

Outcome of Mucormycosis in Liver Transplantation: Four Cases and a Review of Literature

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Mucormycosis is a rare but highly invasive fungal infection that occurs in transplant recipients. The literature contains descriptions of 12 cases of mucormycosis after orthotopic liver transplantation (OLT). This report describes the fatal courses in four patients at our center who developed mucormycosis after liver transplantation.

Of 51 liver transplant recipients who received grafts between December 1993 and April 2003, 4 (7.8%; 3 males and 1 female) developed mucormycosis. The primary liver diseases in the four cases were Wilson's disease, autoimmune hepatitis, primary biliary cirrhosis, and cryptogenic cirrhosis. Three of the transplants were harvested by another team and shipped to our center.

We concluded that selection of poor transplant candidates, prolonged antibiotic therapy and/or hospitalization prior to OLT, and breaks in aseptic technique during harvesting, shipping, and during operation are the main reasons for the high incidence of mucormycosis in our OLT patients.

Keywords: Liver transplantation, Fungal infection, Mucormycosis, Mortality, Outcome, Shiraz, Iran

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Mucormycosis is a rare opportunistic fungal infection that is most often caused by species of the genera *Rhizopus*, *Rhizomucor*, and *Cunninghamella*. This type of infection is usually associated with hematologic diseases (leukemia, lymphoma, myeloma), [1,2] diabetes mellitus [1,3,4] renal failure [2,3] solid tumors [2], and organ transplantation [2,5-13], particularly cases that aggressive immunosuppression is used [10]. The most frequent form of presentation is rhinocerebral, followed by pulmonary, skin and soft-tissue, disseminated, and gastrointestinal forms, respectively.

The fungal agent typically invades the vessel walls, as the initial site of invasion, causing infarctions and ultimately necrosis with black and white pus. This characteristic is unrelated to the site of primary infection therefore; Mucormycosis continues to be associated with high mortality rate.

Our literature search revealed 12 cases of mucormycosis after OLT. This report describes fatal courses in patients who developed this fungal infection after OLT at our center.

Patients and Methods

Liver transplantation program has been established at Shiraz Nemazee Hospital in 1993. For 5 years, from December 1993 to March 1998, our team performed 8 liver transplantations and the 1-year survival rate for these patients was 37.5%. From March 1998 to April 2003, we performed 43 OLTs, and the 1-year survival rate for the 28 patients who were followed for at least a year was 82.1%. Out of the overall 51 OLTs (from 1993 through 2003) 8 cases were living-related liver transplantations, and the other 43 undergone cadaveric OLT. For all 51 cases, the baseline immunosuppression was a triple-drug regimen of steroids, azathioprine and cyclosporine. In some patients in which cyclosporine toxicity developed or in patients with good financial support, Tacrolimus (FK506) was used

instead of cyclosporine. Acute rejection was managed with intravenous boluses of methylprednisolone (Three boluses of methylprednisolone which were given in the dose of 15 mg/kg per each day). Here we describe four fatal cases of mucormycosis that occurred in liver transplant recipients at our hospital.

Case 1

A 45-year-old man with Wilson's disease underwent OLT in October 1999. The transplant was harvested by another team in Tehran, Iran and then shipped to our center. We performed OLT using the piggybag technique with duct-to-duct anastomosis, and with placement of a T-tube in the common bile duct. Postoperatively, the patient did well for 5 days, but he then developed lung infiltrations on chest X-ray (CXR), respiratory failure and impaired renal function. Accordingly, FK506 toxicity and acute tubular necrosis (ATN) were our two differential diagnoses. Therefore, the dose of FK506 was reduced. Blood testing showed increased serum liver enzyme levels, and the patient exhibited flapping tremor. In light of these problems, pulse therapy with methylprednisolone (15 mg/kg/day for three doses) was administered. By the end of the second week post-surgery, the patient's condition had deteriorated even further. Bile flow had completely stopped, and ultrasonography demonstrated significant heterogeneous echogenicity and multiple hypoechoic foci in the liver parenchyma, high-resistance flow in the hepatic artery, ascites, mildly increased cortical echogenesity in both kidneys, and pleural effusion. Echocardiography was unremarkable. The patient died 15 days after OLT. A complete autopsy revealed massive hepatic infarction, and vascular thrombosis, mucormycosis and *Candida* spp. in the liver (Figure 1), stomach, lung and heart.

