MICROBIAL AETIOLOGY OF CONJUNCTIVITIS IN URBAN CHILDREN

THESIS

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CHAPTER I

INTRODUCTION

Introduction

Conjunctivitis is the commonest eye disease in Egypt, where it presents a major problem of public health. The chief menace of acute infective conjunctivitis lies in the corneal complications which may cause blindness (Nakhla, et al., 1970).

In areas where trachoma occurs as an endemic blinding disease, it is often associated with bacterial conjunctivitis which occurs in spring and autumn as epidemics (Wilson, 1932, W.H.O., 1973) Mawell-Lyons, 1953; Huet, 1958). It is accepted that trachoma is due to infection with a member of the Chlamydia group of organisms, but when it occurs in absence of bacterial conjunctivitis as is the case now in American Indians, the disease rarely leads to major visual loss (Hoshiwara, et al., 1971).

It has long been known that trachoma and associated bacterial infections of the outer eye are extremely wide - spread in Egypt and in the past accounted for a major part of visual disability (Thygeson and Dawson, 1959; Hanna et al., 1959).

A previous study by Said et al.(1969a) showed that 4.7% of the rural population and 1.5% of the urban population in Northern Egypt has significant visual disability. At least half the loss in the rural area and one-third in the urban area was due to purulent ophthalmias and infectious diseases (Said et al.,1969a). A series of studies carried out by Assad et al. in Taiwan (1971) emphasized the prevalence of active infectious trachoma in childhood, the occurance of complications in later life, and the differences in the prevalence of disease from community to community (Assaad et al., 1971a; Assaad et al.,1971b).

In a severely affected Tunisian village, all children developed active trachoma by the age of two years but the prevalence of active inflammatory disease declined steadily by the age of 15 years. Thus most infectious cases (i.e. most likely transmit the infection) occured in children under 10 years of age (Dawson, et al.,1976). This is supported by a study that was carried out in two small rural Egyptian communities. Trachoma still affected almost all children by age 2 years; it was very severe and often associated with bacterial infections of the eye (Said et al., 1980). Active infectious trachoma has practically disappeared in urban areas, so that Egyptian Ophthalmogists rarely see active trachoma, but "acute ophthalmias", thought to be the major cause of infectious blindness in Egypt(Arnson,1969).

HISTORY

Neisser in 1879 was the first to describe a bacterial ocular pathogen, the gonococcus. A few years later, while studying the aetiology of Cholera in Egypt, Koch (1883) observed the second organism considered to be a primary pathogen of the eye. This organism was later isolated by Weeks (1886) and came to be known as the Koch-Weeks bacillus (Haemophilus aegyptius).

No annotation of the bacteriology of the conjunctiva could pretend to be complete without a tribute to the two masters of this branch; Axenfeld and Morax. In 1897 Morax isolated a diplobacillus from a case of subacute angular conjunctivitis. This bacterium, identified independently by

Axenfeld, is now familiar as the Morax-Axenfeld diplobacillus, or Moraxella lacunata.

Since the appearance of Axenfeld's book "Die Bakteriologie in der Augenheilkunde" in 1907, a milestone in the history of occular bacteriology was put.

Trachoma has been identified from the earliest human records as the greatest cause of blindness. It was known in China in the 27th. Century B.C., Sumeria in 21 st. Egypt in the 19th. . Greece in the 5th. and Rome in the 1st. Century B.C. (Duke-Elder 1965). It was known in the Islamic world in mediaevial times but attracted little attention in European writings until it became a veritable scourge to both French and British troops in the Napoleonic Campaign in Egypt in 1789 - 1799. In one British battalion of 700 men 90 per cent were known to become infected (Duke-Elder, 1965). With ensuing movements of troops the military ophthalmia spread to civilian populations throughout Europe in conditions of overcrowding and Squalor following the Industrial Revolution and the devastations of war. Whatever the relative roles of Chlamydia and bacteria in the causation of this devastating ophthalmia it was indeed the blinding communicable eye disease historically called Trachoma (Jones, 1975).

NORMAL CONJUNCTIVA

The conjunctiva is the thin transparent mucus lining of the undersurface of the lids from which it is reflected onto the anterior surface of the eye ball. At the lid margin it is continuous with the skin and at the corneal margin it is structurally continuous with the corneal epithelium. It therefore forms an uninterrupted membrane lining the potential space of the conjunctival sac which opens externally through the palpebral fissure at the lacrimal puncta. The mucous membrane is continous with that lining the lacrimal passages, and through this with the nasal mucosa. The conjunctiva may be divided into regions and areas as follows:

1. The palpebral conjunctiva (a) marginal
   (b) tarsal
   (c) orbital.

2. The conjunctiva of the fornix (a) Superior fornix
(b) Inferior fornix
(c) Medial fornix.

3. The bulbar conjunctiva (a) Scleral
(b) Limbal.

4. The plica semilunaris .

CYTOLOGY OF NORMAL OCULAR SCRAPINGS

Axenfeld in the first decade of this century, stated that "the mere examination of the film gives a great deal of information and it is absolutely necessary, in dealing with external and internal disease that the material should be collected at a suitable stage in the disease".

Cellular response reflects some degree of inflammation in the eye and often reveals the presence of infection. Cytological examination of smears from exudate and scrapings obtained from the conjunctivae may contribute to differential diagnosis in various ocular infections (Locatcher - Khorazo and Seegal, 1972).

