single point non-invasive study of nonaffected full biological siblings of patients with schizophrenia, involving administration of a battery of neuropsychological tests to assess the cognitive function in the sibling group. All subjects gave informed consent. The study was carried out from 1st September, 2005 to 1st August, 2006.

Sample consisted of nonaffected full biological siblings of patients with schizophrenia, both new and follow-up cases from district Lucknow, attending the outpatient section of the Department of Psychiatry, C.S.M Medical University on specified days of the week. Siblings

fulfilling the following selection criteria were taken up for the study:

The subjects were between the ages of 18 – 55 years and gave informed consent. The subject should have had at least 8 years of formal education, according to indian standards, and had no history of bipolar affective disorder, obsessive compulsive disorder, psychosis other than schizophrenia in the family (clinically assessed for available siblings and on the basis of history for those not available). They must have scored 3 or less on General Health Questionnaire, 12 item version (18).

Exclusion criteria for subjects included history of current or past psychiatric illness, history suggestive of significant physical disorder which can cause cognitive impairment such as seizures, cerebrovascular disorders, dementia, neurodegenerative disorders, systemic illness with known cerebral consequences, significant head injury, current or past history of any substance abuse or dependence, current use of medications known to impaire cognition like tricyclic antidepressants, antipsychotics, antiepileptics, benzodiazepines & lithium and physical problems that would render study measure difficult or impossible to administer or interpret e.g. blindness, hearing impairment, paralysis in upper limbs. The subjects in the control group were selected from the friends of the patients and healthy volunteers who gave an informed consent and fulfilled the following inclusion criteria: aged between 18-55years, should have had at least 8 years of formal education according to Indian Standards, and must have scored 3 or less on General Health Questionnaire, 12 item version. The subjects who had a past or present history of a psychiatric illness, medical disorders associated with cognitive impairment (seizures, cerebrovascular disorders, etc.) and head injury were excluded from the study as were those with present substance abuse or dependence, current use of medications impairing cognition (tricyclic antidepressants, antipsychotics, antiepileptics, benzodiazapines and lithium) and physical problems that would render study measure difficult or impossible to administer or interpret e.g. blindness, hearing impairment, paralysis in upper limbs. On specified days in adult Psychiatry O.P.D. C.S.M.M.U. patients (old and new) diagnosed as a case of Schizophrenia from district Lucknow were screened for the availability of the siblings. The diagnosis of schizophrenia was ascertained on detailed clinical evaluation using ICD-10 DCR. The available unaffected siblings were

ranked in the order of birth. One of the available siblings was randomly taken after applying the random number tables. Informed consent was taken and information regarding details of identification data, demographic profile, past history, negative history, family history, personal history and physical examination was obtained on the semistructured proforma. General Health Questionnaire, 12 item version (18) which is a measure in social science used for general mental well being with score more than 3 considered significant and warranting use of detailed assessment of an existing psychiatric illness, was used. The siblings were then assessed in detail on the Schedules for Clinical Assessment in Neuropsychiatry (SCAN) (19) (Wing et al., 1990) which is a standardized interview for ICD-10 diagnosis, to rule out any psychopathology or psychiatric illness.

Selection criteria were applied. If found eligible he/she was included in the study. If not eligible the next available sibling was randomly taken after applying the random number tables and assessment for participation in the study was done after taking the informed consent. The following computer based cognitive tests were administered by the investigator on the same day of inclusion so as to avoid loss at follow up or on a mutually convenient day if the subject was not willing to be tested on the same day or if there were more than one sibling included on the same day:

1. Wisconsin Card Sorting Test (WCST)

WCST (20,21) is a test of executive function requiring the ability to develop and maintain an appropriate problem solving strategy across changing stimulus conditions in order to achieve a future goal. It is a classical test for dorsolateral prefrontal cortex function (22). There are 4 cards employed in the test with each card having three stimulus parameters-colour, form and numbers. In the WCST, the subject is presented with a question card and the four stimulus cards and is asked to match question card to the stimulus cards but is not told which parameter to use (colour, form or number). However, he is told each time whether the response is right or wrong. After ten correct responses, the criterion for matching is changed without informing the patient. The subject is required to recognize the new criterion and change his responses accordingly. He/she is tested twice for each criteria. The responses and the scores on different parameters of this test are calculated and entered automatically. Intrascorer reliability coefficients for

WCST range from 0.828 to 1.000.

