Introduction
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Infection continues to be a major source of morbidity and mortality in renal transplant recipients and in other immunosuppressed patients (Gallis et al., 1975; Howard et al., 1978 and Peterson and Andersen, 1986).

The most common causes of serious illness and death in transplant patients are bacterial infections (Anderson et al., 1973). Viral and protozoal infections also can cause lethal infections in the immunosuppressed patients (Howard et al., 1978).

Fungal infection, too, can cause disease and death in transplant recipients (Rifkind et al., 1967; Burton et al., 1972; Gallis et al., 1975 and Schroter et al., 1977). Almost all agreed that the risk factors in transplant patients include the use of large doses of corticosteroids, multiple or recent rejection episodes, older age and poor kidney function.

The objective of this work is to screen the renal transplant patients in an attempt to describe the prevalence of fungal infection and to evaluate its relation to graft function, type of immunosuppression and the period after kidney transplantation.

Review of Literature

FUNGI AND MYCOSES

Historically, the fungi have been regarded as relatively insignificant causes of infection, however, during the past few years, the literatures have shown a sharp increase in the number of cases reported with fungal infections (Roberts, 1986).

The relationship of fungi to bacteria, to plants and to animals has been studied and understood in new ways in recent years. Taxonomists placed the fungi along with the bacteria and the algae, in a separate group, the protists, which is subdivided into the prokaryotes and the eukaryotes. This classification was mainly based on the basis of the degree of development of their cellular organization. The bacteria and the blue-green algae are classified as prokaryotic protists. The more advanced protozoa, slime moulds, fungi and algae are eukaryotic protists (Copeland, 1956).

Whittaker (1969) published his modified classification and this is the generally accepted classification at present. The Whittaker five Kingdoms are (1) Monera, the prokaryotes (bacteria. actinomycetes and blue-green algae), (2) Protista, which includes protozoa and other unicellular organisms, (3) Fungi, (4) Plantae and (5) Animalia(Campbell and Stewart, 1980 and Rippon, 1982).

The simplest morphologic form of the fungi is the unicellular, budding yeast. As complexity increases, the fungi grow as a mass of branching, interlacing filaments known as a mycelium. These mycelial forms are called moulds; few types, yeasts, do not form a mycelium but are easily recognized as fungi by the nature of their reproductive processes and by the presence of transitional forms (Jawetz et al., 1989).

So, fungi are classified on the basis of the sexual processes as following:

. Class I Zygomycotina (The phycomycetes)
. Class II Ascomycotina (The ascomycetes)
. Class III Basidiomycotina (The basidiomycetes)
. Class IV Deuteromycotina (The imperfect fungi)

The class Zygomycotina includes organisms which have non septated hyphae and reproduce asexually by spores produced inside of sporangia and reproduce sexually by the production of zygospores and/or oospores. The most common members of this group are *Mucor, Rhizopus and Absidia.*

The class Ascomycotina includes organisms which have septated hyphae and reproduce asexually by production of conidia or reproduce sexually by means of ascospores produced within an ascus. The most common member of this group is *Aspergillus spp.*

The class Basidiomycotina includes organisms which have septated hyphae and reproduce asexually by conidia or sexually by means of basidiospores. The most common members of this group of medical interest are *Cryptococcus neoformans* and Mushrooms (Jawetz et al., 1989).

The major class to which most of the clinically important fungi belong is the Deuteromycotina (Fungi imperfecti). This group of organisms has septated hyphae and reproduces asexually with production of conidia whether their sexual reproduction is not known. The most common members are *Epidermophyton and Candida.*

But clinicians found more value in categorizing the fungi into three categories according to clinical manifestations of disease produced primarily:

(1) The superficial or cutaneous mycoses.

(2) Subcutaneous mycoses.

(3) Systemic mycoses.

The superficial or cutaneous mycoses are fungal infections that involve the hair, skin and nails without invasion of the subcutaneous tissue. The most commonly group recovered is the Dermatophytes. Subcutaneous mycoses are infections confined to the subcutaneous tissue without dissemination to distant sites.

The systemic mycoses fall into two groups. These two groups are delinated by the interaction of two factors: inherent virulence of the fungus and constitutional adequacy of the host. The first group includes true pathogenic fungi: *Histoplasma, Coccidioides, Blastomyces and Paracoccidioides.* Infections caused by these fungi involve the lungs primarily, but they may become widely disseminated and involve any organ of the body. The second group of systemic fungus infections are caused by opportunistic fungi. The organisms involved have a very low inherent virulence and the patient's defences must be abrogated before infection is established (Rippon, 1982).

In recent years, the rise in the incidence of these opportunistic infections has paralleled the use of antibiotics, cytotoxins, immunosuppressive drugs and other factors that result in lowered resistance of the host. Theoretically, any mould or yeast, as well as any of the primary pathogens may be able to produce fungal invasion of human tissue in an individual whose resistance is lowered. The opportunistic fungus diseases include aspergillosis, zygomycosis, cryptococcosis and candidosis. In addition to these, there are a number of rare infections caused by a variety of soil fungi and fungi that constitute our normal cutaneous yeast flora(Campbell and Stewart, 1980; Rippon, 1982; and Roberts, 1986).

OPPORTUNISTIC MYCOSES

Opportunistic fungi are widely distributed throughout the kingdom fungi and may vary with both the site of infection and the geographic locations.

The most common opportunistic fungi encountered in the immunocompromised patients are *Candida spp.*, *Aspergillus spp.*, *Zygomycetes*, *Trichosporon*, *Geotrichum* and *Cryptococcus neoformans* (Larsh, 1977).

Patients known to be compromised by their particular physiologic conditions such as those with cancer or diabetes, those receiving antibiotics or immunosuppressive drugs for long period of time or those undergoing complicated long surgical procedures with kidney, heart or other organ transplants have many physiologic sites that could contract opportunistic mycoses. The opportunistic fungus infection is directly related to the new developments, actually, each time a new drug is introduced, a new surgical procedure is tried, or almost any other new medical technique or advancement is announced, there are corresponding increases in the cases of opportunistic mycoses (AL-Doory, 1980).