The conjunctival scrapings from the normal individuals show:

1. Normal epithelial cells in various stages of normal aging. When stained with Giemsa stain, they show a single violet coloured nucleus surrounded by a light blue zone of cytoplasm. There is some variation in the size and shape of the epithelial cells, which may be in part because of the depth of the scraping. They may be polyhedral as well as round. In addition to the normal epithelial cells, many scrapings contain a few cells that show an abnormality caused by fragmenting nucleus. Another variation of the normal epithelial cells, show intracellular granules that stain green with Giemsa stain. This occurs most frequently in Orientals and peopole with dark complexions and are not associated with disease. Similar intracellular granules staining dark blue may be observed in normal epithelial cells.

2. Goblet cells, though normal to the conjunctiva of the eye, are rarely seen in scrapings from it since they occur mainly in the fornices. They have granules which are less distinct than elementary bodies of the viral cytoplasmic inclusions and lighter in colour(Locatcher-Khorazo and Seegal, 1972).

3. Lymphocytes, are not normally present in the newborn but are demonstrable in all children from 3 to 10 years of age. (Norn, 1962). They are small and medium sized with a single, sharply defined, round, dense violet nucleus surrounded by a varying amount of delicate, pale blue colour cytoplasm when stained with Giemsa stain (Locatcher-Khorazo and Seegal, 1972).

CYTOLOGY OF INFLAMMED CONJUNCTIVA

In inflammed eyes the epithelial cells seen in scrapings from normal eyes are replaced in whole or in part by various polymorphonuclear leukocytes, lymphocytes, large mononuclear cells, plasma cells, and abnormal epithelial cells, a process giving expression to pathologic changes.

The following typical cytological pictures are worthy of note:

1. The polymoprhonuclear reaction

A preponderance of PMN cells is characteristic of an acute bacterial infection, a general phenomenon to which there are two exceptions, the inflammations caused by

Neisseria catarrhalis and Moraxella, neither of which is pyogenic in type. In the very acute stages of bacterial infections, such as a gonorrheal ophthalmia, the leukocytosis may be extraordinarily intense, in the later subacute stages more mononuclear cells are evident although PMNs still predominate. Among the bacteria the staphylococcus excites a particularly profuse PMN reaction.

Apart from the bacterial infections, the PMN reaction is characteristic of infections by Chlamydia and actinomyces and certain other conditions such as erythema multiforme.

2. The mononuclear reaction

The lymphocytes are also present in scrapings in most chronic inflammations but rarely preponderate, and are also the predominant cell in chronic irritative conditions.

3. The eosinophilic reaction

A preponderance of eosinophils is characteristic of allergic inflammations with the exception of bacterial allergies and phlyctenular conjunctivitis. The most intense eosinophilia is seen in vernal catarrh.

4. The basophilic reaction

The presence of basophilic cells often runs parallel with an eosinophilia. These cells are therefore a feature of allergic inflammations especially vernal catarrh but their presence is not so characteristic as the eosinophilia. In no other condition are they found in considerable numbers except trachoma.

5. Plasma cells

Are rarely found in conjunctival scraping or exudates except in trachoma.

6. Changes in the epithelial cells

The conjunctival epithelial cells as found in scrapings may show changes, three types of which are of diagnostic importance:

a. Keratinization: is seen in its marked degree in cases of long standing ectropion or cicatrization as in trachoma. These cells have a characteristic appearance with their nucleus displaced by a large mass which stains a homogeneous blue with Giemsa and is cleared by alcohol.

b. Multinucleated epithelial cells: are a feature of viral as opposed to bacterial infections.

c. Degenerative changes: in the epithelium, a flattening and polymorphism of the cells with poor staining of the nuclei, have been claimed to be characteristic of trachoma, on the other hand they may be apparent in any chronic conjunctivitis of long standing.

In the examination of epithelial cells from scrapings the presence of intracellular bodies in the cytoplasm should be noted:

1. Mitochondria, irregular elongated bodies fine and dust-like in young cells, coarse in older cells, staining a faint blue in Giemsa stain and de-staining with alcohol.

2. Keratin- like granules produced by a coalition of mito-chondria, staining red with Giemsa.

3. Nuclear chromatin is frequently extruded into the cyto-plasm in the trauma involved in scraping.

4. Russell bodies, spherical red-staining bodies, evident in the presance of degenerating plasma cells.

5. Phagocytosed cellular debris, seen mostly in the subepithelial histiocytes especially when necrosis is present, staining a uniform dark blue with Giemsa.

6. Melanin pigment, occasionally in present as deep brown particles when unstained, becoming green with Giemsa.

7. Virus inclusions, present a characteristic picture whether cytoplasmic or intranuclear (Duke-Elder,1977).

BACTERIAL FLORA OF THE NORMAL CONJUNCTIVA

Many authors from a large number of countries have investigated the bacteriology of the normal conjunctival sac, either in order to investigate the nature and incidence of the various micro-organisms harbouring the normal conjunctiva (Cason and Winkler 1954, Makabe, 1971, Locatocher Khorzoe and Seegal, 1972) or to study the prophylaxis of postoperative infections (Zetzsche, 1962, Drewnoik and Wachtel, 1967) or lastly to answer the question of the value of bacterial culture prior to intraocular surgery (Nolan, 1967, Allan Smith et al.,1969).