2. Continuous Performance Test (CPT)

Sustained attention, vigilance and impulse control is assessed by CPT (23, 24). The test requires a participant to respond to a specified target when it is presented spontaneously within a stream of interfering visual stimuli. The task involves monitoring a random series of geometrical figures.

Attention/vigilance involves maintaining a readiness to respond to a particular target stimulus and inhibiting responses to non-targets over a period of time. It requires one to distinguish targets from non-targets, an ability known as sensitivity. The results obtained are in terms of correct responses, wrong responses, missed responses and the reaction or response time. In the test, target stimulus is rare in frequency and presentation latency is brief. A total of 328 stimuli are presented, out of which 28 are for practice. Stimulus duration and inter stimulus latency are 50 milliseconds and 1000 milliseconds respectively.

3. Spatial Working Memory Test (SWMT)

Working memory is a type of explicit memory system involving short term registering of information. Working memory refers to the ability to maintain a limited amount of information for a brief time (seconds). Immediate memory is considered to be a component of working memory.

In SWMT (21), a test of memory for spatial locations, the subject views a brief presentation of black circle on computer screen and then is asked to point the location of circle after a delay of '0' second and '20' seconds, randomly. During the 20 seconds delay, the subject is engaged in a distraction task by asking to repeat continuously a 3 digits number, appearing on the screen, in reverse order.

The result in SWMT is obtained as number of correct responses and number of non-adjacent errors at 0 second and at 20 second delay respectively.

Results

In this study a total of 120 patients of schizophrenia were screened for the presence of siblings. No siblings were available for 25 of these patients. Out of the siblings that were available, 43 fulfilled the selection criteria but 7 did not complete the assessment. Therefore 36 siblings were included in the present study. The subjects in sibling group had a mean duration of education of 10.56 ± 7.72 years as compared to subjects of

control group who had a mean duration of education of

11.50. ± 8.20 years. 75% of subjects in sibling group were males as compared to 72.22% in the control group. The sibling and the control groups were statistically comparable to each other in context of sociodemographic variables such as age, gender, education, marital status, religion, place of domicile (urban or rural), occupation, family structure (joint or nuclear) and monthly family income (p values for all were >0.05).

Comparison of male and female siblings on computer based cognitive tests

The sibling group consisted of 27 males and 9 females. Table-1 shows the comparison of female and male siblings on the computer based cognitive tests (WCST, CPT& SWMT). On comparing the results of male and female siblings on WCST, it was found that there was no significant difference between males and females of sibling group on all the parameters except, percentage non-perseverative errors (p= 0.026). No significant difference was found between the male and female siblings in their performance on CPT (p>0.05). On comparison of results of SWMT, it was seen that there was no significant difference between males and females of sibling group in any of the parameters except, non-adjacent errors after 20 second delay in which the females performed significantly better(p= 0.014).

Comparison of siblings from simplex and multiplex families on computer based cognitive tests

The sibling group was divided on the basis of the number of patients of schizophrenia in their immediate family:

1) **Simplex family:** only one member in the family suffering from schizophrenia
2) **Multiplex family:** >1 member in the family suffering from schizophrenia

Most of the siblings belonged to simplex group (77.78%) i.e. one schizophrenia patient in the family and the rest (22.23%) belonged to multiplex group i.e. >1 schizophrenia patient per family.