Case 2

A 20-year-old man with cryptogenic liver cirrhosis underwent OLT in September 2001. Fifteen years earlier, he had had a patent meso-caval shunt placed as treatment for esophageal varices, and the shunt was still patent. The donor liver was harvested and shipped from Tehran to Shiraz and the operation was successful. Hepatectomy was performed using the piggybag technique, and Roux-en-Y choledochojejunostomy was carried out in the transplanted liver. The patient did well for the first 14 days post-surgery, and liver function test (LFT) during this period

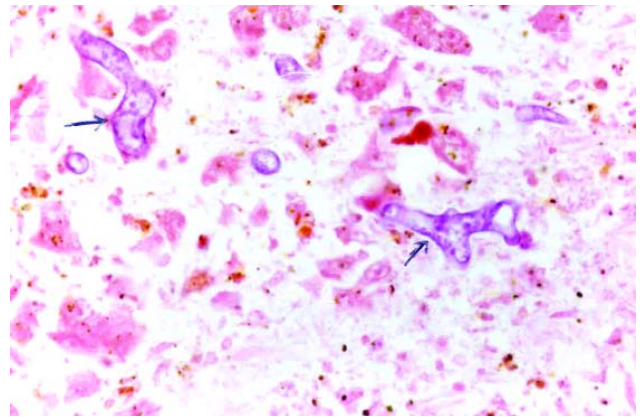


Figure 1. A section of infarcted liver from Case 1 showing mucormycosis in the liver parenchyma (H&E_1000)

revealed improvement. However, his blood urea nitrogen (BUN) and creatinine (Cr) levels were both elevated during this period (60 mg/dl and 2.3 mg/dl), respectively. Cyclosporine was discontinued and FK506 was started for the patient. On the 14th day post-surgery he became febrile and complained of severe pain in the hypochondriac area. CXR showed marked right-sided pleural effusion. Ultrasonography on day 14 showed two subcapsular hypoechoic lesions in the liver parenchyma and these increased in size over the next 2 days (Figure 2). A second laparotomy was carried out 16 days after OLT. This revealed extensive necrosis of the 7th and 8th segments of the liver, and edema, erythema and necrosis of the right hemidiaphragm, abdominal wall, retroperitoneum, and perinephric area. Aggressive debridement was performed and several specimens from all of the exposed regions were taken and submitted for pathology study. Histopathological examinations revealed mucormycosis. The patient was started on amphotericin B on day 15 with 1 mg as test dose and gradually the dose increased from 1 mg/kg/day to ulti-

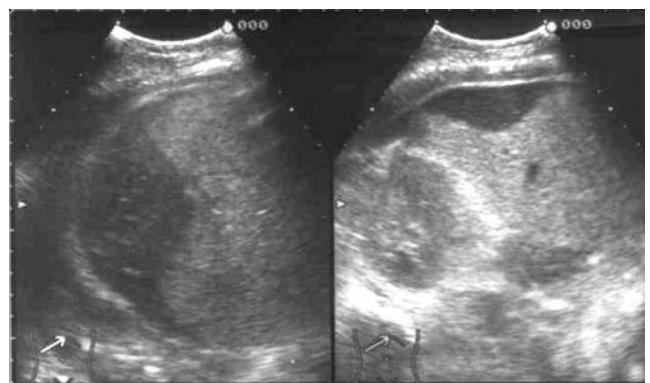


Figure 2. Sonography of the liver from Case 2 shows two hypoechoic lesions (arrow) that represent necrosis caused by mucormycosis.

mately 6 mg/kg/day. But he died of respiratory failure and septic shock on the 20th day post-transplantation.