A wide range of organisms is found in the normal conjunctival sac which vary in their number, their nature and their pathogenicity. The variations depend on several factors. Among those is climate. Staphylococci for example are more prevalent in hot climates, a fact that may explain the occurance of this organism in 62% of healthy conjunctivae in Queensland (Gibson, 1951) or 95% in Egypt (Kamel,1949) and in only 34% in England (Smith, 1954). The habits and the degree of social hygiene in different countries also have a considerable bearing, as well as the prevalence of endogenous and epidemic diseases (Trachoma, Koch-Weeks conjunctivitis). A further interesting fact is the change which has developed with time, it may be that the prolific use of antibiotics has altered the flora in populations wherein such drugs are in common use as well as fundemental changes in hygienic conditions. The age is also one of these factors, for example Staphylococcus epidermidis (41%) in age group 0-5 years (39%) in age group (6-14) and is (45%) in age group 70 years and more (Locatcher-Khorazo and Seegal 1972).

Most of the bacteria harbouring the normal conjunctival sac are saprophytic and non-pathogenic (Duke-Elder, 1977). The Staphylococcus epidermidis and C. xerosis (One species of genus diphtheroids) being much the commonest. This observation is in agreement with the finding of Cason and Wrinkler (1954) 67% but is particularly low as compared to the observation of Fahmy et al.(1974) 82%. Diphtheroids are found in 35 to 38% of cases in the age group of 1-18 years (Locatcher-Khorazo and Seegal, 1972) while Fahmy et al.(1974) found its prevalence was 58%.

Other moicroorganisms are found in the normal conjunctival sac, but in low incidence, such as Streptococci (3%) Micrococci (3%) and Pneumococci (1%)(Fahmy et al.,1974).

SOURCES OF BACTERIAL OCULAR FLORA

At birth the conjunctival sac is normally sterile (Koblanck, 1896) but soon afterwards it tends to become contaminated by organisms either saprophytic or parasitic in character. Locatcher-Khorazo and Seegal (1972) stated that "The eye harbour bacteria from the time of birth throughout life. During his passage through the birth canal the infant acquires some of the microorganisms in the mother's vagina. Although the dramatic example of this is Neisseria gonorrhoea, causing gonorrheal conjunctivitis, the infant routinely picks up bacteria carried in the healthy vagina". Some authors have found a considerable proportion of normal sacs free from bacteria Cason and Wrinkler, 1954 23%, Smith (1954) 47%, but others have denied this, maintaning that practically every conjunctiva exhibits some flora. Considering its exposure to the air and its continuity with the skin it is surprising that it ever remains sterile, among these authors are Orfila and Courden (1961).

The bacteria cultured from the eye usually are similar to those found on the skin and in the upper respiratory tract. Gowen (1934) found that organisms inoculated into the conjunctival sac may be present in the nose in 24 hours; but migration in the reverse sense does not readily occur; he also made a study of the possible role of the eye lids in maintaining the flora of the age: he noted the identity of the flora of the eye and eyelid margins and further pointed out that when the eyes are closed, the upper eyelids overlap the lower lids; thus the upper eyelids are exposed to bacteria of the skin. Gowen believed that the eyelashes play a major role in maintaining the skin flora in the conjunctival sac and the eyes are never devoid of staphylococci, because the source of contamination is always present.

The bacteria commonly found in the air are rarely recovered from the eye. This may be in part because of the high content of lysozyme present in tears. This agent is an enzyme, glucose aminidose that brings about the lysis of micrococcus and some staphylococci by acting on hexosamine bound in a muco polysaccharide fraction incorporated in the cell wall (Meyer, 1954).

It is apparent from the presence of certain microorganisms in the normal conjunctiva that man often carries with him potentially pathogenic organisms in his eyes just as he may be a carrier of such bacteria in his upper respiratory tract. Staphylococcus aureus is the potential pathogen most frequently found and is the commonest cause of postoperative infection and this infection may arise from the patient's own eye (Locatcher-Khorazo and Gutierrez (1956).

CONJUNCTIVITIS

Conjunctivitis is an inflammatory process of the conjunctiva with hyperaemia, papillary hypertrophy and mucopurulent exudate. It is found to be mainly a disease of children, with a maximum incidence at 6 years and under, and it is slightly more common in boys (Nakhla et al.,1970).

Mucopurulent conjunctivitis is diagnosed when eyes show diffuse conjunctival injection, oedema of the conjunctiva, fine subconjunctival haemorrhage, and mucopurulent discharge with or without clinical trachoma. Frequently, mucopurulent discharge appears as a fibrinous sheet covering the exposed surface of the upper lid conjunctiva, easily peeled off with a cotton applicator (Haddad, et al.,1968).

The relationship between trachoma and seasonal epidemics of acute bacterial conjunctivitis has been emphasized in several studies describing the ocular flora of trachomatous individuals (Pages, 1951: Faigenbaum et al., 1956; Kamel 1963). Most authors believe that the bacterial ophthalmias have a deleterious effect on eyes with trachoma. The onset of trachoma in a child has been found to be frequently preceeded by an episode of acute bacterial conjunctivitis, most often due to Koch-Weeks bacillus (Haemophilus aegyptius), but also to Gonococcus and Moraxella (Pages, 1951: Soklar, 1950). Bacterial conjunctivitis occuring in the course of established trachoma has been found to cause clinical aggravation of the trachomatous inflammation, to incite the proliferation of inclusion bodies, and to increase the severity of corneal complications (Pages, 1951, Feigenbaum, et al., 1956; Jabejion, 1951; Poleff and Viennot-Bourgin 1951). It has been suggested that one of the principal mechanisms of topical chemotherapy in severe endemic trachoma is the suppression of bacterial conjunctivitis which reduces the blinding complications of trachoma (Vastine, et al.,1974). The presence of trachoma may aggravate the bacterial conjunctivitis (Pages 1951 Feigenbaum, et al.,1956). Thus Koch-Weeks conjunctivitis is said to be more severe, and to invade the corneal epithelium more frequently, in persons with trachoma (Pages, 1951). It is possible that a bacterial infection could cause a clinical exacerbation in an individual who is an asymptomatic carrier of the trachoma agent. Certainly the most common form of acute trachoma is an individual with chronic trachoma of low activity who contracts an acute bacterial conjunctivitis (Wood, et al., 1967).