Table 2 shows the comparison between siblings from simplex and multiplex group on various computer based cognitive tests. On comparison of their performance on WCST, it was seen that multiplex group performed significantly poorer on all the parameters except in trials administered, percentage perseverative responses and percentage perseverative errors. Although the above parameters did not show significant

Table 1: Comparison between male and female siblings on Computer based cognitive tests (WCST, CPT & SWMT)

Parameter	Females Mean ± S.D. (n = 9)	Males Mean ± S.D. (n = 27)	Comparison of Means
WCST			
Trial Administered	118.89 ± 11.86	125.26 + 6.92	t = 1.98, p = 0.056
% of total number of errors	34.33 ± 15.19	40.78 ± 11.96	t = 1.30, p = 0.200
% persevarative errors	14.89 ± 7.97	22.07 ± 8.06	t = 1.99, p = 0.055
% non-perseverative errors	19.22 ± 8.13	18.56 ± 6.30	t = 2.32, p = 0.026
% perseverative response	18.33 ± 9.48	25.22 ± 8.82	t = 0.25, p = 0.800
% conceptual level response	53.89 ± 21.30	46.56 ± 15.40	t = 1.12, p = 0.270
Categories completed	3.78 ± 2.27	3.22 ± 1.52	t = .83, p = 0.411
Trials to complete 1st category	29.44 ± 20.67	36.70 ± 29.84	t = .67, p = 0.505
CPT			
Wrong response	22.78 ± 24.39	24.04 ± 14.69	t = 0.18, p = 0.853
Missed response	15.89 ± 6.45	13.89 ± 11.26	t = 0.50, p = 0.618
Mean response time (in sec.)	2.87 ± .59	2.73 ± 1/06	t = 0.36, p = 0.718
SWMT Immediate response (zero second delay)			
Correct responses	23.00 ± 1.22	21.78 ± 1.69	t = 1.98, p = 0.055
Non-adjacent errors	0.56 ± 0.72	.67 ± 0.784	t = 0.37, p = 0.710
SWMT After 20 sec. delay			
Correct responses	20.67 ± 2.34	19.30 ± 2.10	t = 1.64, p = 0.110
Non-adjacent errors	0.33 ± 0.50	1.04 ± 0.75	t = 2.58, p = 0.014

Table 2: Comparison between siblings from simplex and multiplex families on WCST

Parameter	Simplex group Mean ± S.D. (n = 28)	Multiplex group Mean ± S.D. (n = 8)	Comparison of Means
WCST			
Trial Administered	122.43 ± 9.54	128.00 + 0.00	t = 1.63, p = 0.111
% of total number of errors	36.29 ± 12.12	49.25 ± 10.91	t = 2.72, p = 0.01
% persevarative errors	19.29 ± 8.79	23.75 ± 6.94	t = 1.32, p = 0.196
% non-perseverative errors	16.89 ± 5.32	25.13 ± 7.37	t = 3.53, p = 0.001
% perseverative response	22.43 ± 9.16	27.25 ± 9.69	t = 1.29, p = 0.203
% conceptual level response	52.46 ± 16.13	34.13 ± 12.23	t = 2.96, p = 0.005
Categories completed	3.93 ± 1.51	1.38 ± 0.52	t = 4.65, p = 0.000
Trials to complete 1st category	27.61 ± 14.76	60.38 ± 45.32	t = 3.34, p = 0.002
CPT			
Wrong response	21.25 ± 14.44	32.38 ± 23.85	t = -1.65, p = 0.108
Missed response	11.93 ± 7.92	23.00 ± 13.12	t = -2.99, p = 0.005
Mean response time (in sec.)	2.77 ± 1.02	2.75 ± 0.75	t = 0.06, p = 0.951
SWMT Immediate response (zero second delay)			
Correct responses	22.57 ± 1.29	20.38 ± 1.77	t = 3.91, p = 0.000
Non-adjacent errors	0.50 ± 0.67	1.13 ± 0.84	t = -2.15, p = 0.039
SWMT After 20 sec. delay			
Correct responses	19.64 ± 2.49	19.63 ± .74	t = .020, p = 0.984
Non-adjacent errors	0.71 ± 0.76	1.38 ± 0.52	t = -2.29, p = 0.028

difference but multiplex group performed poorer than simplex group even on these parameters. On comparison between simplex and multiplex group on CPT, it was seen that multiplex group significantly made more wrong responses (p=0.01) and significantly had more missed response (p=0.005). There was no significant difference between simplex and multiplex group in mean response time, however simplex group took more mean response time in seconds as compared to multiplex group. The siblings from multiplex group performed poorly on SWMT, as shown by the significantly less correct responses (p=0.000) and more non-adjacent errors (p=0.006) committed by them at zero second delay. The multiplex group made significantly more non-adjacent errors after 20 second delay (p=0.023) but no significant difference was observed in total correct responses after 20 second delay though multiplex group made less correct responses as compared to simplex group (p=0.984).