It is difficult to term any fungus strictly non pathogenic, any fungus that can survive at 37°C could cause disease

if there is appropriate host conditions. Among the most common opportunistic fungi are the species of *Candida, Aspergillus, Cryptococcus neoformans, Rhizopus and Mucor* (AL-Doory, 1980).

I. Candidosis (Candidiasis)

a) Candida as an opportunistic pathogen:

Members of the genus *Candida* are indigenous human yeasts that colonize the skin and mucous membranes of normal individuals but produce infection only when natural resistance is compromised (Braude, 1981).

The genus *Candida* comprises many species of medical interest, the most important of these is *Candida albicans (C. albicans), but Candida tropicalis (C.tropicalis)* has become a serious cause of disseminated infection in patients undergoing treatment for haematologic malignancies or receiving bone marrow transplantation (Wingard et al., 1979). The other species encountered in human disease are designated *C. krusei, C.guilliermondii, C. parapsilosis, C. pseudotropicalis and C. stellatoidea.*

Candidiasis is the most frequently encountered opportunistic fungal infection and the repeated recovery of different species of yeasts from multiple specimens from the same patient usually indicates colonization. The simultaneous recovery of the same species of yeast from several

body sites including urine is a good indicator of disseminated infection and the subsequent development of fungaemia (Hunter and Cooper, 1985).

In disseminated invasive candidiasis, the route of infection is usually from the gastrointestinal tract. Another route of entery is by direct intravenous egress, usually via indwelling intravenous catheter, also through injections of contaminated intravenous illicit drugs where the source of the infection is not clear, it could be the skin, the needle and syringe, or the drugs or water used to dilute them (Musial et al., 1988).

It is clear that when the inoculation of organism via the intravenous route is large enough, the normal number of functioning leukocytes can not always prevent dissemination and growth of *Candida* species, particularly *C. albicans and C.tropicalis* (Armstrong, 1989).

b) Morphological and cultural characteristics

Members of the *Candida* genus characteristically present both as yeast cells and as pseudohyphae. Pseudohyphae are chains of budding cells that fail to detach, so that they develop into a branching network that resembles true hyphae. The yeast cells of *C.albicans*, the principle pathogenic form of the genus, are oval and Gram positive with size vary from 2 x 3 um to 8.5 x 14 um (Rippon, 1982).

On cornmeal agar or potato-carrot-bile agar, *C. albicans* reproduces in four different forms: (1) true mycelia, (2) pseudomycelia (3) blastospores and (4) chlamydospores.

The blastospores grow in round clusters at intervals along the pseudomycelia. Terminal chlamydospores growing at the end of the hyphae are the most distinctive features of *C. albicans* and are rarely produced by any other species of *Candida* (Braude, 1981).

In 1962, Mackenzie stated that one of the most valuable tests for rapid presumptive identification of *C.albicans* is the germ tube test. This test is by dilution of a yeast colony in 0.5 to 1 ml of rabbit or human serum then incubated at 37°C for 2 to 4 hours, thenafter, a drop of the inoculated serum is examined microscopically for germ tube formation.

The differential characteristics of *Candida spp.* are shown in (Tables 1 & 2).

Table 1. Gross and Microscopic Appearance of the Different Candida Species (AL-Doory,1980).

Species	* Colonies on agar	Yeast cell morphology	Germ tube	Chlamydospores
C.albicans	creamy	ovoid	+	+
C.stellatoidea	creamy	ovoid	±	rare
C.tropicalis	creamy	ovoid	0	0
C.krusei	flat, dull, dry	cylindrical & crossed	0	0
C.pseudotropicalis	soft, smooth, white	elongated & parallel	0	0
C.guilliermondii	thin, flat, glassy	ovoid or cylindrical	0	0
C.parapsilosis	creamy	ovoid	0	0

* 3 days growth on sabouraud's dextrose agar at 25°C.

Table 2. Carbohydrate Fermentation and Assimilation Reactions of Different Candida Species (Rippon, 1982).

Species	Fermentation						Assimilation							
	Gl	Gal	M	S	L	T	Gl	Gal	L	M	R	S	C	T
C.albicans	+	+	+	V	0	V	+	+	0	+	0	+	0	+
C.stellatoidea	+	0	+	0	0	0	+	+	0	+	0	0	0	+
C.tropicalis	+	V	+	+	0	V	+	+	0	+	0	+	+	+
C.krusei	+	0	0	0	0	0	+	0	0	0	0	0	0	0
C pseudotropicalis	+	+	0	+	+	0	+	+	+	0	+	+	+	0
C.guilliermondii	+	V	0	+	0	V	+	+	0	+	+	+	+	+
C.parapsilosis	+	V	0	0	0	0	+	+	0	+	0	+	0	+

Gl= glucose; Gal=galactose; M=maltose; S=sucrose; L=lactose; T=trehalose; R=raffinose; C=cellobiose; (+)= utilized; 0=not utilized; V=variable.

II. Aspergillosis

a) Aspergillus as an opportunistic pathogen:

Several species of Aspergilli are among the most frequently encountered organisms in the clinical laboratory; some are potentially pathogenic whereas others are infrequently associated with infection or do not cause infection at all. The aspergilli are widespread in the environment where they colonize grain, leaves, soil and living plants. Conidia of aspergilli are easily dispersed into the environment and humans become infected by inhalation. These organisms are capable not only of causing disseminated infection as seen in immunocompromised patients, but also of causing a separate organ affection including invasive lung infection, pulmonary mycetoma, allergic bronchopulmonary aspergillosis, external otomycosis, mycotic keratitis, onychomycosis, sinusitis, endocarditis and central nervous system infection. Most often, immunocompromised patients acquire a primary pulmonary infection which rapidly disseminates and causes infection in virtually every organ (Roberts, 1986).

