BACTERIAL AND MYCOLOGICAL FLORA IN PATIENTS WEARING SOFT AND HARD CONTACT LENSES

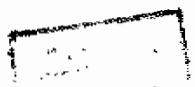
THESIS

Submitted in Partial Fulfilment
of Doctoral Degree in Ophthalmology

By

MAGDA MAHMOUD SAMY

M.B.B.Ch, M.S.



Supervised by

Prof. Dr. Mohamed Hassan Abdel Rehim
Professor of Ophthalmology
Ain Shams Faculty of Medicine

Prof. Dr. Gilbert Smolin
Professor of Ophthalmology
University of California,
San Francisco Medical Center

Prof. Dr. Hassan Hosny Youssef
Professor of Chest Diseases
Ain Shams Faculty of Medicine

Prof. Dr. Youssef Abdel Ghani
Professor of Mycology
Ain Shams Faculty of Science

Faculty of Medicine
Ain Shams University
Cairo

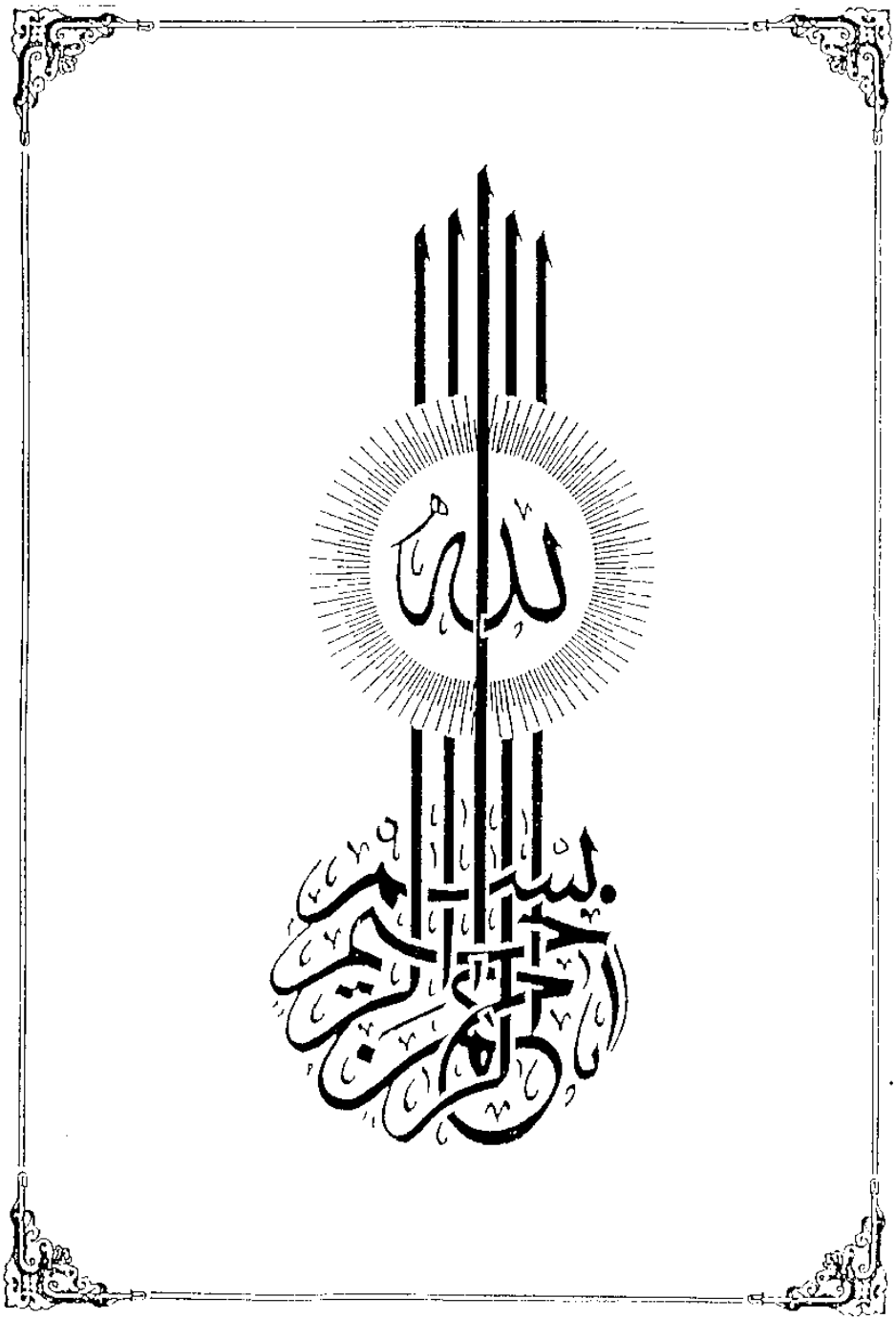
1985

ACKNOWLEDGEMENT

I feel grateful to professor Dr. MOHAMED HASSAN ABDEL REHIM for his supervision and encouragement throughout the work. The support and pursuit of Professor Abdel Rehim were behind the completion of this joint project between Ain Shams Ophthalmology Department and the Proctor Foundation, University of California - San Francisco. I feel also grateful to all the members of the Ophthalmology Department in Ain Shams University for their encouragement and support.

I would like to thank Professor Dr. Gilbert Smolin who has given a lot of his time and experience during the study. He has been thoroughly supervising every step in this project. Through the facilities provided by Dr. Smolin this study was completed.

The advise provided by Professor Dr. Hassan Hosny and Professor Dr. Youssef Abdel Ghani was a motive for the achievement of the work.



CONTENTS

Literature Citing

A. Bacterial Flora of the Eye .

B. Fungal Flora of the Eye.

C. Infectious Keratitis.

D. Contact Lenses and Infectious Keratitis.

A. THE BACTERIAL FLORA OF THE EYE.

Bacterial flora of the conjunctiva of the normal eye is remarkably similar around the world. There is no difference in the flora recovered from the lid margin and those recovered from the conjunctiva . Cultures taken from persons of all ages in many parts of the world have shown essentially the same findings(1-6).