Discussion

The selection criteria were made stringent to minimize the confounding factors in evaluation of cognitive functions. Such confounding factors could have been extremes of age, psychiatric or significant physical disorders, significant substance use and use of medications associated with cognitive changes. Likewise a minimum level of cognitive functioning was ascertained by including only those siblings who were formally educated for more than 8 years. None of the siblings had taken any benzodiazepine on the day of cognitive assessment or five days prior to that, so that it was ensured that the sibling's psychomotor activity and reaction was not impaired and they were not sedated.

Low mean years of education of the subjects (sibling group: 10.56+2.13 years; control group: 11.50+2.59years) could be due to the fact that this hospital is a government hospital providing medical facilities mainly to people from low socioeconomic status. Also as a whole the literacy rates in India are lower than the western countries. The distribution of subjects according to religion, family structure, domicile occupation and monthly income was consistent with the patient population usually seen in the outpatient department of Department of Psychiatry, CSMMU, Lucknow, UP, India.

Mean age for the sibling group was 25.50+7.72 and this could be due to schizophrenia occurring at younger age group and the greater likelihood of siblings belonging to a similar age group

as the patients.

When females and males in the sibling group were compared among each other for the performance on various computer based cognitive tests by applying Independent t test, it revealed significant differences between the means of percentage non perseverative error where males committed significantly more errors as compared to females. Males also committed significantly more non adjacent errors after 20 second delay parameter in SWMT as compared to females. Considerations such as the later onset of psychosis in female patients and their superior prognosis may give the impression that female patients have cognitive performance superior to males. One neuropsychological study failed to support this conclusion and, without considering syndrome differences, found no sex differences (25). Egan et al., 2000 (26) found no differences between sexes in sibling or control group for any Continuous Performance Test measure. Bozikas et al., 2010 (27) assessed basic cognitive abilities: attention, working memory, abstraction, inhibition, fluency, verbal learning and memory, visual memory, visuospatial skills, and psychomotor speed on a battery of neuropsychological tests and found that the degree of cognitive impairment is the same for male and female schizophrenic patients. Further Goldstein et al., 1998 (28) studied sex differences in neuropsychological functions among patients with schizophrenia. In this study it was seen that male patients were significantly impaired across all functions in comparison with normal male subjects and on tests of attention, verbal memory, and executive functions in comparison with female patients though female patients performed significantly worse than female normal comparison subjects only on tests of attention, executive functions, visual memory, and motor functions. Hence it was here concluded that women with schizophrenia may be less vulnerable to particular cognitive deficits, especially those involving verbal processing, than schizophrenic men. Findings of our study cannot be generalized due to small sample size of female siblings as compared to males. Keeping in mind the mixed results of the previous studies, extensive future research is required to be conducted with a larger sample size to reach an unambiguous conclusion to this debate.

In the present study, siblings from multiplex family performed significantly poorly as compared to siblings from simplex families.

On Wisconsin Card Sorting Test (WCST) the sibling from multiplex

group performed significantly poorly as they made more total number of errors which implies that they had more difficulty in understanding the concept of the test as compared to siblings from simplex families (p=0.01). Among the errors, the siblings from multiplex families made significantly more number of non-perseverative errors (p=0.001). The siblings from multiplex families made significantly less number of conceptual responses (p<0.005) further supporting the notion that their understanding of the test was poorer than the siblings from simplex families. The siblings from multiplex group completed significantly more number of categories as compared to those from simplex group (p=0.000). The siblings from multiplex group also took significantly more trials to complete the first category (p=0.002) indicating that they initially had more problem in understanding the test than the siblings from simplex group.