Aspergillus fumigatus (A. fumigatus) is the most common species seen in respiratory tract secretions and is responsible for most cases of pulmonary aspergillosis. Others including *A. flavus, A. niger and A. terreus* are being seen with greater frequency as a cause of infection in the immunocompromised host (Musial et al., 1988).

Repeated recovery of an organism from the same site or its recovery from several different sites may serve as evidence for infection, especially in the neutropenic patient (Aisner et al., 1979).

b) Morphology and cultural characteristics

1- A. fumigatus

A. fumigatus is a rapidly growing mould (2-6 days) which produces a fluffy to granular, white to blue-green colony. Mature sporulating colonies most often exhibit the blue green-powdery appearance. Microscopically, *A. fumigatus* is characterized by the presence of septate hyphae and short smooth conidiophores (up to 300um in length) having a characteristic cell at their base. The tip of the conidiophore expands into a large, dome-shaped vesicle which has bottle-shaped phialides occuring in a single row and around the upper half or two-thirds of its surface. Cultures of *A. fumigatus* are thermotolerant and are able to withstand temperatures up to 45°C (Roberts, 1986).

2- A. flavus

A. flavus, is somewhat more rapidly growing (1-5 days) and produces a yellow green colony. Microscopically, long

conidiophores (up to 1 mm in length or more), globose vesicles and phialides are produced directly from the vesicle surface or from a primary row of branches. The phialides give rise to short chains of yellow-orange elliptical or spherical conidia that become roughened on the surface with age (Roberts, 1986).

3- A. niger

A. niger produces mature colonies within 2-6 days. Growth begins initially as a yellow colony that soon develops a black, dotted surface as conidia. Microscopically, *A.niger* exhibits septate hyphae, long conidiophores (1.5 to 3 mm in length) that support spherical vesicles which give rise to large metulae and smaller phialides from which long chains of brown, rough-walled conidia are produced (Roberts, 1986).

III. Zygomycosis

a) Zygomycetes as an opportunistic pathogen

Fungi in the class Zygomycotina produce serious infection in the immunocompromised host, but the rate of infection is often less than *Candida or Aspergillus species*. Members of this group are ubiquitous in nature, often found growing on bread, fruit and leaves in addition to soil.

Infection is usually a result of inhalation or sometimes, traumatic inoculation of the organism into the skin. Zygomycosis is most often seen in patients having diabetes mellitus, haematologic malignancies, neutropenia, extensive burns or organ transplants and usually associated with those receiving cytotoxic, corticosteroid therapy or sometimes in drug addicts. Pulmonary zygomycosis occurs frequently enough that must be highly suspected and treated immediately, rhinocerebral zygomycosis most commonly in patients with uncontrolled diabetes and ketoacidosis (Musial et al., 1988).

b) Morphology and cultural characteristics

Colonies produce a fluffy, white to gray or brown hyphal growth that diffusely covers the surface of the agar within 24-96 hours. It is impossible to distinguish between the different genera and species of *Zygomycetes* based on their colonial morphologic features since most are identical in appearance. Microscopically, the *Zygomycetes* produce large ribbon-like hyphae that are irregular in diameter and non-septate. The identification of the three most common *Zygomycetes; Mucor, Rhizopus and Absidia* is based on the presence or absence of rhizoids and the position of the rhizoids in relation to the sporangiophores and the morphology of sporangiophores. *Mucor* is characterized by sporangiophores that are not branched and have at their tips around

sporangium filled with sporangiospores. *Mucor* does not have rhizoids or stolons and this distinguishes it from the other genera of *Zygomycetes*. *Rhizopus* has unbranched sporangiophores with rhizoids which appear at the point where the stolon arises. *Absidia*, an uncommon isolate in the clinical laboratory, is characterized by the presence of branched sporangiophores with rhizoids which originate between sporangiophores (Greer and Rogers, 1985).

IV. Cryptococcosis

a) Cryptococcus neoformans as an opportunistic pathogen

Cryptococcosis, primarily an infection of the immunocompromised host, is produced by *Cryptococcus neoformans (Cr. neoformans)*, a member of the class Basidiomycotina, the group to which the mushroom belong. This organism is widely distributed in nature and is most often found in association with the excreta of pigeons. It is thought that exposure to *Cryptococcus neoformans* is common and that primary route of entery is by inhalation. Pulmonary infections are usually self-limited but if the fungus disseminates via the blood stream to the central nervous system, it results in meningitis or less often, in a focal granulomatous lesion in the brain called a cryptococcoma. Cryptococcosis is the most common disseminated fungal infection seen in patients

with AIDS. Other sites that may be involved include the skin, bone and rarely, the kidney, liver and genitourinary tract (Armstrong, 1989).

b) Morphology and cultural characters

The genus *Cryptococcus* has 17 recognized species and *Cr. neoformans* is the only known pathogenic species and the only species which can grow at 37°C within this genus. Colonies grow rapidly and appear slimy. Colouration ranges from white to creamy yellow in most, through whitish yellow to light pink in some. The reverse side of colonies is usually colourless. Microscopically, cells appear spherical to ovoid and occasionally elongated or polymorphic with capsule formation depending on the medium. Species of *Cryptococcus* do not form pseudohyphae, do not produce ascospores, not have carotenoid pigment but they hydrolyze urea (AL-Doory, 1980).

V. Trichosporonosis

a) Trichosporon species as an opportunistic pathogen

Species of *Trichosporon* are widely distributed in nature and may also be found in respiratory secretions, stool, urine and on the skin of healthy persons(Haupt et al., 1983).

Trichosporonosis as an infetcion of the immunocompromised host has been recognized only during the past 17 years.

Disseminated infection is seen primarily in patients with haematologic malignancy and profound neutropenia (Hoy et al., 1986). However, infection has been found in patients underlying renal and bone marrow transplantation (Haupt et al., 1983).