Staphylococcus epidermidis and diphtheroids are regarded as the most constant inhabitants of the lid and conjunctiva. The carrier rate of Staphylococcus epidermidis reported from different sources varies from 33.8%(7) to 75.7%(8), while that of diphtheroids varies from 18%(4) to 83%(9).

Factors that account for this variation in the percentage include the locality where the investigation was carried on, the type of culture media and the method of culturing.

Other strains of Staphylococcus, Streptococcus hemolyticus, Moraxella species, Streptococcus pneumoniae, Haemophilus aegyptius, Neisseria meningitidis, and a variety of other organisms may also be found in the normal flora of the eye. All these organisms with the exception of diphtheroids can become pathogenic to the eye(10).

The normal conjunctival flora has been demonstrated to contain anaerobic organisms(11-16). The obligate anaerobe Propionibacterium acnes has been cultured as the predominant anaerobe in 49.5% of eyes studied by Mc Natt and his colleagues(16). Other investigators(13,14) have isolated Lactobacillus, Eubacterium, Clostridium, Bacteroides, and Actinomyces species in small percentage of normal eyes. These anaerobes although considered to be harmless commensals, can become invasive and produce diseases under favorable conditions. For example Propionibacterium acnes has been identified as cause of postoperative bacterial endophthalmitis(15).

The conjunctiva is sterile at birth but shortly thereafter becomes invaded with various saprophytic bacteria(10,17). An infant routinely picks up bacteria during passage through the birth canal. An example of this is Neisseria gonorrhoeae which causes conjunctivitis in the newborns. Staphylococcus epidermidis, Staphylococcus aureus, Streptococcus species and Escherichia coli also can be transmitted to the infant's eye during birth(17).

Other sources of bacteria in the eye may be the skin of the face,the upper respiratory tract and/ or the skin of the hand. Occasionally, Gram-negative bacilli which are more common in the gastrointestinal and urinogenital tracts are isolated from the eye(10,17).

