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Opportunistic Deep Fungus Infections in Children

A Histopathological Study*

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Mycotic diseases may pathologically be divided into superficial and deep fungus infections. In the superficial type, the skin, oral cavity and vagina are affected. Deep fungus infections are more dangerous than superficial types and invasion occurs beyond those areas.

Deep fungus infection may be seen as a primary infection (infection occurring in healthy individuals)[1] but it is frequently a secondary (opportunistic) condition in which the fungus becomes a pathogen due to a reduced host resistance.[2-5]

The purpose of this histopathological study is to evaluate the incidence, types of fungi, organ involvement, various predisposing diseases and conditions in children with opportunistic deep fungus infections seen at the histopathology laboratory of Hacettepe Children's Hospital, between June, 1964 and September, 1970.

Materials and Methods

The material was selected from 800 personally-studied cases autopsied between June, 1964 and September, 1970. For various reasons the material was not consecutive. A total of 25 cases of deep fungus infection were detected. In addition, 2 more cases were included of which diagnoses were made during life. The patients died within 2 months

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of diagnosis but permission for autopsy could not be obtained. Cases were recorded for study when a fungus could be seen or was suspected to be present in the deep-seated tissue sections stained with hematoxylin and eosin (H&E). All histopathological material was fixed in 10 % formalin solution and embedded in paraffin.

In addition to routine H&E staining for the demonstration of fungi in sections, special stainings with periodic Acid-Schiff reaction (PAS), Gridley's Stain for fungi, Gomori's Methenamine-Silver Nitrate technique (Groccott's application to fungi), and toluidin blue metachromasia were carried out when necessary. The fungi were identified by their morphological characteristics in tissue sections. The criteria for histopathological diagnosis of fungi have been well-documented in the text-books of mycology[6] and pathology[7-8] and will not be repeated here.

Following a pertinent diagnosis of fungus infection, clinical and pathological findings were detected from patient's charts and autopsy protocols. The patients' age, sex, type of fungus, site of involvement, predisposing diseases and conditions were studied and pertinent findings are summarized in Table I.

Observations :

A total of 27 cases were found. The incidence in autopsied cases was 3.2 %, 5 types of fungus infections being detected. There were 18 cases of candidia, 4 cases of Aspergillus, 3 cases of mucor and one of each cryptococcus and penicillium infections. Observations on specific types of infections are recorded here and the salient features of all of them are discussed below.

Aspergillus Infections : Four of the 27 fungus infections were aspergillosis. (Figures 1 - 3). Case No. 1 was published previously.[9] The ages ranged from 8 to 12 years and all were boys. In 3 of the 4 cases, the lungs were involvedand the histopathologic reactions were necrotizing bronchopneumonia in two and, abscesses in one case. Granulomatous reactions with epitheloid cells, giant cells and eosinophils without necrosis were present in the liver in another case. In one case the gastro-intestinal tract was involved, together with the lungs. Clinically, Case No. 4 consisted of allergic disease in the patient. The predisposing disease was leukemia in 3 of the 4 cases who all had leukopenia and were treated with steroids and antibiotics. In some of these cases methotraxate, purinethole or endoxan were also given. One of the 4 cases (Case No. 2) had liver