In our study, on the test for attention, vigilance and concentration abilities – the Continuous Performance Test (CPT), the siblings from multiplex group made significantly more wrong responses i.e. the errors of commission (p=0.010), more missed response i.e. errors of omission (p=0.005) as compared to siblings from the simplex group. Findings in our study are supported by findings by Tsuang et al., 2006 (29) who compared the CPT performance of siblings from multiplex and simplex families and found that siblings from multiplex families exhibited worse performance on the degraded CPT and less proficiency in processing the perceptual load than those from simplex families. It can therefore be said that sustained attention along with perceptual load processing is more impaired in the siblings of schizophrenic patients with high familial loading and that this finding might be useful for future genetic dissection of schizophrenia.

Our findings after comparison between siblings from multiplex families and siblings from simplex families on SWMT are supported by reports from studies done earlier. Tuulio-Henriksson et al., 2003 (30) found an effect of familial loading (i.e., multiplex families versus simplex families) on a test of backward visual span. Faraone et al., 2000 (31) also showed greater impairment in individuals from multiplex versus simplex families on immediate and delayed story recall and immediate visual recall. Byrne et al., 2003 (32) showed a negative relationship between genetic liability (i.e., more than one affected first-degree relative > one affected first-and second-degree relative > affected second degree relatives) and delayed story recall, semantic verbal

fluency, and inhibition response errors on a word completion test.

The results of the study should be interpreted in view of the following limitations. Due to the constraints of a time bound study and because of the stringent selection criteria, the sample size was small and hence the results are subjected to Type II error. The small sample size makes it difficult to apply results to generalized population. The study is a cross-sectional study and to further clarify the state trait controversy longitudinal studies are needed. Cognitive functioning can depend on environmental (e.g. shared deviant rearing environment or gene-environmental interaction) and genetic factors. However, in the present study, any difference in the cognitive abilities is attributed to the genetic factors alone.

Despite the above limitations the present study has a few strengths when compared to many other studies in this area. Many confounding factors such as age, gender, significant physical illness, psychiatric conditions, substance use, and medication affecting cognition (e.g. benzodiazepines) were taken care of thus enabling the maximum attribution of the results to genetic sharing between the sibling and the index proband. The computerized version of these test were an added advantage as they ensured greater reliability, objectivity and standardization, less confrontational and formal approach. Moreover, it allowed the clinical assessors to have more time to focus on the patients rather than upon the presentation of the test material and data.

Our inclusion of only adult siblings as first degree relatives for assessment offers advantages (33) as compared to children at high risk study or including parents of those suffering from schizophrenia because having a parent with schizophrenia might interfere more with cognitive development than having a sibling with schizophrenia and adults may have already reached the peak age of risk for schizophrenia, having a parent with schizophrenia might interfere more with cognitive development than having a sibling with schizophrenia, age-linked stratification bias is reduced. Our siblings will not be affected by general decline in cognition seen with age which would have been the case if parents of those who are suffering from schizophrenia would have been included.

Conclusion

In the present study female sibling group performed significantly better than males on one parameter of WCST and one parameter of SWMT thus concluding that females may have better prognosis or greater resilience

to cognitive impairment than males. Also, the siblings from multiplex families perform poorer as compared to siblings from simplex families on various parameters of WCST, CPT and SWMT and hence support the finding of greater effect of genetic loading on cognition in siblings of schizophrenia patients. However these findings must be corroborated by further studies in future possibly with a greater sample size so that these findings could be applied to the general public.