The organisms responsible for the conjunctivitis have been studied by several authors. The gonococcus is known to be a common cause of conjunctivitis in Egypt (Wilson, 1935; Lyons and Amies, 1949). Moraxella which is known to produce subacute angular blepheroconjunctivitis rather than acute diffuse inflammation, was found by Smith (1954) in a small proportion of healthy conjunctivae, but has been reported in 1% of cases of acute conjunctivitis (Jones et al., 1957).

Staphylococcus has been isolated from the conjunctival sac in 2% of normal individuals (Smith, 1954), but Sadoughi 1948) thought that it was usually a contaminant. It was obtained in pure culture in acute conjunctivitis by Sanyal(1929), and Freindenwald (1929).

The Koch-Weeks bacillus (Hemophilus aegyptius) was the commonest pathogenic organism isolated (40%) from patients with acute conjunctivitis in Assiout(Nakhla et al., 1970); it was isolated in all seasons except winter. The haemolytic Streptococcus was the next most frequent organism (30%). This and the pneumococcus were more commonly encountered in winter season. The pneumococcus was isolated in 11.5% of cases. It is difficult to determine how far it should be regarded as pathogen, but it was found alone in 21 of 24 cases from which it was isolated. Gonococcus was the fourth in frequency (7.5%). Moraxella was found in about 5%. Staphylococcus aureus was also cultured in 5.1%. of cases.E. Coli was isolated in only about 0.5% in one of them it was found in pure culture (Nakhla et al.,1970).

In two rural Egyptian villages about 30 kilometers south east of Alexandria, Ophthalmological and microbiological studies were done. The typical trachoma inclusions were found in the cytoplasm of conjunctival epithelial cells in 15% of Giemsa stained smears. In addition, bacteria were plentifully evident in smears particularly Haemophilus-like organisms (23.2%) and large diplococci of Neisseria-like organisms (8%). The initial culture techniques yielded numerous Haemophilus species (28%), Pneumococci (8%), Moraxella species (4%). The highest prevalence of inclusions and Neisseria-like organisms was in 2 to 5 years old (24%) and (13.5%) respectively, who also have the highest prevalence of active trachoma. All ocular pathogens were dramatically less frequent after 9 years of age when the prevalence of severe and moderate intensity trachoma also decreased (Said et al.,1980).

Among Tunisian children with active trachoma (Vastine, 1974) the most frequently encountered potential ocular pathogens were Haemophilus species. In the conjunctival scraping, the typical Giemsa stained cytoplasmic inclusions of trachoma were found in 17%; only those cases with intense conjunctival inflammation had inclusions and mild cases were never inclusions positive in Giemsa stained smears. Moraxella species together with other potentially pathogenic species (Pneumococcus, Beta-haemolytic streptococci, Staphylococcus aureus and gram negative rods were isolated. Many children had moderate to heavy growth of Streptococcus viridans which has been considered by some to be a potential pathogen in the eyes (Fudekowicz, 1963; Scheie and Albert, 1969). Among organisms not usually associated with eye disease, Diphtheroids and Staphylococcus epidermidis occured most frequently (Vastine et al., 1974).

Of the eight bacterial types isolated from the conjunctival sac of children in South Lebanon, Staphylococcus was isolated in 72% Haemophilus (36%) Streptococcus and Pneumococcus (27%) Bacillus subtilis (22%). Coliforms (12%), and Neisseria and Diphtheroids each in 0.3% of cases (Haddad and McPhail, 1969). In a previous study by Haddad and Ballas (1968), Haemophilus was found intracellularly in 3.4%, extracellularly in 2.2% in Giemsa-stained smears of conjunctiva. Also Staphylococcus, Streptococcus and Pneumococcus were found extracellularly in 14% and intracellularly (2.4%) and Neisseria in 0.2% extracellularly (Haddad and Ballas, 1968). The acute conjunctivitis was the commonest type of infection seen at the Institute of Ophthalmology Columbia-Presbyterian Medical Centre(IOCPM.C.). This study had covered the period from 1938 to 1968 and presented data on premature babies (38 cases), full term babies (1,223 cases), and children to 18 years (3.062 cases). In the premature babies, Staphylococcus aureus, Diplococcus pneumoniae and Pseudomonas aeruginosa accounted for 13.7% infections respectively; the Pseudomonas aeruginosa infections were the most severe. This organism was reported and has proved to be difficult to control in patients of all ages. In the Newborn pneumonococci (24.6%) Haemophilus (10%) Staphylococcus aureus (27.6%) and Neisseria gonorrhaeae (25%) topped the list of agents causing acute conjunctivitis. Pneumococci (46%), H. influenzae (34%) and Staphylococcus aureus (34%) also were agents of acute conjunctivitis in children to 18 years of age. Streptococcus viridans (13%) as well played a major role in the conjunctivitis of this group (Locatcher-Khorazo and Seegal, 1972).

EPIDEMIOLOGY OF CONJUNCTIVITIS

Seasonal variation in the occurance of conjunctivitis has been reported from several parts of the world, including

Egypt (Maxwell-Lyons and Amies, 1949) and the Southern United States (Bengston, 1933; Davis et al., 1950; Davis et al., 1952; Dow et al., 1957; Dawson 1960).