Trichosporon beigelii (T.beigelii); the most common cause of Trichosporonosis; has long been recognized as the aetiologic agent of white piedra, an infection of the hair of persons living in tropical and temperate areas but the clinical manifestations of trichosporonosis are diverse and the diagnosis is usually not suspected (Musial et al., 1988).

b) Morphology and cultural characters

Members of the genus *Trichosporon* are yeast like organisms characterized morphologically by the production of blastoconidia and the development of true mycelia and arthroconidia. They do not produce asexual or sexual spores. Identification of *Trichosporon beigelii (T. beigellii)* was based on the following criteria: growth at 37°C, production of urease, assimilation of many carbohydrate and nitrate and absence of fermentation ability. Morphologically, true hyphae with chains of arthroconidia and infrequent blastoconidia were seen on corn meal agar with polysorbate 80 at 24°C but identification of

Trichosporon capitatum (*T. capitatum*) was based on the following criteria: growth at 37°C, lack of urease activity, assimilation of only glucose and galactose, absence of fermentation ability and inability to assimilate nitrate. Morphologically, true hyphae with abundant arthroconidia and blastoconidia were seen on corn meal agar with polysorbate 80 at 24°C (Haupt et al., 1983).

The differential biochemical reactions of Trichosporon species are shown in Table 3.

Table 3. Biochemical Reactions of Trichosporon spp.(AL-Doory,1980).

Species	Fermentation						Assimilation								Urease	Nitrate
	Gl	Gal	M	S	L	T	Gl	Gal	L	M	R	S	C	T	Production	Reduction
T.beigelii	0	0	0	0	0	0	+	+	+	+	+	+	+	+	+	+
T.capitatum	0	0	0	0	0	0	+	+	0	0	0	0	0	0	0	0

Gl= glucose; Gal=galactose; M=maltose; S=sucrose; L=lactose;

T=trehalose; R=raffinose; C=cellobiose; (+)= utilized;

0=not utilized.

VI. Geotrichosis

a) Geotrichum candidum as an opportunistic pathogen

Geotrichum candidum (G. candidum) is the only known pathogenic species of *Geotrichum*. It has been reported as the aetiologic agent for oral, gastrointestinal, bronchial and pulmonary infection (AL-Doory, 1980).

b) Morphology and cultural characters

G. candidum is always included with the yeasts because of its similarity to *Trichosporon* in the formation of arthrospores. Its colonies appear white to buff coloured and the reverse side is creamy. Microscopically, hyphae appear septate and hyaline and are composed of chains of rectangular arthrospores that readily break apart (AL-Doory, 1980).

VII. Other uncommon fungal infections

A variety of fungi other than those discussed before have been recognized as causing infection in the immunocompromised host. *Paecilomyces lilacinum* has been associated with cellulitis in the immunosuppressed patients (Jade et al., 1986). *Torulopsis candida* (Fungaemia), *Penicillium* species (generalized subcutaneous abscess) and *Pityrosporum* species (Folliculitis) (Musial et al., 1988).

RENAL TRANSPLANTATION

Renal transplantation has become the treatment of choice for most patients with end stage renal failure (Morris, 1984).

The most common kidney disease indicating renal transplantation is glomerulonephritis, followed by pyelonephritis, interstitial nephritis, cystic kidney disease and multisystemic disease, of which diabetes mellitus is the major example. Over recent years, it has become apparent that no disease represents an absolute contraindication to transplantation (Briggs, 1984).

Renal transplantation as a treatment modality for patients with end stage renal disease is now showing a slow but steady improvement in results. This is due to a better understanding of both basic immunobiology and measures for medical and surgical management of these patients (Carpenter, 1989).

The recent knowledge in organ preservation, histocompatibility tests in vitro involving pretransplant immunologic responses, immunosuppression and other aspects of patient management have greatly improved the likelihood of transplant functional success (William, 1988).

Organ transplants can be generally divided into two types : (1) Those from a living donor (related or non related) and (2) those from a cadaver donor. Increasing degrees of genetic similarity between donor and recipient confer upon organ transplants increasing chances for successful transplant function (Clane, 1985).

Glassock (1989) stated that the goal of renal transplantation is to achieve maximal rehabilitation of patients with end stage renal failure and can be accomplished by prolonged patient and graft survival with relative freedom from complications related to immunosuppression and minimal risk of morbidity or mortality from surgically related events. Yet, for all but a small number of patients with end stage renal failure, renal transplantation remains the the treatment of choice.

Immunosuppression:-

The management of renal transplant recipient is divided into three areas of primary concern; (1) The persistant effects of uraemic state and the physiological compensations; (2) suppressing the immune system which if allowed to operate uncheked, would result in rejection of the graft; and (3) the complications of the post transplant state which are the direct result of the surgery and the immunosuppressive drugs (Samouel et al., 1985).

The era of clinical transplantation began in the late 1950s and early 1960s after the discovery of azathioprine as an effective immunosuppressive agent in experimental animal and man (Schwartz et al., 1960 and Calne et al., 1962).

During the period of (1960-1969), there was very little background experience in clinical organ transplantation to guide the care and management of immunosuppressed patients. It was painfully discovered that in order to achieve prolonged graft survival and function, kidney transplant recipients have to be maintained on potent immunosuppressing medications with high risk of secondary infectious complications and mortality, then came the improvement of patient care and survival in (1970-1979), during that time, a combination of prednisone and azathioprine became the conventional post transplant immunosuppressive treatment (Dagher, 1986).

In 1980s, there has been significant progress in understanding of the biology of the immune response against graft related antigens, which was accompanied by improving the graft survival due to several factors; pretransplant blood transfusion, blood and tissue typing, use of ciclosporin and use of monoclonal antibodies (Dagher, 1986).

Immunosuppression may be non specific or specific where the former is the generalized suppression of the immune

response with agents chosen especially for effects on rejection reaction. Conversely the specific immunosuppression is the suppression of the immune response to donor alloantigens without suppression to other antigens (D.H. Sachs, 1987).