Bacteria commonly found in the air rarely colonize on the ocular tissue. These may be because airborn organisms (1) are introduced in small numbers,(2) find the oculoadnexal tissue unfavorable for growth, and (3) are rapidly flushed away by the tear film.

The normal bacterial flora is not modified by age except for a rise in the incidence in diphtheroids in persons over 50 years of age(17). There is also no difference in the bacterial flora between the healthy eyes of males and females, nor seasonal variations in the carriage rate(17). The flora obtained from the right and left eyes is almost always identical(17,18).

Eyes obtained from the eye bank carry the same flora found in life in the normal eye with an exception . All bacteria are more numerous than in the living eye(19-21).

Patients receiving immunosuppressives have the same bacterial flora as normals , although the number of positive cultures is higher(22).

Infectivity is deterimined chiefly by (1) the virulence of the organism, (2) the host resistance, and (3) whether the organism is colonized or transiently localized to the tissues.

Virulence of the organisms can be exerted in a number of ways. Some organisms have antiphagocytic factors as parts of their structures which protect them from host phagocytes. Others possess endotoxins as part of their wall, or produce exotoxins that are toxic to the host cells.

Staphylococcus aureus produces coagulase which deposits fibrin around the focus of infection and prevents entrance of immunoglobulins.

Dissemination of the organisms is accomplished with the help of hyaluronidase (which depolymerizes hyaluronic acid) and of both the fibrinolysins and streptokinase (which are elaborated by most strains of hemolytic streptococcus group A and bring about the digestion of fibrinogen and lysis of fibrin clot). Other enzymes such as proteases, collagenases, amylases, nucleases, staphylokinase are also elaborated by bacteria to enhance their pathogenicity.

Some microorganisms such as Staphylococcus aureus may possibly change to L-forms (viable bacteria minus cell walls) in the presence of antibiotics, re-emerging as fully virulent organisms when the antibiotic is withdrawn.

Many parasites (e.g., Mycobacterium tuberculosis, Listeria monocytogenes, Francisella tularensis) can resist destruction if phagocytosed by macrophages. They all contain large quantities of structural lipid that may protect them from phagocytic digestion within the phagolysosomes(23).

The surface of Staphylococcus aureus organisms is covered with a substance called protein A. The antibody-binding fragment (F ab), which is normally the combining end of the antibody molecule, can not combine with protein A, only the opposite end (the complement-binding fragment F c) combines with it. This reversed attachement blocks phagocytosis by macrophages or polymorphonuclear leucocytes (24,25).

Some organisms have specialized pili (hair-like, proteinaceous antigen) ,

which facilitate adherence of the bacteria to the epithelial cells. Pili are required for colonization.

Lowered resistance predisposes the host to disease produced by his own normal flora or by a number of opportunistic pathogens(26). The host defenses which protect the conjunctiva from bacterial infections include non-specific and specific immune mechanisms.

Apart from the flushing mechanism, tears contain substances which have antibacterial activity(27). Secretory immunoglobulin A (S Ig A) appears to play a key hole in preventing mucosal invasion of several microorganisms. It inhibits the adherence of bacteria to mucosal surface, thus limiting an organism's colonization in the eye(28). Secretory Ig A also produces lysis of bacteria in the presence of complement and lysozme, and augments phagocytosis and opsonizaiton of microorganisms(29).

Lysozyme is an antibacterial substance in the tears that inhibits bacterial activity in the eye. It is an enzyme of low molecular weight which reduces the local concentration of the susceptible bacteria by attacking the mucopeptides of the cell wall (30). Although most Gram-positive bacteria are affeced by lysozyme, Staphylococcus aureus is not, since the lysozyme susceptible site on this organism's cell wall is blocked structurally from lysozyme attack(23).

Lactoferrin,another major tear protein, has bacteriostatic properties presumably due to its ability to make certain metals unavailable for microrganisms(31).

Bacteria can be removed by phagocytosis. They first adhere to polymorphonuclear leucocytes and macrophages and then engulfed by a phagosome. The

phagosome fuses with lysozymal granules to form a phagolysosome in which the bacteria are killed by the low PH and by hydrolytic enzymes.