In the Middle East, conjunctivitis is more common among children during the harvest season (July and August) and the fig season (August and September), often being referred to as "harvest conjunctivitis" or "fig conjunctivitis". A somewhat similar phenomenon observed in cotton-growing areas is occasionally referred to as "cotton-pickers conjunctivitis" (Bengston, 1933).

Haddad et al.(1969) found that the highest rate of conjunctivitis was in September (38%) and October (37.5%); next in frequency of conjunctivitis was Winter (13%), then Summer (11%), and the least rate was in Spring (5%).

The Koch-Weeks bacillus was seen to have two peaks, one in Autumn (34%), and the other in Spring (24%), the former being higher; it is the commonest organism at all seasons, except in Winter when it was replaced by Beta-haemolytic streptococci (35%) and it also had a higher incidence in Winter (20%) than in Summer (16%); Nakhla, et al., (1970). Haddad et al.(1969) were in agreement, that the highest prevalence of Haemophilus was in Autumn (50%); but next to Autumn Winter (35%), then Spring (33%).

The beta haemolytic streptococcus had a higher incidence in winter (35%) than in Autumn (25%). The Summer prevalence was 9% while in spring it was 12%. Pneumococcus was cultured more in Winter (15%).While Staphylococcus aureus nearly not present in Winter, had the peak in Summer (6%). (Nakhla,et al.,1970).

Distribution of mucopurulent conjunctivitis was also age related ranging from 30% in preschool children to 12% in children of age group 5-9 years. In these two groups, the rate of Haemophilus was 58% in the first and 34% in the latter (Haddad, et al.,1969). Conjunctivitis was seen to be mainly a disease of children, with a maximum incidence at 6 years and under, and it is slightly more common in boys. Haemophilus was cultured in 16% and 34% from children of age 0-1 year and 1-6 years respectively, while haemolytic streptococcus was isolated in 19% and 32% of children of the same age group in the same respect (Nakhla, et al.,1970). This supported the results of Said et al.(1980) who recovered Haemophilus from 37% of children of age group 0-2 years and 36% of children 2-5 years old, when trachoma had its highest activity.

In neonates, Chlamydial ophthalmia is acquired during birth through an infected cervix. The disease is usually mainfest between the 4th. and 10th. day of life and may vary from a severe exudative ophthalmitis clinically indistinguishable from gonococcal ophthalmia to little more than "sticky" eye (Ridg way, 1978). Of 103 cases of ophthalmia neonatorum in San Francisco, thirty-three yielded both infectious agents (Rees, et al.,1977).

There were significant differences in the pathogenic organisms found in the eyes of girls, as compared to boys. Girls had pathogens on lids or conjunctivae in 75% of the culture, significantly higher than the 59% of boys who carried pathogens. Of the girls, 54% carried Streptococcus viridans while only 16% of the boys showed this organism. This difference is highly significant. Micrococci and Moraxella were also found more often in girl's eyes (Wood et al., 1967). In upper Egypt, the famales were more than males in their frequency of conjunctivitis, 28% of girls carried Koch-Weeks bacillus, while 25% of boys showed this organism (Nakhla et al., 1970). Rowe et al. (1979) found that most of the infants with ocular discharge had palpebral injections. In 32% of these infants Chlamydia trachomatis was recovered as the causative organism, it was also recovered from 4% of infants with a history of ocular discharge.

Marked differences may occur in the general level of severity of trachoma and in the incidence and distribution of disabling complications and sequelae, between neighboring districts. These differences were thought to depend largely on the type and distribution of associated infections of the conjunctiva (WHO, 1962).

The blinding hyperendemic trachoma is still the greatest cause of preventable blindness, with staggering prevalence in rural communities of the less fortunate developing world. WHO estimates that at present trachoma affects 500 million people and that some 2 million have been blinded by it (Tarizzo, 1973).

The condition is hyperendemic when it occurs in eyes subjected to repeated infection by both Chlamydia and bacteria, this disease complex is termed "blinding hyperendemic trachoma" of eye to eye transmission (Jones, 1975). It was clear from studies such as carried out in Gambia, that trachoma can be hyperendemic with some ensuing visual damage but without causing blindness (Sowa, Collier and Blyth, 1965). This type of trachoma generally occurs in the absence of manifest bacterial infection causing mucopurulent conjunctivitis with seasonal epidemic exacerbations. It was clear that inclusion conjunctivitis agent alone can cause hyperendemic trachoma; but it seems that bacterial infection was required in addition to give rise to blinding hyperendemic trachoma, the major public health problem that has always plagued man living in certain primitive conditions (Jones, 1975). It is thus clear that improving the conditions of life can abolish the risk of blindness from trachoma. Nevertheless, such improvements were slow, costly and difficult to effect in the poorer rural world, so we were still confronted by the gross phenomenon that trachoma and its complications remain today the most important cause of preventable blindness (WHO, 1973).

TRANSMISSION OF INFECTION WITHIN THE POOL OF OCULAR PROMISCUITY

To look more closely at the phenomenon of ocular promiscuity and its role in transmission of infection, we need to determine the reservoir of infection in the community, from which infectious organisms are shed, and to consider the ways in which organisms may be transferred to other individuals. The main reservoir lies in the affected children, especially the babies and pre-school children who have the most copious discharge (Jones, 1975), with the possibility that some affected adults mainly females, may shed sufficient agent to infect others.