A. Non Specific Immunosuppression (Conventional immunosuppression):

1. Steroids:

The fact that steroids affect the immune system had been shown since 1920. The mechanism by which steroids exert their immunosuppressive action is not fully understood however, there is abundant experimental evidences that steroids are immunosuppressive for both cell mediated responses and humoral immunity. Azathioprine when conjoined to steroids act synergistically to achieve adequate immunosuppression, which is not the case in using either drug alone in high doses (Olson et al., 1984).

2. Azathioprine:

Azathioprine is one member of a group of drugs known collectively as thiopurine. It is the nitroimidazole derivative of 6-mercaptopurine which acts on the cellular level by suppressing the humoral antibody synthesis (Strom et al., 1981).

3. Cyclophosphamide:

This drug is used in renal transplant to substitute azathioprine when the later have to be withdrawn due to hepatotoxicity. Cyclophosphamide has more myelosuppressive effect than azathioprine and long standing use ends with haemorrhagic cystitis as well as gastrointestinal upsets (Strom et al., 1981).

B. Specific Immunosuppression:

1. Ciclosporin (Cyclosporin A):

Ciclosporin is an exciting new immunosuppressive drug which has proven to be a potent agent in a wide variety of experimental models of tissue transplantation and promises to be a valuable addition to the immunosuppressive armamentarium in clinical organ transplantation (Morris, 1984).

Ciclosporin was extracted from the fermentation of broth culture of the fungus *Trichoderma polysporum*. In spite of its toxicity and many drug interactions, there are some undoubted advantages from the use of ciclosporin; it is a highly potent immunosuppressive with relative specificity for T-lymphocytes; lacking myelotoxicity; does not affect the phagocytic function with availability of maintaining its blood level to avoid the overdosing toxicity (R.Wenger, 1982).

Lastly and most important, the effect on the immune system is reversible. If the drug is stopped, the effect is reversed very quickly (T. Beveridge, 1981).

2. *Anti-lymphocyte globulin* (A.L.G.):

A.L.G. is prepared from the serum of an animal that has been immunized with tissue from another species. A.L.G. has been tested in numerous experimental procedures and proved to have profound suppressive effect upon cellular mediated immunity. The problem with A.L.G. that it is a biological product and its potency can vary enormously depending upon the type of animal used, the type of antigen rejected, the route of administrations and the purification of the globulin (Jaffers and Cosimi, 1984).

3. *Monoclonal antibodies:*

The recent development of hybridoma technology has made it possible to obtain large quantities of antibody against single determinant (monoclonal antibody). The most currently available monoclonal antibodies have been produced in mice. The currently available monoclonal antibodies are produced by Ortho Pharmaceutical Corporation as OKT_3, OKT_4 and OKT_8. Each of these antibodies has been raised against specific subpopulation of peripheral blood leukocytes (Jaffers and Cosimi, 1984).

4. Ionizing irradiation:

The rational use of radiation to facilitate kidney transplantation is twofold. First, lymphocytes and particularly the small thymus influenced cells that are responsible for cell mediated attack on the graft are killed by small dose of radiation (Trowell, 1976). Second, radiation is anti-inflammatory acting indirectly by slowing leukocyte migration so that fewer cells reach the graft and increasing the graft acceptance (Speirs, 1976).

INFECTIOUS COMPLICATIONS IN RENAL TRANSPLANT RECIPIENTS

Renal transplant patients are susceptible to infection for three main reasons:-

(1) They are uraemic and therefore already immunosuppressed, are usually anaemic, and may suffer from coagulation defects and the effects of protein restriction (Goldblum and Reed, 1980).

(2) They have undergone a major surgical operation involving vascular and urological procedure and the transplanted organ may also be contaminated during harvesting. Furthermore, their surface defences are breached by urinary catheters, intravenous cannulae and peritoneal dialysis catheters (Morris,1985).

(3) They receive immunosuppressive drugs, which have broad effects on immune competence. The immunosuppressive treatment affects both specific and non specific defence mechanisms (Cohen and Pinching, 1982).

So, they are more liable to infection than normal persons, the most common sites and infections are :

(A) Urinary tract infection :

Urinary tract infection which is the most common infection in renal transplant recipients. Fortunately, it also

carries the lowest mortality. Nearly all of the mortality seems to occur in the first month and is associated with urinary leaks and obstruction, perinephric abscesses, wound infections, or septicaemia. Early and compulsive investigations for these disorders, leading to early diagnosis and therapy, may avoid a high mortality (Franklin and Dale, 1989).

(B) Lung infection:

Pulmonary infections occur in 10 to 25% of renal transplant recipients, carry a mortality of 40% to 60% and are the most common cause of infectious mortality. Pneumonias can be especially problematic because of the many possible causative organisms, the atypical presentations seen in immunosuppressed patients and the high rate of mixed or superinfections (Ramsey et al., 1980).

(C) Septicaemia:

The prevalence of bacteraemias seems to be falling. They appear to be secondary to urinary tract infection, gastrointestinal sources and wound or catheter infection (Peritoneal, vascular, bladder). The reduced incidence of bacteraemia is probably related to meticulous surgical technique, sterile catheter management, early removal of vascular and bladder catheter, and early diagnosis (Franklin and Dale, 1989).

(D) Gastrointestinal tract infections:

Stomatitis and oesophagitis in renal transplant recipients are most commonly caused by *Herpes simplex* virus or *Candida albicans*. Although the diagnosis is often based on clinical grounds, endoscopy with appropriate biopsies and cultures should be considered when oesophageal involvement is suspected (Peterson and Andersen, 1986).

(E) Cutaneous infections:

Wound infections in renal transplant recipients were considered major infectious disease complication, but now have become an exceedingly uncommon problem in many centers. The reduced incidence in these types of wound infections appears to be due to improved surgical techniques and use of perioperative antibiotic prophylaxis (Tillegrad, 1984).

Vesicular skin eruptions due to *Herpes simplex* virus or *Varicella zoster* virus are common complications of renal transplantation. Ulcerative, chronic draining or nodular skin lesions should always raise the suspicion of atypical mycobacterial infection (Wolfson et al., 1985), nocardiosis or fungal disease e.g. crytococcosis, histoplasmosis, aspergillosis or disseminated candidiasis (Peterson and Andersen, 1986).