Case 3

A 36-year-old man with primary biliary cirrhosis underwent OLT in November 2001. He was admitted to the medical ward 3 days prior to the operation due to hepatic encephalopathy and elevated BUN and Cr (60mg/dl, and 1.6 mg/dl, respectively) and had received antibiotic therapy (Ciprofloxacin, 500 mg orally, twice daily and paromycin 250 mg orally every 6 hours). Donor liver belonged to a cadaver in Shiraz. After OLT, he exhibited oliguria in the first 2 days post-surgery so the intravenous cyclosporine dose was reduced. However, over the next 8 days, he developed persistent polyuria with BUN 50-90 mg/dl and Cr 0.3-1.0 mg/dl and conclusively a course of methylprednisolone bolus therapy was initiated for the patient. The patient's level of consciousness deteriorated over the first 10 days after OLT, but brain computed tomography on day 10 showed nothing abnormal. On day 12 post-surgery, he was re-intubated and placed on ventilatory support. Serial CXRs obtained from day 10 to day 12 post-surgery showed left paracardiac infiltration followed by diffuse bilateral lung infiltration. On day 12, examination revealed that the patient was febrile, ultrasonography showed a hypoechoic lesion in the transplanted liver, with deterioration of LFT. On day 13 post-transplantation, a second course of methylprednisolone was administered for acute rejection. Two days later, tracheostomy was performed and examination and culture of bronchoalveolar lavage (BAL) fluid revealed no fungal infection organisms. The liver lesion continued to enlarge and the patient failed to improve, so laparotomy was performed 15 days after OLT. This revealed necrosis of segment 8 of the transplanted liver. The lesion was debrided, and mucormycosis was detected on pathological examination of the specimen. Treatment with amphotericin B was initiated, but another laparotomy was performed 4 days later due to additional deterioration of the patient's condition. This showed diffuse mucormycosis involvement of the liver and peritoneal cavity. The patient died within 12 hrs of this third operation.

Case 4

A 16-year-old female with autoimmune hepatitis underwent OLT in January 2001. She was hospitalized and treated for spontaneous bacterial peritonitis (SBP)

and hepatic encephalopathy 12 days before the operation and she had received antibiotic therapy (imipenem, 500 mg IV every 6 hours for 8 days). Her treatment regimen included cyclosporine (3 mg/kg/day for 3 months), methylprednisolone (4 mg IV every 8 hours for 8 days), imipenem (500 mg IV every 6 hours for 8 days) and fluconazole (100 mg twice daily for 6 days). The donor liver was harvested and shipped from Tehran to Shiraz. Orthotopic liver transplant was performed using the piggybag technique and duct-to-duct biliary anastomosis, and all went smoothly. The patient developed infiltration of the left upper lobe of the lung on the 2nd postoperative day, and thus could not be weaned off the ventilator. The infiltration extended to both lungs, and she developed a persistent fever and respiratory failure (Figure 3). Testing of BAL fluid was negative for fungal infection. The patient died on the 8th day after OLT due to septic shock and respiratory and renal failure. Pathological examination of the liver and lung graft tissue showed mucormycosis in the lung (Figure 4) and pericentral coagulative necrosis in the



Figure 3. CXR of Case 3 shows infiltration of both lungs.

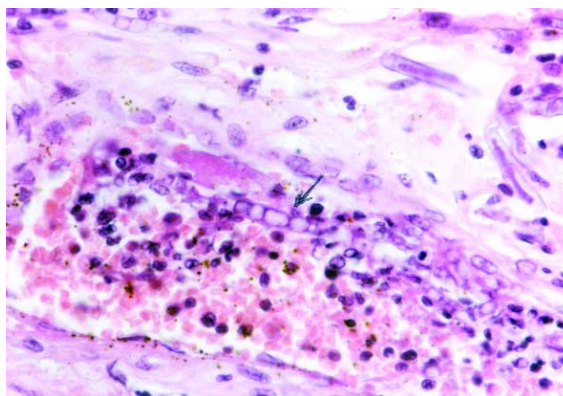


Figure 4. A section of the lung from Case 4 shows mucormycosis hyphae invading the vascular wall, (arrow) (H&E _1000).

transplanted liver, which was suggestive of liver ischemia.

Discussion

Fungal infections are still important causes of mortality and morbidity after transplantation, and most occur within the first 2 months post-operative. A wide variety of fungal species are encountered in OLT patients, but *Candida spp.* and *Aspergillus spp.* are the most common [5,14]. Fungi of the subclass Zygomycetes in the order Mucorales are associated with high morbidity and mortality as well, and are also significant concerns in this patient group. Zygomycetes are filamentous phycomycetous fungi, and many species are potential human pathogens.