As a part of acute infammatory response, the neutrophils are mobilized in the conjunctival epithelium in response to specific chemotactic stimuli, which play an important role in the defense against pyogenic bacteria(27).In addition, the increased capillary permeability brings about a massive transudation of bactericidal factors contained in the serum such as C-reactive protein, properdin and complement system.

The specific immune defenses include humoral immunity and cell-mediated immunity (CMI).

Humoral immunity plays an important role in the defense against microorganisms. Opsonizing antibody and the third component of the complement are necesary for the adherence of polymorphonuclear leucocytes to bacteria.The complement omponents can destroy bacteria directly or by causing chemotaxis of the neutrohils.

Antibodies can also neutralize the soluble exotoxins released by certain cteria.

Cell-mediated immunity (CMI) plays an important role in combating microganisms. When a T-lymphocyte becomes sensitized to a bacterial antigen, it reases lymphokines which can invade the macrophage and kill the ingested organisms. e sensitized T-lymphocytes also release chemotactic factors for neutrophils, sinophils, and basophils. Factors that help aggregation and activation of macphages at the site of insult are also released by the sensitized T-lymphocytes 2).

B.THE FUNGAL FLORA OF THE EYE.

Fungi are vegetable organisms of low order. Most of them are considered to have their normal habitation in soil and vegetable matter.

Fungi are structurally more complicated than bacteria, since they are larger and vacuolized, and usually have sexual reproductive mechanisms. They are usually multicellular, filamentuos structures, although yeasts and some branched fungi are unicellular. A hypha is an individual filament, which may be divided by septae. A mass of hyphae constitutes the mycelium. Hyphae can be seen clinically in some cases of keratomycosis, fungal endophthalmitis and associated with fungal invasion of soft contact lenses(33-37,39).

Most fungi recovered from patients with keratomycosis have also been isolated from the normal eye. Such findings are compatible with the hypothesis that fungi found in some ocular infections may have been carried by the patient(14,17).

The low incidence of fungi in newborns supports the fact that fungi are airborn into the conjunctiva(41).

The incidence of fungi in clinically normal eyes of adults has been reported from various areas of the world(42-57). The percentage of cases showing postive cultures varies from 0% to 52% (Table 1).

A warm moist climate, a rural agricultural environment and advanced age may be associated with an increase incidence in the number of positive fungal cultures (58).

Fungi in the normal eye appear to depend on random seeding from the environment and appear to be transient(40). The lids are more likely to contain fungi than the conjunctivas.

Cultures taken from the conjunctivas of immunosuppressed patients did not show increase in the number of positive fungal culture(22).

Table 1 : The worldwide Incidence of Fungi Isolated from Healthy Eyes.

Authors	Year	%	Region	City
Fazakas(42).	1935	24.3	Hungary	Debrecen
Fazakas(43).	1938	25.6	Hungary	Debrecen
Fazakas(44).	1953	25.5	Hungary	Debrecen
Mitsui & Hanabusa(45).	1955	18.4	Japan	Kumamoto
Hammeke & Ellis(41).	1960	10.3	U.S.A.	Arkansas
Jank & Schwab(46).	1961	11.1	Austria	Vienna
Azevedo(47).	1962	0	Brazil	Rio de Janeiro
Agarwal & Khosla(6).	1963	6	India	New Delhi
Ovsepian & Osipian(48).	1965	2.5	U.S.S.R.	Erevan
Ainley & Smith(49).	1965	27.9	England	Oxford
de Ocampo & associates(50).	1965	3.5	Philipine	Manila
Vasquez de Pargo & Pereiro(51)	1965	52	Spain	Santiago
Nema & associates(52).	1966	22.2	India	Alligarh
Sinha & Das(53).	1968	13.4	India	Calcutta
Williamson & associates(54).	1968	2.9	Scotland	Glasgow
Locatcher Khorazo & Benham(55)	1968	4.4	U.S.A.	New York
Wilson & associates(40).	1969	6	U.S.A.	Miami
Gugnani & associates(56).	1977	37.1	Nigeria	Enugu
Ando & Takatoi(57).	1982	6.6	Japan	Kanagawa

Although different fungi are found in different geographic areas, the most commonly isolated ones are Penicillium, Aspergillus, Fusarium, Candida, and Cephalosporium species.