(F) Musculo-skeletal infections:

Musculo-skeletal infections are caused by a variety of pathogens in renal transplant patients. In addition to common bacterial organisms such as staphylococci and Gram negative bacilli, unusual pathogens such as the atypical Mycobacteria, *M. tuberculosis* and fungi must be considered. Cultures and biopsy specimens should be appropriately processed for these organisms and therapy is guided by the results of these diagnostic procedures (Peterson and Andersen, 1986).

FUNGAL INFECTION FOLLOWING KIDNEY TRANSPLANTATION

Renal transplant recipients are subjected to a variety of alterations in anatomy and physiology that increase susceptibility to infections. These include disruption of normal cutaneous barriers; steroid, antibiotic therapy, renal failure and acidosis. These factors result in abnormal phagocytic function, depressed humoral and cellular immunity, and increased susceptibility to pathogenic and opportunistic organisms (Gallis et al., 1975).

Although most infections recognized after renal transplantations are bacterial (Eickhoff, 1973), however, a considerable numbers of patients were reported to have systemic fungal infections and in many cases, the diagnosis was made late or even may not done except at the post mortem examination (Tapia et al., 1973).

The most reported fungal infections in renal transplant recipients have been of the opportunistic variety. Rifkind et al. (1967), were the first to review systemic fungal infections in a series of transplant patients. They found systemic fungal infections in 23 of 51 autopsy reports; in this series, only one case was recognized before death. Combined bacterial, viral or parasitic infections are still the rule in transplant patients who die with fungal infections. Death however, can be the result of isolated fungal infections.

Since Rifkind's initial report, a high incidence of fungal infection has been noticed by kidney transplant groups (Burton et al., 1972; Gallis et al., 1975 and Howard et al., 1978). Almost all agreed that the risk factors in transplant patients include the use of large doses of corticosteroids, multiple or recent rejection episodes, poor transplant function and older age. The controversy about the effect of these factors in predisposing for systemic fungal infections has been settled yet. For example, Gallis et al. (1975) found no relationship with renal function or leukopenia to infections. Ramsey et al. (1980) noticed that only 5 out of 54 patients treated with immunosuppressive drugs to have invasive pulmonary fungal infection. In similar study, Ho and colleagues (1984) found that one out of 81 patients treated with immunosuppressive drugs had fungal infection. While Howard et al. (1978) noticed that patients with poor renal function, previous rejections or recent courses of high dose steroids therapy were more susceptible to lethal infections which followed their initial bacterial or viral infections.

Previous reports in this area have been of two types: the first group of reports (Rifkind et al., 1967 and Gallis et al., 1975) include a general survey of fungal infections. In these studies, the common fungi observed were *Cryptococcus, Candida and Aspergillus*, with occasional

reports of *Phycomyces*, *Chromomyces* or soft tissue infections with *Phialophora gougeroti*. The second group (Burton et al., 1972) described infections with specific fungus in transplant recipients, in which it was assumed that a source of *Candida* infection is frequently present and may serve as a reservoir for later or concomitant systemic infection.

Fungal infection may occur as a primary infection or as a result of reactivation of previous fungal infection by immunosuppressive medications (Brooks and Remington, 1984). The severity of infection has a linear relationship with rejection episodes considering the high doses of immunosuppressive drugs used in cases of renal transplants (Bach et al., 1973). Furthermore, it may be necessary to discontinue all immunosuppressions in order to overcome various infections especially fungal infections (Gallis et al., 1975). The discontinuation of immunosuppressive drugs as well as aggressive treatment with antifungal agents, offer the best hope for preventing severe morbidity or mortality from fungal infection in renal transplant recipients (Jones et al., 1982).

Materials & Methods

MATERIALS AND METHODS

This study was carried out on one hundred renal transplant recipients and twenty normal control cases in the Urology and Nephrology Center, Mansoura, Egypt.

I. Materials

A- Patients:

One hundred renal allograft recipients (77 males and 23 females) ranged in age from 11 to 58 years (mean ± S.D. = 32.1 ± 8.6) with a post-transplantation period ranged from 1 to 86 months (mean ± S.D.= 23.6 ± 19.7).

All patients have been given immunosuppressive drugs; 49 patients with prednisolone and azathioprine; 14 patients with prednisolone and cyclosporin A and 37 patients with prednisolone, azathioprine and cyclosporin A. A group of 20 normal control cases (14 males and 6 females) ranged in age from 20-35 years (mean ± S.D. = 27.4 ± 3.3) were also included.

B- Specimens:

Urine, stool, sputa and sera were collected from both patients while they attended outpatient clinic for follow up after kidney transplantation and control cases. Skin scraping was performed on two patients showed clinically suspected skin fungal infection.

C- Media:

1) Sabouraud chloramphenicol agar (Ajello et al., 1963)

This medium consists of:

Peptone	10 gm
Dextrose	40 gm
Chloramphenicol	0.5 gm
Agar	15 gm
Distilled water up to	1000 ml

The pH was adjusted at 5.6

2) Sabouraud liquid broth (USP, 1965)

This medium consists of:

Peptone	10 gm
Dextrose	40 gm
Distilled water up to	1000 ml

The pH was adjusted at 5.6.

3) Potato-Carrot- Bile medium (Paraltou and Marcelou, 1956)

This medium consists of

Peeled and grated potato	20 gm
Peeled and grated carrot	20 gm
Agar	25 gm
Fresh bovine bile	150 ml
Water up to	1000 ml

Potato and carrot were boiled until complete cooking then, filtered by gauze. The medium was sterilized in the autoclave at 120°C for 10 minutes. This medium is selective for chlamydospore formation of *Candida albicans*.

4) Sabouraud tetrazolium chloramphenicol (Segretain et al., 1974).