TABLE I

Fungus Infections	Case No.	Age	Sex	Site of Involvement	Material for Study	Histological Reaction	Predisposing Diseases	Predisposing Conditions
Aspergillosis	1	10 y	M	Lung and pleura	Autopsy	Necrotizing bronchopneumonia	Leukemia	Leukopenia Antibiotics Steroids
"	2	10 y	M	Liver	Incision Biopsy of liver	Granulomas with eosinophils	Obstructive Biliary cirrhosis	Died of liver failure
"	3	8 y	M	Lung and ulcer of stomach	Autopsy	Necrotizing Bronkopneumonia and abscess	Leukemia	Leukopenia Antibiotics Treatment Steroids
"	4	12 y	M	Lung	Autopsy	Lung abscess	Leukemia	Leukopenia Antibiotics Treatment Steroids Allergic reactions
Candidiasis	5	8 Day	M	Oesophagus	Autopsy	Epithelial erosion with acute inflammation	Tricuspid atresia bronchopneumonia	Naso-gastric tube
"	6	4 Mo.	F	G. I. tract and lung	" "	Epithelial erosion with acute inflammation	Malnutrition enteritis and sepsis	Acidosis, Dehydration
"	7	2 Mo.	F	G. I tract	"	Epithelial erosion with acute inflammation	Gastro-enteritis, Bronchopneumonia	Acidosis
"	8	20 Day	M	Oesophagus and lung	"	Epithelial erosion with acute inflammation	Congenital adrenal hiperplasia	Acidosis
"	9	3 Mo.	M	G. I. Tract	"	Gastric ulcer and acute inflammation	Gastro-enteritis Bronchopneumonia	Naso-gastric tube
"	10	13 Day	M	G. I. Tract	"	Gastric ulcer, acute inflammation	Ileal atresia, post-operative enteritis with ulceration	Naso-gastric tube
"	11	2 Mo.	M	G. I. Tract	"	Acute inflammation and intestinal ulcers	Gastro-enteritis Bronchopneumonia	Acidosis, Dehydration
"	12	3. 5Y.	M	Oesophagus	"	Acute inflammation	Gastro-enteritis Bronchopneumonia	–
"	13	2.5 Y.	M	G. I. Tract and lung	"	Acute inflammation and ulcers	Malnutrition Gastro-enteritis Bronchopneumonia	Acidosis Dehydration
"	14	4 Mo	M	G. I. Tract and lung	"	Acute inflammation and ulcers	Gastro-enteritis Pneumooptis-carini pneumonia	Acidosis Dehydration
"	15	6 Mo	F	G. I. Tract and Lung	"	Acute inflammation and epithelial erosion	Malnutrition, Enteritis, Thymic dysplasia	Acidosis Dehydration
"	16	14 Y.	M	G. I. Tract	"	Ulcer and inflammation	Typhoid fever	Leukopenia Antibiotic Acidosis
"	17	10 Day	M	Oesophagus	"	Acute inflammation	Enteritis and Bronchopneumonia	Naso-gastric tube
"	18	14 Mo	M	Oesophagus and cardia	"	Acute inflammation	Thymic dysplasia, Malnutrition renal disease, cytomegalic inclusion disease	Uremia Dehydration Hypercalcemia
"	19	15 Mo	M	G. I. Tract	"	Acute inflammation	Malnutrition Gastro-enteritis Bronchopneumonia	Dehydration Acidosis
"	20	3 Mo	F	Oesophagus and urinary bladder	"	Necrotizing inflammation of urinary bladder	Renal disease and malnutrition	Acidosis Catheterization of urinary bladder
"	21	10 Y	M	Oesophagus and stomach	"	Acute inflammation	Nephropathy	Steroid treatment
"	22	1.5 Y	F	Lung	Partial autopsy	Acute inflammation	Chronic bronchitis and interstial pneumonia	Antibiotic treatment Suctioning of mucus from bronchi
Cryptococcosis	23	4 Mo	M	Generalized without central nervous system involvement	Autopsy	No	Malabsorption syndrome	A Avitaminosis
Mucormycosis	24	4.5 Y	M	Brain	"	Necrotizing encephalitis with thrombosis	Renal disease malnutrition	Uremia Acidosis
"	25	7 Y	F	Lung, Thyroid, Spleen	"	Necrotizing bronchopneumonia and thrombosis	Leukemia	Leukopenia Antibiotics Steroids
"	26	12 Y	M	Lung	"	Necrotizing bronchopneumonia	Leukemia or lymphoma	Leukopenia Antibiotics Steroids and endoxan
Penicillosis	27	8 Y	F	Blood fluid, pleural, possibly in lung?	Bloody pleural fluid aspiration during life	Cultures of fungi and positive skin test.	Leukemia Bronchopneumonia	Leukopenia

Figure 1
Aspergillus in lung, H+E x 250. Fungi are seen but not as good as silver stain which is shown in Figure 3.