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Implant Infection : Pathophysiology, Diagnosis and Treatment*

R.C. Mohanti

ABSTRACT

The incidence of implant infection is increasing due to frequent use of internal fixation. The incidence varies from 1% - 3.5%. The pathological changes that occur in implant infection are marked by glycocalyx formation, bacterial colonisation and adhesion. Prevention of infection consists of prophylactic antibiotics and taking care of environmental factors. The author's observation reveals *Staph.aureus* is the common organism and infection rate can be reduced by taking care of perioperative and intraoperative factors. Presence of Biofilm makes treatment difficult. Diagnosis of imminent infection is very important and requires high index of suspicion. Implant infection treated early can reverse the changes. Established infection can be treated by thorough debridement, and external fixators or exchange nailing. Aseptic peri- and intraoperative enviroment and careful surgery will lead to reduction of implant infection.

Keywords : Infection, implant, glycocalyx.

Introduction

The subject of implant infection is of contemporary relevance in view of the frequent for arthritic joints with implants are also on the rise. In developing countries like India, the infrastructure being inadequate as well as asepsis being doubtful, it is necessary to pay attention to infection occurring after implant surgery.

It has been reported by various authors that the infection rate is significant

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even in closed nailing. Approximately two million fracture fixation devices are inserted annually and two million total hip replacements (THRs) are performed in U.S.A., out of which 5000 (1%) get infected. The infection rate in total knee replacement (TKR) is 1.5-2.5% and in revision hip surgery 3.4% (1). Charnley reported infection rate 9% in 1964, but the rate came down to 1% in 1996 after he introduced laminar flow system (2, 3). Winguist *et al* (4) reported infection rate in closed nailing 1% and in open nailing 2-9%. Khan *et al* (5) reported from Pakistan 5.7% infection rate. There are very few reports on implant infection from India. Sandhu *et al* (6) reported infection rate 8.8%, Mohanti & Sahu reported 12% (7). Mukhopadhyaya (8) observed 6000 cases of bone and soft tissue surgery and observed that while infection rate in soft tissue surgery was 0.5%, in bone surgery it was 15%. Mohanti *et al* studied the incidence in 2008 and reported it to be 3.8% (9).

Pathophysiology

Any infection in bone after surgery follows a particular course. The pathological changes that occur in implant infection take a different course. The implant influences the infection in two ways: (a) while inserting the implant a closed fracture is converted to an open fracture (b) the implant being a foreign body interacts with the host tissue environment in various ways. The environment consists of soft tissue changes resulting from fracture as well as the environment changes that occur due to the surgical wound. The fractured bone and implant also contribute to the changes in tissue environment.

Implant Interaction and Bacterial Colonisation and Adhesion

The implant to serve its purpose has to remain in the body for a variable period. In case of replacement for rest of the life. Inside the body, the material of implant interacts with the host cells and bacteria. This interaction either leads to integration or failure. Once the implant is in place, there is a race between the host cells and bacteria to colonise on its surface. The cells and bacteria along with serum protein and cellular debris form a film on the implant is known as 'Glycocalyx'. The formation of clycocalyx is an important step in the implant infection process and the predominance of host immune cells or load of bacteria decides teh onset of infection. If the host cells predominate,

biointegration of implant occurs, conversely if bacteria predominate it leads to infection. Bacterial glycocalyx enhances bacterial proliferation, interferes with phagocytosis and causes further aggregation of bacteria. The immunocompromised state enhance bacterial proliferation. After some time the bacterial colonies are envoloped by a substance which is known as Extracellular Polymeric Substance (EPS). This covering protects the bacteria from the effect of antibiotics. It also limits the usefulness of culture.

Since, bacterial are the main cause of infection, the causative organism and its sensitivity has been studied by various authors. Staphylocacous aureus is the most common infecting organism, while Trampaz (10) and Khan (11) have reported 50% cases are due to Staph.aureus, Mohanti and Sahu (7) and Mohanti *et al* (6) have reported Staph.pyogenous 24.2% and Pseudomonas 18.8% to be the causatie organisms. Sensitivity of these organisms have changed over the years with the introduction of newer antibiotics. In 1980s, it was ampicillin, gentamicin and chloromycetin (6, 7). Ciprofloxacin, linezolid and cefoperazone-salbactum are found to be effective in the past decade (5, 9, 10).