Mucormycosis is the common name given to several different diseases caused by fungi of the order Mucorales; however, mucormycosis is also referred as phycomycosis and zygomycosis [15]. Zygomycosis are ubiquitous in the environment, and infection can result from inhalation, direct contact, or ingestion of spores. They are not usually commensals, but nosocomial exposure can lead to colonization and invasive disease [16,17]. The reported incidence rates of zygomycosis complicating solid-organ liver transplantation range from 0% to 9% [9]. Diagnosis of mucormycosis is usually delayed because the condition tends to be misidentified as a bacterial infection. Once mucormycosis is suspected or confirmed by fluid culture or biopsy [16], immediate treatment is vital. This includes reduction of immunosuppressant doses, administration of liposomal amphotericin B, and repeated surgical debridement [3,8-11]. The latter often requires resection of necrotic tissue until the surgical margins are free of infection. Many authors have reported that cultures from suspicious sites are usually negative for fungi, and this was the case in all of our patients. The diagnosis of mucormycosis should be made by tissue biopsy rather than by fungal culture [3,8-11].

Mucormycosis can be categorized as rhinocerebral, pulmonary, gastrointestinal, cutaneous or disseminated. A review of the literature revealed only 12 previously reported cases of mucormycosis after OLT [5,6,8,9,10-12,18-20] (Tables 1 and 2). Ten of these patients were males, and the mean age of the 12 individuals was 40 years (range, 19-60 years). Our four cases were three males and one female, and the mean

age was 29 years (range, 16-45 years). The incidence of mucormycosis in our 51 OLT cases was 7.8%. The time of onset after OLT varies widely, with a reported range of 3-2920 days [5,6,8,10,12,18]. The mean time of onset in our four cases was 13 days post-surgery (range 8-15 days) (Table 2).

After OLT cutaneous mucormycosis most frequently develops at the site of the laparotomy wound. In one case in the literature, this form of infection developed at a drain exit site and another patient developed cutaneous mucormycosis above the eye [5,10,18]. Rhinocerebral mucormycosis initially develops in the nasal sinuses and extends to the paranasal sinuses and the ethmoidal and sphenoidal cells [18]. The rhinomaxillary form should be suspected in any transplant recipient who exhibits fever, maxillary swelling, and edema and opacification of the sinuses

Table 1. Characteristic of previously reported patients who survived mucormycosis after orthotopic liver transplantation (OLT).

Author/Re	Age (yrs) /sex	Infection site	Time of onset after OLT	Amphotericin B	Surgery
8	53/M	Maxillary sinusitis	1095 days	5-6 gm	Debridement
10	38/M	Cutaneous (wound)	7 days	1.5 gm	Debridement
18	50/M	Cutaneous (wound)	40 days	4.5 gm	Debridement & excision
18	48/M	Cutaneous (drain exit)	60 days	3.5 gm	Excision
18	46/M	Maxillary sinusitis	110 day	5 gm	Debridement, maxillectomy
19	45/M	Gastrointestinal (gastric ulcer)	20 days	4 mg/kg/d	Excision

Table 2. Characteristic of previously reported patients who did not survive mucormycosis after orthotopic liver transplantation (OLT). The four last cases are the cases presented in this report.

Author/Re	Age (yrs) /sex	Infection site	Time of onset after OLT	Amphotericin B	Surgery
5	NR	Cutaneous (wound)	7 days	NR	NR
6	19/F	Disseminated	NR	Yes	No
9	28/M	Gastrointestinal (gastric perforation)	2920 days (5 days after re-OLT)	6 mg/kg/d	Esophagectomy, hemigastrectomy, debridement
11	35/M	Gastrointestinal (cecal perforation)	23 days	No	Cecal wedge resection
12	60/M	Cutaneous (above eye)	7 days	Yes	Excision
20	32/M	Rhinocerebral	3 days	Yes	No
Case 1	45/M	Disseminated	14 days	2 mg/kg/d	No
Case 2	20/M	Gastrointestinal (liver)	14 days	Yes	Intensive debridement of liver
Case 3	36/M	Disseminated	15 days	Yes	Intensive debridement
Case 4	16/F	Pulmonary	8 days	No	No

NR: Not Reported

on computed tomography scanning [3,8,9]. The surgical treatment for rhinomaxillary mucormycosis consists of early extensive debridement [8], and partial maxillectomy may be necessary in severe infections. Cerebral mucormycosis is a severe, usually lethal infection. It is caused by spread of fungal invasion from the paranasal sinuses to the retro-orbital region, and further extension through the orbit into the brain [3,19]. Thrombosis of the cavernous sinus and death are frequent outcomes in these cases.