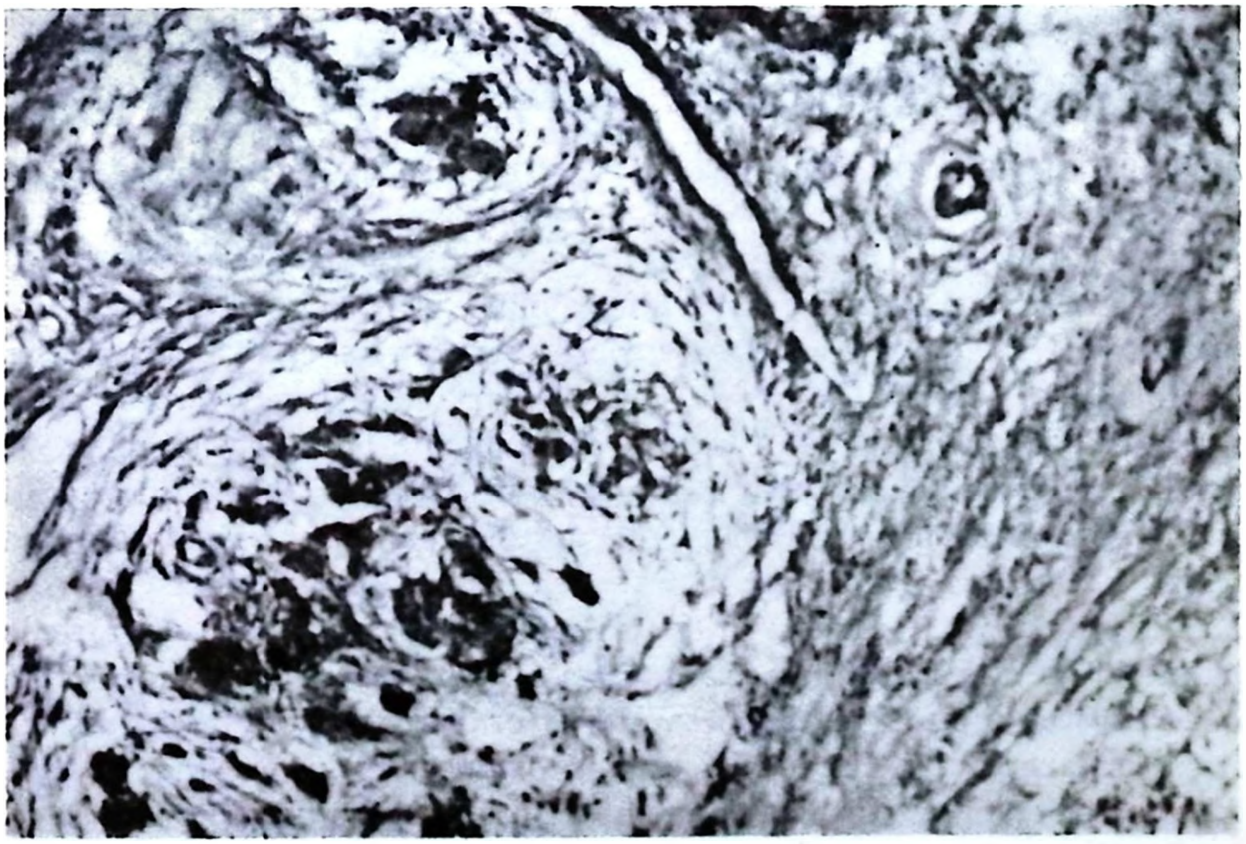

Figure 2
Aspergillus granuloma in liver, P.A.S. x 100

failure due to obstructive biliary cirrhosis. (This case was referred to Profs. I. N. Dubin and R. Knight of the Women's Medical College of Pennsylvania, Philadelphia, U. S. A.).

Candida Infections: Candidiasis was the most frequent infection. (Figures 3 and 4).