It consists of:

Peptone	10 gm
Dextrose	40 gm
Chloramphenicol	0.5 gm
Triphenyltetrazolium	0.01 gm
Agar	15 gm
Distilled water up to	1000 ml

The pH was adjusted at 5

5) Medium for sugar fermentation (Collee et al., 1989)

This medium consists of

Peptone	15 gm
Andrade's indicator	10 ml
Sugar to be tested	20 gm
Distilled water up to	1000 ml

Andrade's indicator was prepared from 0.5% aqueous acid fuchsin with 1 mol/litre NaOH to turn the colour of the solution yellow. The six sugars used were; glucose, lactose, sucrose, maltose, galactose and trehalose.

D- Stains:

1) Lactophenol cotton blue stain (Ajello et al., 1963)

It consists of:

Phenol crystals	20 gm
Lactic acid	20 ml
Glycerol	40 ml
Distilled water	20 ml
Methyl blue	0.075 gm

Dissolving the phenol crystals in the liquids by gentle warming and then adding the dye.

2) Gram's stain

Conventional Gram stain was prepared and conducted as mentioned by Collee et al.(1989).

E- Reagents For Indirect Immunofluorescent Technique

(Segretain et al., 1974).

1) Phosphate buffered saline

- Stock solutions:

i. NaCl	85 gm/L. of distilled water
ii. KH_2PO_4	90.7 gm/L of distilled water
iii. K_2HPO_4	116.2 gm/L of distilled water

- Working solution:

Stock solution (i)	100 ml
Stock solution (ii)	2.33 ml
Stock solution (iii)	11.67 ml
Distilled water	886 ml

pH was adjusted at 7.4

2) Mounting fluid

It consists of:

$NaHCO_3$	0.071 gm
Na_2CO_3	0.016 gm
Distilled water	10 ml
Glycerol up to	100 ml

pH was adjusted at 8.9

3) Antihuman immunoglobulin conjugated with fluorescein

was commercially available from Behring, West Germany.

II. Methods

A- *Sampling*:

1) Urine

Midstream urine was collected aseptically in sterile, screw capped 50 ml disposable plastic tubes (Inter Med, Nunc, Denmark) and cultured within one hour after collection.

2) Stool

Faecal material was collected in 128 ml sterile, wide-mouthed cups with screw caps (ELKay, England) and cultured within one hour after collection.

3) Sputum

Sputum collected by deep cough after patient instructed to vigrously wash mouth with water and collected in disposable sterile Petri dish (InterMed, Nunc, Denmark) and cultured within one hour after collection.

4) Sera

2 ml sera were obtained from each patient and each control case, then were stored frozen at-20°C till being tested for fungal antibodies by indirect immunofluorescent antibody technique.

5) Skin scraping

The skin lesion was thoroughly disinfected with 70% ethanol and scraped with blunt edge of sterile scalpel blade and placed in a sterile Petri dish for culture.

B- Specimen processing

1) Urine

The urine samples were centrifuged at 2000 rpm for 15 minutes; the supernatant fluid was discarded and leaving 1 ml of urine with the remaining sediment, mixed with vortex mixer for few seconds then, 0.5 ml of sediment was inoculated on two Sabouraud chloramphenicol agar plates, one plate was left at room temperature and the other was incubated at 37°C, the plates were checked 3 times weekly for fungal growth and discarded as negative, if no growth appeared after 4 weeks (AL-Doory, 1980).

2) Stool

Faecal specimen was processed by emulsifying about 1 cm^3 of the specimen in 10 ml of sterile distilled water, to which 1 ml of 1 mg/ml chloramphenicol solution, was added, after mixing, specimen was allowed to sit at room temperature for 1 hour, then centrifugated at 2500 rpm for 15 minutes, the supernatant was discarded leaving about 1 ml

of sediment, which was mixed by vortex mixer and 0.5 ml of sediment was inoculated on two Sabouraud chloramphenicol agar plates, one plate was left at room temperature and the other at 37°C , then the plates were checked 3 times weekly for fungal growth and discarded as negative, if no growth appeared after 4 weeks (AL-Doory, 1980).

3) Sputum

When sputum was mucoid or sticky, 0.5 ml sterile saline was added to each 0.5 ml of the sputum to assist in breaking up clumps, then by inoculating 0.5 ml of the sputum on two Sabouraud chloramphenicol agar plates, one plate was left room temperature and the other at 37°C. The rest of specimen was used for lactophenol cotton blue wet mount for direct microscopic examination of fungus. The plates were checked 3 times weekly for fungal growth and discarded as negative, if no growth appeared after 4 weeks (AL-Doory, 1980).

4) Skin scraping

The tip of a sterile probe was moistened by submerging it into the agar plate and was used to transfere small pieces of the skin scraps to the agar plate, then the agar plates were incubated at room temperature and 37°C then, were examined twice weekly for fungal growth and discarded as negative if no growth appeared after four weeks (Campbell et al., 1980).

C- Identification and Characterization of Fungal Isolates

i. **Macroscopic appearance of the colony:**

Size, shape, surface, colour and consistency of colonies and the reverse of the plate for each fungal species were examined.

ii. **Microscopic examination of the colony:**

a- Film stained with Gram's stain.

b- Film stained with lactophenol cotton blue stain: this is the most routine mount used in mycology laboratory. A drop of lactophenol cotton blue is placed on a clean glass slide and by using a flamed firm needle, a small amount of colony is cut from the culture preferably to take a small amount of agar with a piece of colony, then, the material was gently teased with the aid of a flamed dissecting needle and a cover glass was pressed gently over the preparation then, by examination under the microscope with lowered light at low power (X10) and then at high power (X40) (Campbell et al., 1980)

iii. **The Isolated Yeasts Were Characterized and Identified As Candida spp. By:**

1) Germ tube formation (Chaffin and Sogin, 1976)

It was performed by inoculating 0.5 ml of human serum with a loopful of organism and incubating for 2 hours at

37°C, then a drop of the sediment was placed on slide with a drop of lactophenol,overlaid with a coverslip and examined microscopically for the presence of germ tube which appeared as blue filamentous protrusion emerging from blastospore parent. This was positive for *C. albicans*.