Pulmonary mucormycosis usually occurs in immunosuppressed patients and can sometimes be evidence of previous bacterial [2,7,14] or cytomegalovirus pneumonia [20]. Diagnosis is suspected when a patient exhibits fever cough, dyspnea, hemoptysis, and radiologic findings (infiltrate, pleural effusion, and abscess) and it is confirmed by culture or lung biopsy. Pulmonary wedge resection may be sufficient, but sometimes lobectomy is required to eradicate mucormycosis confined to the lung. This form of the disease is associated with 65% mortality [12]. Only three cases of gastrointestinal mucormycosis after OLT have been reported to date [9,11,19]. Despite aggressive surgical treatment two of these patients died. As the name suggests, the disseminated form of mucormycosis can involve various organs, specifically the lung, heart, brain, liver and/or kidney. To date, premortem diagnosis of this serious infection has been possible in only 50% of cases, and only 2 of 10 reported patients with this form have survived [6].

In the 12 previously published cases of mucormycosis after OLT [5,6,8,9,10,12,16], the overall mortality rate was 50% and the three patients with the cutaneous form, two with maxillary sinusitis, and one with the gastrointestinal form survived the illness (Table 1). The best results are achieved in cases where there is prompt diagnosis and immediate aggressive treatment. High dose of amphotericin B must be administered in a very short period of time, and all infected tissue must be surgically resected. Two of our four cases were the disseminated form of mucormycosis, one was the gastrointestinal form, and one was the pulmonary form. All these cases were fatal (%100 mortality), which is in the line with previously reported mortality in the literature for these forms of mucormycosis (Tables 1 and 2).

With respect to clinical forms of mucormycosis after OLT, the most frequent presentation in our 4 patients and the 12 previously reported cases were the

skin-subcutaneous form with the frequency of 5 out of 16 (31.3%), followed by the gastrointestinal form (frequency of 4 out of 16, 25%) and the disseminated form (3 out of 16, 18.8%). The next most frequent presentations were the maxillary sinus form (2 out of 16, 12.5%), followed by the more severe rhinocerebral form (1 out of 16, 6.3%), and the pulmonary form (6.3%) (Tables 1 and 2). The type of infection relates to the mode of transmission, with inhalation of spores leading to the rhinomaxillary- cerebral forms, direct inoculation leading to the cutaneous form, ingestion leading to the gastrointestinal form, and hematogenous seeding leading to pulmonary and disseminated forms [3,8,9].

Predisposing factors noted in literature review

Authors have noted a number of underlying diseases and potential predisposing factors for the development of mucormycosis after OLT. These include diabetes mellitus, especially in the setting of acidosis (ketoacidosis) [10,12], cholestasis [21], hypertransfusion [21], acute rejection [9,10], treatment with high-dose of steroids or OKT3 [10], renal failure [10,12], bacterial infection [6,11,12], and re-transplantation [9]. Diabetic ketoacidosis is considered a risk factor because species of the order Mucorales grow best in acidic high-glucose medium [22]. Additionally, steroid therapy in diabetics is known to inhibit macrophages activity and impairs chemotaxis of polymorphonuclear leukocytes [23].

Predisposing factors noted in our review

As mentioned, our liver transplantation program was started in 1993, so our experience with these procedures is relatively limited. The incidence of mucormycosis in our 51 OLT cases to date is 7.8%. The senior surgeons on our team have participated in more than 1000 renal transplantations, and none of these recipients developed mucormycosis. Prior to liver transplantation, one of our four patients had hepatic encephalopathy and SBP, and another had encephalopathy alone. Both of these individuals received antibiotic therapy before OLT. After surgery, two patients developed respiratory failure, and one of them developed renal failure as well. None of the four patients was diabetic, but all required at least one course (range 1-2 gr/day) of high-dose methylprednisolone for acute rejection. The operation time in our four cases ranged from 6 to 14 hrs. All the transplant-

ed livers in these cases were perfused within 14 hrs of harvesting; however, we noted that three of the four cases of mucormycosis occurred in transplanted livers (a total of seven) that had been harvested and shipped to our center from Tehran. Also, three of four cases developed over four consecutive months, which may indicate break in septic techniques. We conclude that selection of poor transplant candidates, prolonged antibiotic therapy and/or hospitalization prior to transplantation, and breaks in aseptic technique during harvesting, shipping, and/or transplantation are the main reasons for the high incidence of mucormycosis in our OLT patients.

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