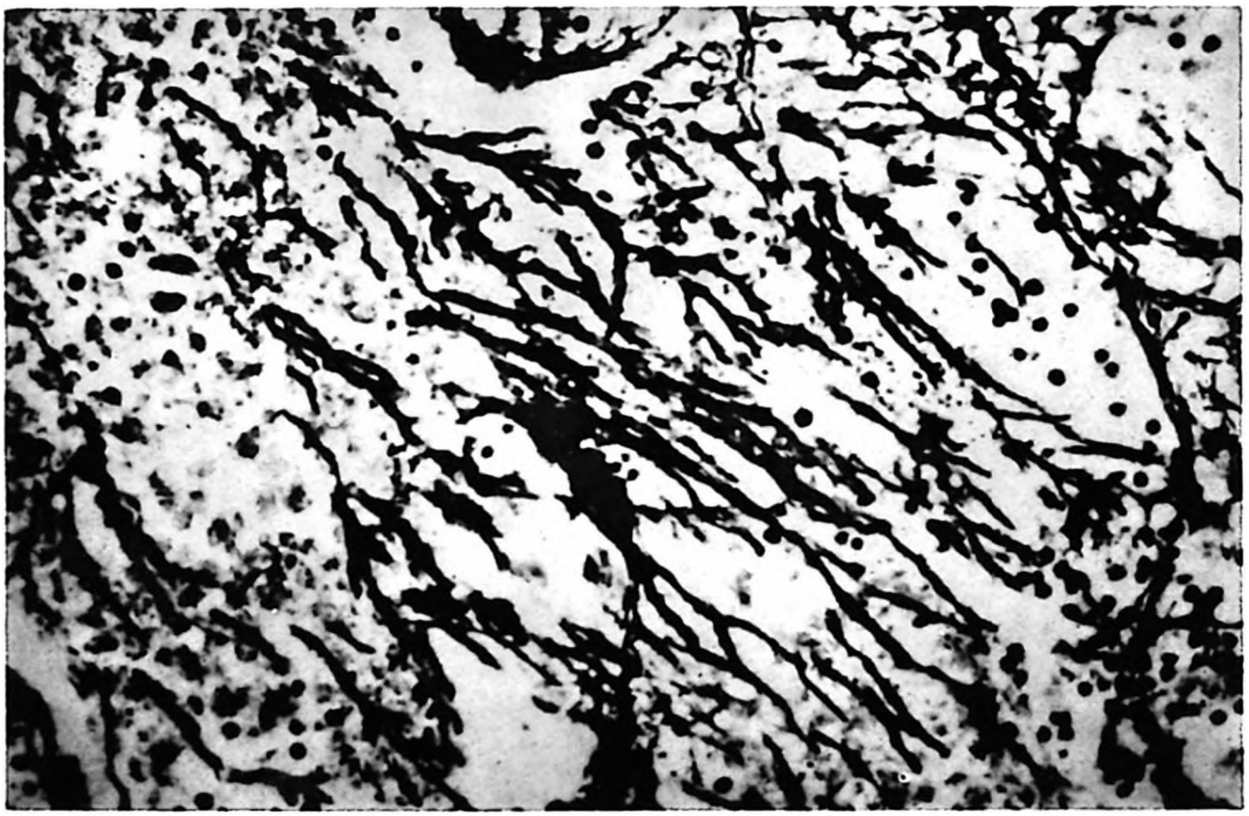

Figure 3
Aspergillus in lung, Methamine-Silver x 250. Thin non-septate and bifid hyphea with regular contours.

There were 18 cases, representing 66.6% of the entire group. The patients' ages ranged from 8 days to 14 years. In all cases, except cases numbered 16 and 21 who were 14 and 10 years old, the ages were less than three and half years. Five cases were female and the rest were male. It must be noted, however, that the sex and age distribution in this material is the result of the reflection of the postmortem permissions obtained which, in the majority of the cases, were infants and younger children. In addition to this, the majority of the hospital admissions were male children. In all cases the gastro-intestinal tract was involved; in 6 cases candida was limited to the oesophagus or cardia and present in the small or large bowel, in 11. Ulceration of the intestine was seen in 4 of 11 candida enterocolitis cases but no perforation was present. However, perforation of intestinal ulcers with secondary peritonitis due to candida has been reported in a few cases in the literature.[10-12]

Figure 4

Candida in G. I. tract. Silver stain x 250. Hyphea and yeast cells are seen.

The lungs were involved in 6 cases and, when the lung was involved, the oesophagus or gastro-intestinal tract was also involved in all cases except one, where examination of the gastro-intestinal tract was not performed because of the limitation of post-mortem examination permission. This finding suggested aspiration of fungi to the lung from the upper gastro-intestinal tract. Predisposing diseases were gastro-enteritis in 12 cases and malnutrition in 6 cases. In 4 of these, malnutrition and gastro-enteritis were coexistent. The other predisposing diseases were kidney disease in 3 cases and congenital adrenal hyperplasia and typhoid fever in one of each cases respectively. Other predisposing conditions were acidosis in 10 cases, dehydration in 7 cases. In 6 of the 18 cases traumatic manipulations were noted. These were naso-gastric tubage or catheterization for the purpose of diagnosis, feeding or treatment. The role of modern drug therapy in candida and other fungal infections has been well established,[4 13-14] and is outside the scope of this study. It will not be discussed here.

Cryptococcosis: There was one case (Case No. 23) of generalized cryptococcal infection without nervous system involvement. The organism was present in the lungs, liver, spleen, kidneys, adrenals, gastro-