The mechanisms by which infectious material may be transferred from eye-to-eye within the pool of ocular promiscuity, include:

1. Direct contact between children at play, etc.
2. Spread by fingers: in rural areas children and adults

customarily sleep on mats in a single room. Exudate from the eye contaminates the mats and provides an ideal hand-to-eye transmission route.

3. Spread on cloths used to wipe faces, Taylor and Associates (1958), in studying eye infections in the Punjab, suggested that the most important vehicle for the spread of eye infections is the village woman's shawl, a general purpose garment used for cleaning the faces of children. A similar garment is used in Arabia (Bobb, et al., 1969). Survival time of the trachoma agent on cloth is ample to permit dissemination by this route (Sowa, et al.,1965).

4. Transmission by flies, in many areas where eye-seeking flies are not prevalent. In such areas, trachoma is in general a much less important cause of blindness than in those areas of the Middle East, North Africa, and Australia where eye-seeking flies are found in great profusion, seasonal epidemic conjunctivitis is rife and blinding hyperendemic trachoma is the prime cause of blindness (Jones, 1975).

There is a great deal of circumstantial evidence implicating flies in the transmission of trachoma and bacterial conjunctivitis recently reviewed by Greenberg (1973). The case against flies was established for transmission of bacterial conjunctivitis: the peaks of prevalence of flies coincide with the times of epidemics of conjunctivitis, the organisms have been grown from flies caught in the vicinty, and control of flies produced a striking reduction in seasonal conjunctivitis, as compared with villages with no fly control (Lyons and Abdine, 1952; Ponghis, 1957).

The case against the role of flies in trachoma has not yet been generally regarded as proved. Some of the best-documented studies of trachoma have shown that it can be hyperendemic, as in Gambia, without any important factor of transmission of infection by flies (Sowa and others,1965). The ocular and nasal discharges from children with trachoma contain infective Chlamydia. In an experiment, flourescein placed in the eye of one child with trachoma, was seen to have been transmitted rapidly by flies to other nearby children, and Chlamydia had grown from flies caught near the children in this house (Darougar and Associates, 1971).

The eye-seeking flies, Musca sorbens and Musca domestica, which suck up ocular and nasal discharges and vomit them arround the eyes and noses, and into the pre-corneal tear film of nearby individuals have been shown to provide precisely the requirements for ocular promiscuity: frequent, unrestricted, and indiscriminated mixing of infective ocular discharges.

It is of interest to consider briefly the interactions of heat, dust and flies, with bacteria, viruses and chlamydia. It is a striking phenomenon, in villages with blinding hyperendemic trachoma, that flies cluster arround the eyes, nose, and mouth of any person with ocular or nasal discharge. Such discharges may well increase the fly clustering. Moist babies and small children with their almost continuous succession of upper respiratory and ocular infections, whether bacterial, viral or chlamydial are a prime target. Windborne dust especially if containing coarse particles. Commonly leads to ocular irritation and conjunctival inflammation and so to discharge that attracts flies (Jones, 1975).

CHAPTER II

AIM OF THE WORK

Aim of the work

The purpose of this work is to study the ocular pathogens involved in acute conjunctivitis in children attending the opthalmology out-patient clinics in Alexandria. This will include:

1. A search for Chlamydial inclusions in Giemsa-stained conjunctival scrapings.

2. A search for bacterial pathogens by cultures on blood agar and chocolate agar.

CHAPTER III

MATERIAL AND METHODS

Materials and Methods

Materials

This study was carried out on a series of 184 children of age group under 10 years, of both males and female sex. Each patient had a thorough clinical examination; only those diagnosed as moderate and severe conjunctivitis were selected at random for this study among the attendants of the outpatient clinic of Ophthalmology Department in Alexandria University Hospital and the out-patient clinic of the Ministry of Health Ophthalmic Hospital in Alexandria.

Methods

I. Clinical examination

The clinical severity of the mucopurulent conjunctivitis was estimated according to the following scale:

- None or minimal conjunctivitis:

  Normal conjunctiva or minimal hyperaemia and follicular reaction in lower conjunctiva, no discharge.

- Mild conjunctivitis:

  Some hyperaemia, minimal mucoid discharge or thread of purulent discharge in inferior cul-de-sac.

-Moderate conjunctivitis:

Marked hyperaemia, purulent discharge on lid and inferior cul-de-sac.

-Severe conjunctivitis:

Marked bulbar hyperaemia with swelling of lids and peri-orbital area, copious purulent discharge(Vastine,*et al.* 1974).

II. Bacteriological examination

Both lower conjunctival sacs (but not the lower lid margin) of each patient were swabbed with a single cotton-tipped applicator moistened with nutrient broth. Swabs were streaked immediately on two plates, one of human's blood agar, the second of VCN chocolate agar. One half of each blood agar plate streaked with the initial swab and a second sterile dry applicator was then passed through the initial streak and used to inoculate the remaining half. Staphylococcus epidermidis was applied on to three spots of the streak.

Each blood agar plate was incubated aerobically at

37°C. for 48 to 72 hours before being read (Vastine, et al., 1974), the VCN chocolate agar plates were incubated at 37°C. for 24-48 hours in a jar with increased carbon-dioxide tension. The latter was produced by placing a candle stub in the jar with the cultures, lighting it and closing the jar (Burrows, 1949).

The VCN chocolate agar

Consists of:

- Ordinary chocolate agar.
- To each 1000 ml. of the medium we added the following antibiotics Vancomycin 3000 micrograms, Colistin methane sulphonate 7.500 micrograms, and Nystatin 12,500 units (Cruickshank, 1975).