Preventive Measures

Antibiotic prophylaxis and control of various environmental factors are necessary to prevent infection in implant surgery. The role of prophylactic antibiotics has now been established in implant surgery after some initial debate about its beneficial effect. The most common organism is Staphylococcus aureus and coagulase negative Staphylococci. Sensitivity of these organisms have been studied by Sandhu *et al* (6) and Mohanti and Sahu (7), and has been discussed in preceeding paragraph. Trampaz *et al* (10) used ciprofloxacin and linozalid. However, Mohanti *et al* (9) found the organism sensitive to cefaperazone plus salbactum. Discovery of newer antibiotics has lead to changes in sensitivity. At present cefazolin or cefuroxime are the antibiotic of choice (11). Some authors have found rifampicin useful in treating resistant strains (10). Aminoglycosides can be used in fixation of open fractures. The best time to administer prophylactic antibiotics is 30-60 minutes before incision. One intraoperative dose should be given in case of prolonged procedure.

Regarding post operative antibiotics regime, western literature suggest 2-3 post operative dose. However, in India most surgeons recommend use of antibiotic to continue the cause atleast upto 5 days after surgery, if not more. One of the local methods of prophylactic antibiotic is the use of antibiotic impregnated cement, though it makes the treatment of implant infection difficult once the infection sets in.

Environmental Factors which can influence infection

The environmental factors which influence infection can be :

(a) Patient related factors; e.g. anaemia, diabetes, smoking, etc. and local: unhealthy skin, site, etc.

(b) Hospital related factors; e.g. O.T. personnel, traffic, talking, material, type of O.T.

(c) Intraoperative factors; e.g. procedure, duration, decision hemostasis, drain, implant.

(d) Skill of Surgeon and his team.

There are many other factors predisposing infection other than the factors mentioned above. In India, most of patients are anaemic. Though the surgeons wait to control diabetes before operating, he the same is not done he can not wait for correction of anaemia. Besides, the asepsis of OT material, number of OT personnel, OT traffic and talking are common problem in operation theatres in India. These factors are commonly ignored by the OT administration. Extensive dissection, lack of hemostasis, lengthy procedure predispose to infection. It has been reported that when duration of procedure is less than 1 hour the infection rate is 1.3%, while when it is more than 3 hours, it is 4% (12) Drains predispose to infection and it should be closely monitored. The quality of implant plays an important role. This is a problem in India where standardisation of implant is lacking and substandard implants are available in the market. Lastly but not the least, the skill of the surgeon and his team plays an important role which is often overlooked. The planning, decision making, meticulous dissection, choice of implant, hemostasis and closure are all in the surgeon's hand and all surgeons are not same.

Our Experience

As it has been already mentioned, there are very few studies on implant infection done in India. We have made two studies, one in 1982 and another is

an ongoing study from 2008-09. The main findings are shown in Table 1.

The carrier state was observed from examination of swab from skin, nose and throat of patients. In both the series 30-35% were positive for organism like Staph.aureus. There were one case each of proteus and Pseudomonas in the 1982 series. However relationship could not be established between wound infection and carrier state. Geeta Mehta (13) has also reported similar findings. Most of the infection with increased preoperative hospital stay. Sandhu *et al* (6) have also observed that long pre-operative hospital stay increases the chance of infection. Similarly, we have also observed that duration of surgery influences risk of infection. Sawyer *et al* (12) have observed that infection rate is 1.3% when duration is less than one hour while it is 4.4% when duration is more than 3 hours. Sometimes various specialities operate in the same operation theatre. In our series of 1982 the operations were less than one hour while it is 4.4% when duration is more than 3 hours. Sometimes various specialities operate in the same operation theatre. In our series of 1982 the operations were carried out in operation theatre where General surgical