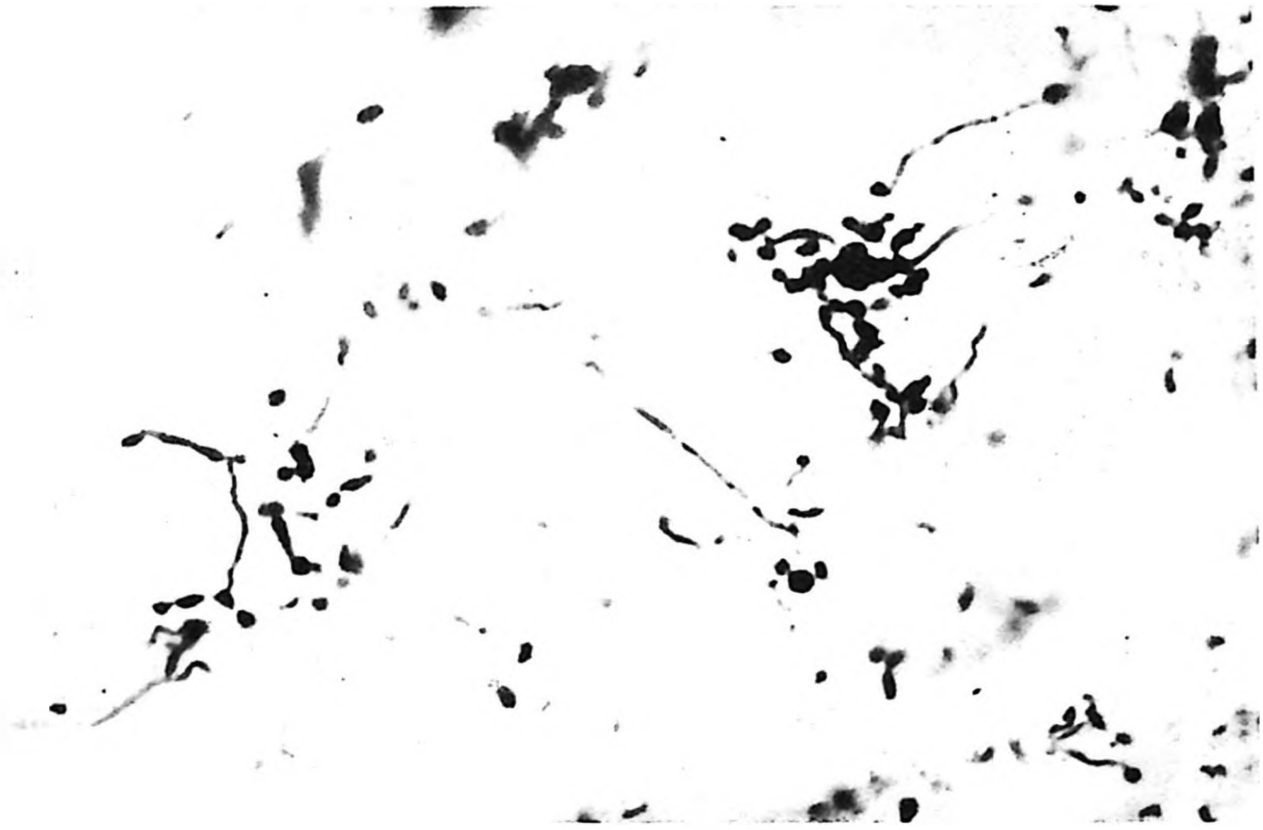

Figure 5
Candida in lung. Gram stain x 400. Septate hyptate hyphea and yeast cells are seen within the alveoli.

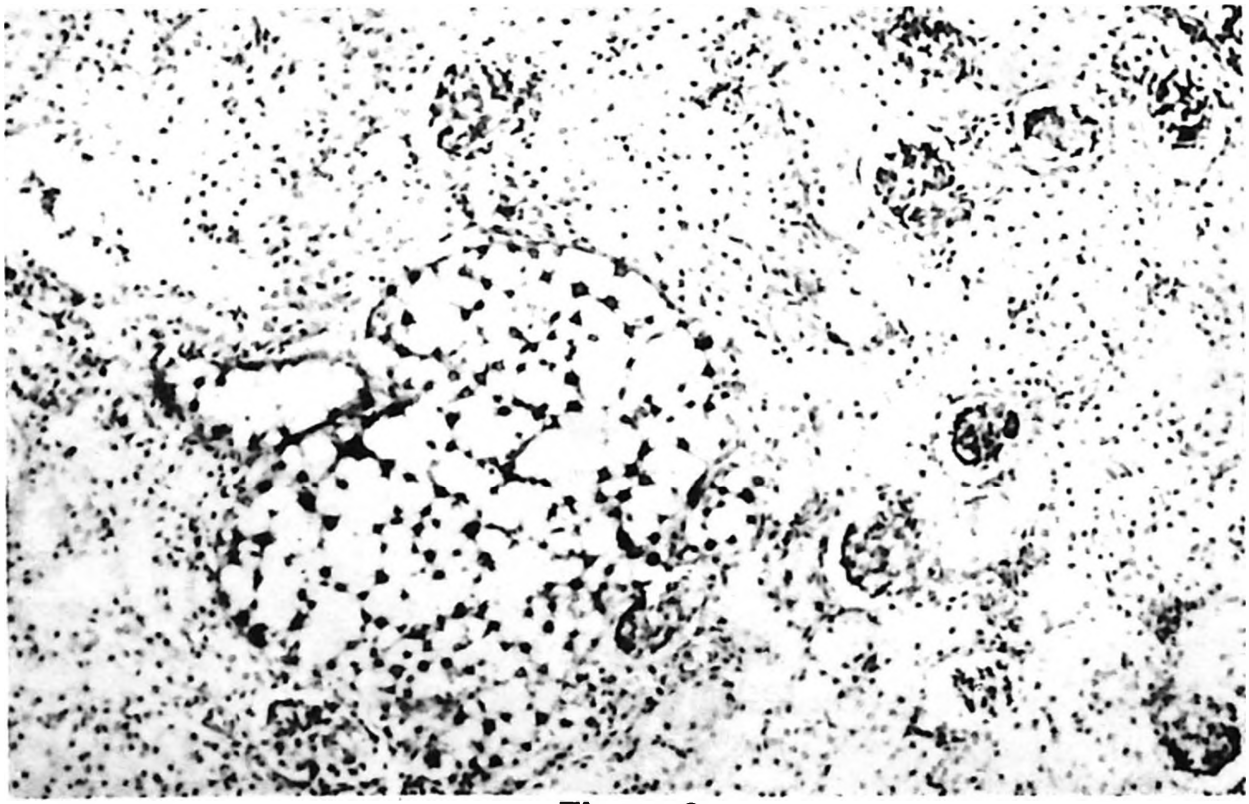

Figure 6
Cryptococcus in kidney. Toluidin blue metachromasia x 250. Mass of round bodies showing purple-red metachromasia of capsuler material.