2) Test for Chlamydospore Formation (Paraltou and Marcelou, 1956).

The isolated *Candida* was inoculated on to Sabouraud's chloramphenicol agar at 37°C for 24-48 hours, then sub-cultured on potato-carrot-bile medium tubes (PCB medium) with several stabs in the bottom of the tube then one final stab nearer to the top. The cover of tube media was not tighten to allow aeration which is essential. The inoculated tubes medium were left at room temperature for 24-48 hours, the presence of chlamydospores was checked by taking off agar piece from a zone showing filamentous growth and crushed between the slide and coverslip on a drop of lactophenol blue and examined. Chlamydospores appear as terminal or lateral, round or oval spores with a diameter of 6-12 um with thick wall, distinguishable from yeasts and other spores with diameter of only 3 to 4 um. If no spores appear after 48 hours incubation, another subculture for 48 hours into PCB medium was done. Presence of chlamydospores was characteristic for *C. albicans*.

3) Sabouraud Tetrazolium Chloramphenicol medium

(Segretain et al 1974).

It is a medium for differentiating *Candida* species, particularly *Candida albicans* from other yeasts, but culture on this medium does not replace other techniques used for yeast identification. On this medium *C.albicans* strains grow easily producing smooth raised glistening creamy-textured colonies but showed no or limited ability to reduce the indicator salt. *C. stellatoidea* produces orange-pink colonies, *C. tropicalis* produces maroon-red colonies, *C. pseudotropicalis* gives salmon pink colonies, *C.parapsillosis* gives rose pink colonies, *C.krusei* gives white to pale pink colonies and *C.guilliermondii* gives red colonies.

4) Sugar fermentation tests

The medium for sugar fermentation was prepared by dissolving the peptone and Andrade's indicator in one litre of water then 20 gm of the sugar to be tested was added; the six sugars used were; glucose, lactose, sucrose, maltose, galactose and trehalose; 3 ml amounts of each sugar media were dispensed in standard test tubes containing an inverted Durham tube and the sterilization was done by steaming at 100°C for 30 minutes on 3 consecutive days,

then, the inoculum suspension of test organism was added into each tube and incubated at 37°C for 72 hours. Fermentation of the sugar was shown by the indicator turning red, indicating that acid has been produced and by formation of gas bubbles in Durham tube (Collee et al., 1989). The interpretation of results of identification was carried out according to Table (4).

Table (4): Summary of Differential Characteristics of Candida species.

Candida species	Germ tube	Chlamydospores formation	Tetrazolium medium	Sugar fermentation					
				Gl	Gal	M	S	L	T
C.albicans	+	+	Creamy white	+	+	+	V	0	V
C.stellatoidea	±	Rare	Pink	+	0	+	0	0	0
C.tropicalis	0	0	Red-purple	+	V	+	+	0	V
C.krusei	0	0	Dull white	+	0	0	0	0	0
C.pseudotropicalis	0	0	Pink	+	+	0	+	+	0
C.guilliermondii	0	0	Red	+	V	0	+	0	V
C.parapsilosis	0	0	Pink-red	+	V	0	0	0	0

Gl= glucose; Gal=galactose; M=maltose; S=sucrose; L=lactose;

T=trehalose; (+)=positive; (0) negative; (V)= variable.

D) Detection of Fungal Antibodies by Indirect Immunofluorescent Technique.

I. Antigen preparation

. *Aspergillus antigen*

Aspergillus culture was prepared on Sabouraud liquid broth and shaked for 7 days at 37°C by electrical shaker at 20 rounds per minute, then collected to be mechanically disintegrated by pestle in a mortar containing sterile sand to break mycelium into short pieces under aseptic precautions. Washing was done several times by sterile distilled water followed by centrifugation at low speed to sediment the sand particles, the supernatant containing Aspergillus hyphae was diluted with sterile distilled water. Smears of Aspergillus antigen solution were placed on each circle of a glass slide, dried and heat fixed (Kaplan and Kaufman, 1961).

. *Candida antigen*

Candida culture was prepared on Sabouraud liquid broth and shaked for 7 days at 37°C by electrical shaker. Then, by centrifugation and washing with sterile distilled water for 3 times. The sediment was taken to make Candida suspension with sterile distilled water. Smears were placed on each circle of a glass slide, dried and heat fixed (Drouhet, 1965).

II. Performance of Indirect Immunofluorescent Antibody test (Cherry et al., 1960).

1- Clean dry glass slide was divided on its surface into multiple circles by diamond pencil.

2- On each circle of the slide, a drop of antigen suspension was placed, dried and heat fixed.

3- A series of double fold dilutions of the test and control sera were prepared in phosphate buffered saline (PBS).

4- One drop of each diluted serum was added on each circle and the slide was incubated in a humid Petri dish for 30 minutes at 37°C.

5- The slide was then rinsed in two changes of PBS,5 minutes each, then left for air dry.

6- One drop of antihuman immunoglobulin labelled with fluorescin dye (one in ten dilution) was added on every circle of the slide.

7- The slide was reincubated in a humid Petri dish for another 30 minutes at 37°C , then washed with PBS for 10 minutes.

8- The slide was plot dried carefully and mounted in buffered glycerol saline.

9- The slide was covered and examined under fluorescent microscope *(Leitz, W. Germany)*.

10- Positive immunofluorescence was indicated by bright green fluorescent colour against black background.
The highest dilution of serum which gave positive fluorescence was taken as the end point of that serum.

STATISTICAL ANALYSIS OF DATA

Our data were analysed using the following tests:

(1) T-test for unpaired data:-

This was used to test dependency of quantitative data on dichotomous qualitative variable.

(2) Chi square test:-

This was used to test dependency of a qualitative variable on another qualitative variable in cross tabulation.

(3) Fisher exact test:-

This was used for the same purpose of testing association between two qualitative variable in a two by two contingency table when the expected value in any of the four cells was less than 5.