Table 1 : Characteristics observed in implant infections in Indian studies

	Mohanti & Sahu 1982	**Mohanti et al 2008-09**	**Comments**	**Reference**
Carrier state Skin swab Nasal swab	30-35% Positive	30-40% Positive	Staph aureus Pseudomonas Proteus, E.coli	Mehta (2) Staph aureus, Staph.epidermis
Pre-op. hospital stay, Duration of surgery	15-20 days 1-2 hrs.	15 days 102 hrs.	Risk of infection↑ Risk of infection↑	Sandhu *et al* (6) Sawyer (12) < 1 hr - 1.3% < 3 hrs. - 4.4%
Type of OT	Combined OT	Specialized OT		
Pre-op. & Post-Op. Antibiotics	Ampicillin Chloromycetin	Cefoperazone + Salbactum	Rifampicin Vancomycin?	Trampaz (10)
Infection rate	12%	3.8%	Variable	

emergencies were done and as a result infection rate was very high. While in our series of 2008 infection rate was reduced as the theatre was used only for clean orthopaedic surgery. Antibiotic sensitivity as observed by the author has been discussed in preceeding pages. The infection rate in the 1982 series was very high (12%). This is due to operation being done in a combined OT. This was brought down in 2008 with the use of a separate operation theatre for clean Orthopaedic surgery.

Diagnosis

Every orthopaedic surgeon after putting the implant is afraid of one possibility, that is infection. Most other complications can be solved, but infection once established is difficult to threat. Some authors classify clinical features of infection as Type - I, II, III (14). Others classify as early, delayed and late (15), However, in order to prevent damage and retrieve the situation a very early diagnosis should be made and if possible infection controlled and changes reversed. We have grouped the symptoms as

(a) Imminent - 3rd - 4th day

(b) Early - 5th - 10 days

(c) Late - 10 - 20 days

It is the diagnosis of "imminent infection" which is most important. To diagnose imminent infection, surgeon has to have a "high index of suspension" and alert to the telltalle signs of infection.

Signs of Imminent Infection

(a) Pain and fever persisting beyond 72 hours. (Analgesics should be stopped after 48 hrs.)

(b) General well being and comfort of the patient disturbed.

(c) Throbbing pain at the site

(d) Persistence of distal edema.

If the above signs are present one should become alert and closely monitor the patient. The wound should be inspected after 72 hrs, with a close watch for tension and edema around and in between the sutures. Induration around the wound, tenderness around the area, and presence of discharge must be recorded for. These signs suggest imminent infection. The discharge should be sent for culture and sensitivity. At this stage, the laboratory investigations play only a supportive role with leucocytosis, ESR > 40, CRP > 10 mg/ltr are suggestive of infection. Aspiration cytology and culture can be

done.

Treatment of imminent infection

If the pain, fever persists beyond 4-5th day the patient should be taken to the operation theatre, remove the stitches, clean the wound, irrigate with antibiotic solution, if necessary set up suction irrigation and close the wound. Change the antibiotics if necessary. With these steps one can control the infection and reverse the process.

Treatment of established infection

Sometime, after 10-12 days when the stitches are removed there is a burst of pus discharge and partial wound dehesion. In such a situation, the wound should be opened up and debridement should be done till a healthy bleeding area is left behind. If possible close the wound with antibiotic beads or suction irrigation. Proper antibiotics should be given for atleast 3 weeks.

Treatment of deep infection

Sometimes patients come late weeks or months after with swelling and discharging sinus, X-Ray shows signs of osteomyelitis. After culture sensivitiy test, radical debridement, seqrestrectomy and some times corticotomy should be carried out. At this stage the surgeon is faced with a dilemma i.e., to remove the implant or not. For treatment of infection implant should be removed, but for healing of fracture fixation is necessary. The stability of fixation should be assessed. If fixation is stable keep the implant along with irrigation and prolonged antibiotics. Then wait and watch. If the union progresses, remove implant after union. If fixation is unstable, remove implant, apply external fixator and follow it up with exchange nailing.

Some of these patient may be left with skin loss, exposed implant, and they will need reconstructive procedure.

Conclusion

(a) An accurately reduced and internally fixed fracture but infected is a disaster.

(b) Presence of "Biofilm" makes the treatment of implant infection difficult.

(c) Awareness of factors predisposing infection and careful surgery can provide **"Safety with Excellence".**

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