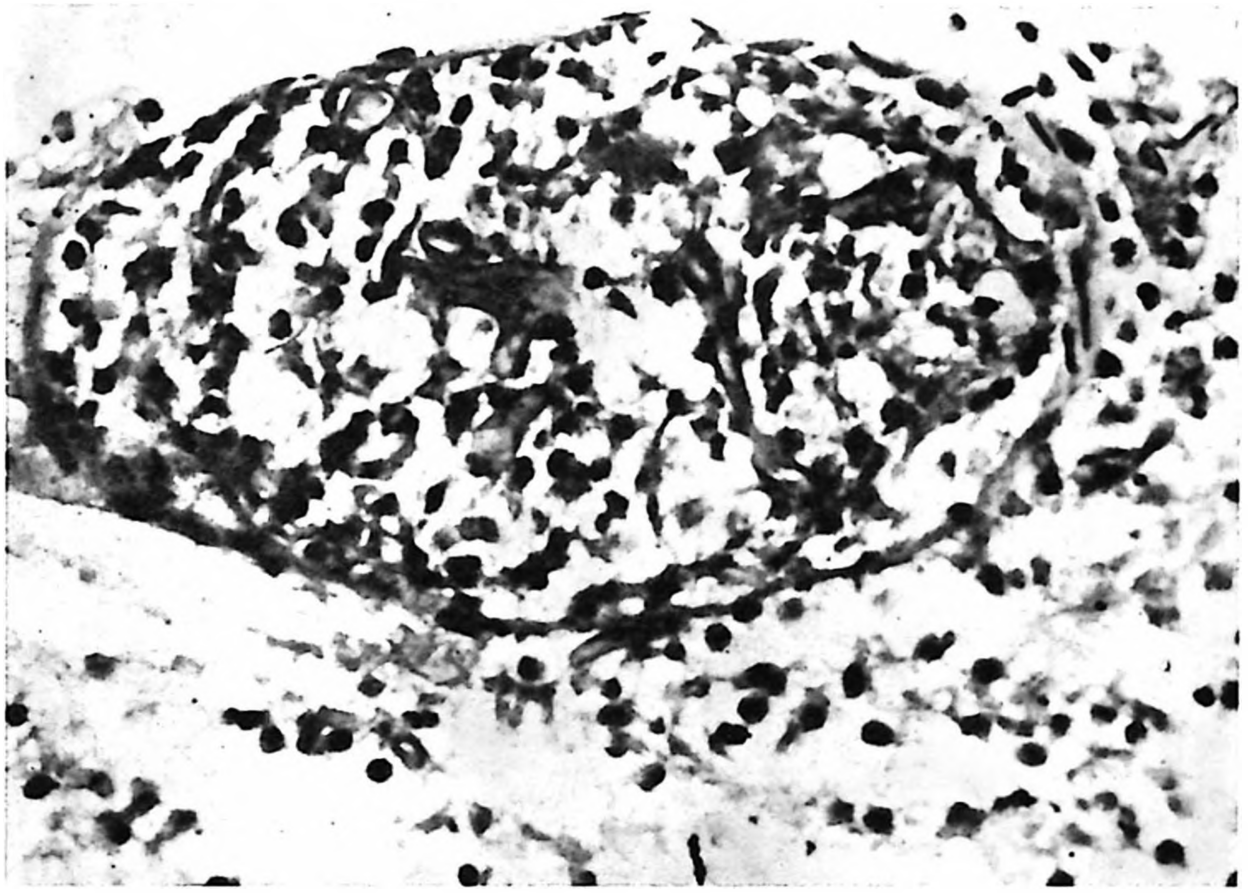

Figure 7
Mucor within the thrombosed vessels H+E x 250.

intestinal tract and lymph nodes. In general no inflammatory reaction against the presence of large numbers of organisms of forming masses of round or ovoid thick walled bodies was present. (Figure 6). This case has been previously reported.[15] The predisposing disease was malabsorption syndrome, the predisposing factor believed to be A avitaminosis.

Mucormycosis: There were 3 cases of mucormycosis. Two of the patients were male and one was female. (Figures 6 and 7). The ages ranged from 4.5 to 12 years. The disease was limited to the lung in one (Case No. 26), to the brain in another (Case No. 24). The latter has been published.[16] In another case (Case No. 25), the infection involved the lungs, thyroid and spleen. In all these cases inflammatory reactions were of the necrotizing type and vascular thrombi were present. The predisposing diseases were leukemia or lymphoma in 2 and, in the 3rd case, renal disease and malnutrition were present. The predisposing conditions were leukopenia, antibiotics and antitumor therapy in two cases and uremia and acidosis due to kidney disease in the 3rd case.