Granding growth on culture plates

The amount of bacterial growth on a plate was graded as follows:

No growth	None to two colonies
Light growth	Three to 30 colonies
Moderate growth	Thirty to 100 colonies
Heavy growth	More than 100 colonies to completely confluent growth.

This grading system was applied to each individual species on the plate and to overall bacterial growth(Vastine, et al. 1974).

Identification of organisms

In addition to identifying the organisms by their colonial characteristics and Gram staining we used special preparations or tests when indicated.

1. Colonial appearance

Haemophilus: Satellitism, is highly characteristic of Haemophilus. In cultures on blood agar plates, streaked with staphylococcus aureus, staph. epidermidis or staph. citreus relatively large colonies of Haemophilus, with a slightly elevated central portion are seen. These larger colonies (1 ml. or more in diameter) always developed in the immediate vicinity of a colony of staphylococcus.

2. On blood agar it forms tiny transparent, pin-point colonies, sometimes flat, and tending to become confluent, sometimes more convex and with less tendency to confluence.

Neisseria

Two different types of gonococcal colonies can be observed: Type I gives a large, irregularly round, flattened, translucent colony with an undulate edge; type II gives a smaller, round, low convex or raised, yellowish white, opaque colony, with a slightly uneven surface and an entire or faintly lobate edge. Type I colonies are usually observed when pus from acute gonorrhoea is plated out a type II colonies are usually found in old laboratory cultures, or occasionally in chronic gonorrhoeal lesions.

STREPTOCOCCI

Growth on ordinary nutrient media is generally poor; on media enriched with blood, serum, or glucose it is more rapid. Colonies on blood agar seldom exceed 1 mm in diameter after 24 hours at 37°C, and further incubation results in no increase. The changes produced in blood agar may be:

Beta-Haemolysis: The colony is surrounded by a sharply defined, clear and colourless or slightly pink zone in which red blood corpuscles cannot be seen.

Alpha-Haemolysis: A greenish discolouration of the blood, 1-3 mm in width surrounds the colony. Clumps of intact erythrocytes can be seen with it.

MORAXELLA

After 24 hours at 37°C on blood agar, the colonies are round, up to 1 mm. in diameter raised greyish, and translucent . Colonies of some strains are surrounded by a fairly wide zone of incomplete haemolysis.

2. Microscopic appearance

HAEMOPHILUS

Gram - negative rods, very small, 1-1.5 micrometer by 0.3-0.4 micrometer, with rounded, sometimes rather pointed ends. In some cultures these coccobaccillary forms are the only forms seen. Usually, among these predominating short forms are found a proportion of longer bacilli and a few long thread forms. In other cultures, the coccobacilli may be relatively scanty, or altogether absent, and longer and somewhat stouter rods may predominate. Other strains, again, may present an entirely different picture, the bacilli being thin, long, wavy or curved and sometimes lying together in tangled mass.

NEISSERIA

The members of the group are all Gram-negative cocci but they differ considerably in their morphology and arrangement. The same organism may vary considerably according to environmental conditions, thus, in the body the meningococcus and the gonococcus present an almost typical arrangement in the form of diplococci with flattened or slightly concave adjacent sides, but in culture they may appear as oval or spherical cocci without the typical diplococci arrangement. Most of the members of the group are arranged in pairs, tetrads, or small groups.

STREPTOCOCCI

Gram positive, more or less spherical in shape and are arranged in chains or pairs, the length of chain varies, it depends to some extent on the medium in which the organism grown, but some streptococci characteristically form long chains where as others are mainly diplococci.

MORAXELLA

In films, it appears as Gram negative rods, 2-3 micrometer long and 1 micrometer broad, with parallel or slightly convex side and rounded ends. The bacilli occur in pairs placed end-to-end, and sometimes in short chains. They are found free in the conjunctival secretion, or within PMNs and desquamated epithelial cells (Topley and Wilson, 1976).

3. Special preparation and tests

Optochin discs: for Pneumococcus, Coagulase test for staphylococci, Oxidase test for Neisseria (Cruickshank; 1975).

GOAGULASE TEST

Since, for practical purposes, Staph. aureus is defined as the species consisting of the coagulase-positive strains of Staphylococci, the test for coagulase production is a conclusive identifying test for the species (Gruickshank,1975).

The slide coagulase test

Was done as follows:

Place a drop of Saline (0.9% NaCl) solution or water on a clean microscopic slide, with the minimum of spreading, emulsify a small amount of solid culture, e.g. one or two colonies, in the drop of saline to form a smooth milky suspension. Dip an inoculating loop into undiluted human plasma warmed to room temperature and stir the adhering traces of plasma into the drop of bacterial suspension on the slide. Coarse clumping becoming visible to the naked eye within 5-10 seconds is a positive result (Williams and Harper, 1946).

OXIDASE TEST

This test depends on the presence in bacteria of certain oxidases that will catalyse the transport of electrons between electron donors in the bacteria and a redox dye-tetramethyl-P-phenylene-diamine. The dye is reduced to a deep purple colour.

The test is used for screening species of Neisseria, Alcaligenes, Aeromonas, Vibrio and Pseudomonas which give positive reactions. (Steel, 1961).

Plate method

Cultures are made on a suitable solid growth medium. A freshly prepared 1% solution of tetramethyl-P-phenylene-diamine dihydrochloride is poured on to the plate so as to cover the surface, and is then decanted. The colonies of oxidase positive organisms rapidly develop a purple colour within 60 seconds after that the result is negative even if the colour had appeared (Cruickshank, 1975).