It has been shown experimentally that both antibiotics and steroids increase the number of fungi isolated from the healthy eyes(45,52,59). Mitsui and Hanabusa(45) found that the incidence of fungi in the conjunctival sac after topical steroid therapy was 67%, while in control cases it was only 18%.

There are 100,000 named species of fungi, about 200 are either primary pathogens or occasional opportunistics. Although the primary pathogens are able to produce disease in a healthy host, the opportunistics require a great defect in the normal defense to become established(60-62). Most of fungi are saprophytic and can be rapidly destroyed by the defense reactions of the host(59).

The pathogenicity of fungi is related to various factors. Hypersensitivity reactions occur frequently with fungal infection and may result from sensitization to specific fungal antigens.

Fungi may elaborate or indirectly generate substances that have toxic effect on tissues such as mycotoxins. These include trichothecenes which are produced by many Fusarium and Acremonium species. At low dose, a single application of these toxins results in a prolonged inflammatory response, while higher doses cause tissue necrosis. A number of trichothecenes have been shown to be cytotoxic or cytostatic to many kinds of animal cells in culture(63).

Aflatoxin, a toxin produced by Aspergillus species , can cause inhibition of DNA and RNA polymerase as well as impairment of protein synthesis.

Pathogenicity of Candida albicans is thought to be due to the production of proteolytic enzymes that cause lysis of the host tissue, which allow Candida to penetrate and invade this tissue(64,65).

Sobel and associates(66) have demonstrated that the adherence of Candida albicans to epithelial cells is aided by a surface protein that binds to the epithelial cell receptors.

Diamond and associates(67) demonstrated a substance liberated by Candida and inhibits the contact of human neutrophils with Candida pseudohyphae. This inhibitory substance may be a cell wall component or a part of a mucus layer secreted by the organism, which would be analogous to the capsule, an important virulent factor in many pathogenic bacteria.

From the high prevelance of fungi in the enviroment and the low incidence of mycotic diseases, it obvious that most humans are highly resistant to fungal invasion.

The cell-mediated immune defense system plays the major role in protecting the host from fungi(68).Evidence of the importance of cellular immune reactions include the facts that fungal infections are most frequently found in patients with depressed cellular immunity and tissue infected with fungi shows intense mononuclear and granulomatous reactions(69). Humoral antibody immunity also plays a role(70). During fungal infection both cellular and humoral immune responses are stimulated.

C. INFECTIOUS KERATITIS.

Infections of the cornea may lead to serious visual impairment and are considered to be one of the major causes of blindness throughout the world.

The cornea is a unique anatomic structure . It consists of a hard, homogenous , avascular tissue which is incapable of regeneration, except for epithelial cells, fibroblasts of the stroma and Descemet's membrane.

The predominant feature of corneal infection is necrosis, often resulting in ulcer formation. Damage unless extremely mild, leaves a permanent scar on the cornea.

There are two types of keratitis in which bacteria have been considered, marginal and central. Marginal keratitis is common in bacterial conjunctivitis and blepharitis. It is rarely caused by the bacteria themselves but it is rather a secondary toxic or hypersensitivity response. This form of keratitis is not severe and usually heals with the conjunctivitis or blepharitis.

Central infectious keratitis generally follows corneal trauma and is due to direct invasion by infectious agents. The process may be severe and can end with endophthalmitis and panophthalmitis.

The central part of the cornea is most exposed. It is thin, poorly nourished,poorly supplied with antibodies and more liable to trauma and subsequent contamination.

In the preantibiotic era, the common organism in central infectious keratitis was the pneumococcus. Now Gram-negative bacilli are the main agents of this disease.

Infectious strains that were formerly considered non pathogenic can now cause disease. These are known as opportunistic organisms and may be highly resistant to antibiotics. In addition anaerobes have now begun to be recognized as a major cause of serious corneal infection, particularly in drug addicts(10).