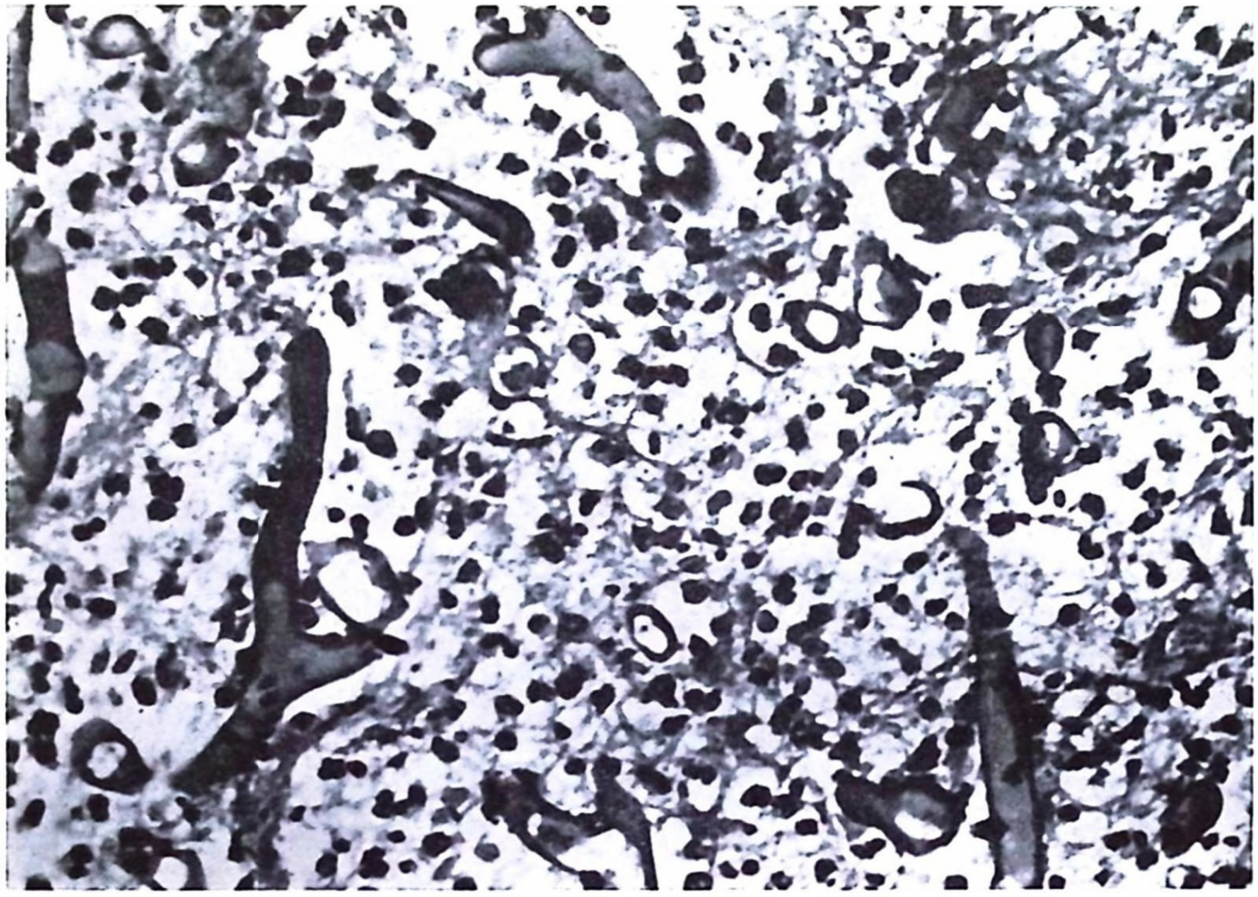

Figure 8

Mucor, H+E x 400, showing large, non-septate, irregularly branched hyphea.

P e n i c i l l o s i s : In this histopathological study, this was the only case where the diagnosis depended upon mycological culture studies and positive skin tests. The fungus was originally seen in aspirates of bloody pleural fluid as a septate hyphae and round bodies. No specific morphological diagnosis was possible but later mycological culture studies identified the organism as penicillosis (mycological studies and diagnosis were made by Prof. Dr. Hayati Erkmen, Mycologist, Department of Bacteriology, University of Ankara, Turkey). This infection occurred in an 8-year old female child with leukemia and pulmonary infiltrate which terminally also developed staphylococcal pneumonia. The patient died about 2 months after the diagnosis and permission for post-mortem examination was not obtained.

Discussion

Deep fungus infections may be divided into primary and secondary (opportunistic) types. Primary infections occur by pathogenic fungi in a healthy individual, such as actinomycosis, blastomycosis, coccidioidomycosis, histoplasmosis, maduromycosis, nocardiosis and

sporotrichosis, in contrast to opportunistic fungus infections which occur in patients with a predisposing disease or condition.[1-5] The terms' *opportunistic fungus infection*, *predisposing disease* and *condition* in this study have been used following the advice of committees of the international symposium which suggested that they designate ubiquitous saprophytes and 17 occasional pathogens that invade the tissues of man and animal with (1) predisposing diseases, such as diabetes, leukemia, lymphoma, cancer, and aplastic anemia or (2) predisposing conditions such as agammaglobulinemia, neutropenia, splenectomy and roentgen therapy and the use of steroids and antileukemic and antibiotic drugs. In this study of 27 cases of deep-seated opportunistic fungus infections, 5 types were found. The general incidence in autopsied cases was 3.2 %. There were 18 cases of candida, 4 cases of aspergillosis, 3 cases of mucor and one of each cryptococcus and penicillium infection. In only two of the 27 cases has the diagnosis been made while the patients were alive; in the remaining 25 cases, diagnoses have been made at post-mortem examination. These findings reflect the dangerous course of the deep-seated mycosis. They occurred in all ages ranging from 8 days to 14 years old. The majority of the candida infections tended to occur in infants and younger children and other mycotic infections at a later age.

Both sexes were affected; male frequency appeared to be higher but this impression is false as previously mentioned, and is a reflection of the post-mortem examination and hospital admission in general. The most frequent infection was candida with 66.6%, the second aspergillosis 14.8 % and the third mucormycosis 11.1%, cryptococcosis and penicillosis were infrequent. Candidiasis has been reported as also being the most infectious in patients with leukemia[18] and with some form of cancer.[3] Any organ may be affected, but in general the gastrointestinal tract and lungs were most frequently involved. The involvement of other organs included the liver, spleen, thyroid and brain but was much less frequent. The different types of fungus infection appeared to have preferential sites of involvement; Candida was most common in the gastrointestinal tract, including esophagus, and others, whereas aspergillosis and mucor frequently involved the lungs.

As expected no specific type of histologic reaction for each type of fungus infection was noted, but reactions varied in intensity, with little or none in cryptococcosis suppurative and necrotizing inflammation in others. Thrombosis was frequent in mucor, non-necrotizing granuloma occurred in one of the four aspergillosis. Generally, the

histopathologic reaction suggested an acute course of the fungus infection in children.

A variety of predisposing diseases was seen but, in the first group the majority occurred in patients with gastro-enteritis with or without malnutrition, and malnutrition associated with other diseases. Malnutrition was seen in other cases with deep mycosis, but was mostly noted in candida infections. Thymic dysplasia was seen in two cases of candida but not in other mycoses. The presence of thymic dysplasia was a direct evidence of cell-mediated immune deficiency. Thymic atrophy is a regular finding in children dying from malnutrition which probably leads to immunological deficiency later in the course of the disease such as is seen in patients with certain forms of malignancy.[19] Malnutrition as an important factor in fungal infections of infants and young children or developing countries was noted by Pedreza in Columbia.[14] The second most frequent group of predisposing diseases was lymphoreticular malignancies, especially leukemia. Candida infection was frequent in the first group of disease compared with the second group when aspergillus and mucor infections were frequent. The third in frequency of predisposing diseases, was congenital or acquired renal diseases. The last and infrequent predisposing diseases were obstructive biliary cirrhosis, congenital adrenal hyperplasia and malabsorption syndrome.

Predisposing conditions: Various conditions were noted. These were leukopenia, antibiotics, steroids and antitumor therapy, acidosis, dehydration, uremia, liver failure and A avitaminosis. However, in this study, the frequency of acidosis, dehydration and traumatic injury played a significant role in candida infections. Trauma, as a possible portal of candida, is also known to occur.[20]

Summary

Twenty-seven opportunistic deep-seated fungus infections in Turkish children were studied. The incidence in autopsied cases was 3.1 %. Five types of fungus infections were found. They were aspergillosis, candidiasis, cryptococcosis, mucormycosis and penicillosis. The most frequent infection was candidiasis which occurred mostly in infants and young children with gastroenteritis and malnutrition. Thymic dysplasia, as a direct evidence of deficiency of cellular immunity, was seen only in candidiasis. Thymic atrophy seen in cases of malnutrition probably played a similar role later in the course of the disease. Dehydration and acidosis were frequent and trauma was also significant in

candidiasis and, in this type of infection, the gastro-intestinal tract was usually involved. In contrast, candidiasis, aspergillosis, and mucormycosis were seen in older children (mean age 9 years old) and were frequent in lympho-reticular malignancy, especially leukemia; in the majority of the cases the lungs were involved. A variety of other less-frequently encountered predisposing diseases such as renal and hepatic failure, congenital adrenal hyperplasia and malabsorption syndrome with A avitaminosis were found.

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