Optochin sensitivity test

The discs used were ready made discs of Difco production code No. 659986.

The bacteriological findings were reported in a simple bacteriological sheet.

III. Cytology

Value of cytological smears:

- Rapid provisional diagnosis;
- Aid to selection of special media as for Moraxella.

Technique of taking scrapings from conjunctiva

Scrapings from the tarsal portion of infected conjunctiva is taken. The area preferred is near the retrotarsal conjunctiva, and about 2 mm. away from the lid margin to avoid bacterial contamination. The patient is made to look down to evert the upper lid, as the trachoma follicles have a predilection for the upper tarsal conjunctiva, particularly in the acute stages.

Once a sufficient quantity of clinical material is there at the edge of the scraper, it is transferred to a microscopic slide which is already prepared. The excess fluid adherent to the scraping is eliminated by touching the scraper at a point away from the area which is selected for the smear.

Preparation of the smears

The scraper is held at an angle with the surface of the microscopic slide as in the case of blood films and the scrapers is drawn so that a thin tissue smear of unicellular thickness is obtained. The slides were allowed to dry in air for 15 minutes to 6 hours before being fixed for 5 minutes with absolute methanol at room temperature. They were refixed in methanol before being stained with Giemsa stain (Vali, 1974).

Giemsa staining method

This method gives excellent permanent preparations if a reliable brand of stain is used. Giemsa stain is prepared by dissolving 0.5 gm of powder in 33 ml of acetone-free, absolute methanol. The solution is mixed thoroughly, allowed to sediment, and stored at room temperature for use as stock. Dilution of this stock stain are made with commercial cytologic buffers, in a ratio of 1 part of stock Giemsa solution to 40 or 50 parts of diluent. Any pH between 6.8 and 7.2 is accepted.

The smear is air dried, fixed with absolute methanol for at least 5 minutes, and again dried. It is then covered with the diluted Giemsa stain (freshly prepared the same day) for 1 hour. The slide is then rinsed rapidly in 95% ethanol to remove excess dye and to enhance differentiation; it is then dried and examined microscopically (Schacter and Dawson, 1978).

The smears from each eye were examined microscopically for at least 10 minutes. Smears with numerous inflammatory cells were usually examined for longer period of time

(30 minutes). Since the separation of the epithelial cells that show a single violet-coloured nucleus surrounded by a light blue zone cytoplasm, appeared to be an important feature, this finding was recorded if present in more than half the low-power fields examined. Among the other cellular features recorded in this study were:

a. Polymorphonuclear neutrophils (PMNs).

b. Small and medium sized lymphocytes which are with a single sharply defined, round, dense violet nucleus surrounded by a varying amount of delicate, pale blue cytoplasm when stained with Giemsa stain.

c. Plasma cells, some what larger than lymphocytes, have a characteristic eccentric nucleus with distinctive distribution of chromatin. The cytoplasm is opaque, abundant and stains blue.

d. Blastoid cells and other stem cells.

e. Leber cells, the largest cell of the leucocytic series seen in ocular scrapings. It contains a single nucleus, which stains less deeply than lymphocytes, and which may be round or irregularly oval. The zone of cytoplasm surrounding

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e. Leber cells, the largest cell of the leucocytic series seen in ocular scrapings. It contains a single nucleus, which stains less deeply than lymphocytes, and which may be round or irregularly oval. The zone of cytoplasm surrounding the nucleus is wider than that of the small lymphocyte. They are phagocytic and engulf not only bacteria but also debris.

f. Multinucleated epithelial cells where the cytoplasm contains keratin granules. Vacuoles in these cells may be few or numerous and since they do not stain, they appear as clear round spaces.

The observation also includes the presence and number of typical trachoma (Chlamydia) inclusions in epithelial cells. Inclusions appeared as discrete masses in the cytoplasm consisting of particles ranging from the small red to purple staining elementary bodies to the larger dark blue staining initial bodies. Elementary body inclusions were usually large and contained many particles. Initial body inclusions were smaller and contained fewer particles.

The results from each eye were tabulated separately: (Yoneda, et al.,1975).

A number of structures are commonly confused with chlamydial inclusion and could be a source of error in the interpretation of smears. These include:

<u>Pigmented granules</u>

Melanin granules were black or blackish-green in these Giemsa stained smears. The individual particles were irregular in size, Clumps of these granules rarely formed a close-packed cap over the nucleus of the cell closely resembling a trachoma inclusion.

<u>Nuclear extrusions</u>

Appeared in the cytoplasm as irregular masses attached to the nucleus, they resembled the nucleus in colour texture.

<u>Goblet cells</u>

Had granules which were less distinct than elementary bodies and lighter in colour.

<u>Eosinophilic granules</u>

Appeared as individual or clustered granules on the surface of epithelial cells; they could be distinguished from inclusions by their colour, and by the presence of eosinophilic polymorphonuclear leucocytes and free granules.

<u>Bacteria</u>

Smears taken during acute bacterial conjunctivitis are likely to show numerous PMNs, together with microorganisms involved. The ingestion of bacteria by PMNs. is variable (Locatcher-Khorazo and Seegal, 1972). Multiple types of bacteria were looked for. Among these bacteria were poorly stained slender rods or cocco-bacilli (Haemophilus-like), Diplobacilli (Moraxella-like), diplococci (Neisseria-like), lancet shaped diplococci(Pneumococcus-like) and other densely staining cocci, (Yoneda _et al_.,1975). Staphylococci, and Diphtheriods are present in the normal conjunctivae but usually not in sufficient numbers to be seen readily in conjunctival scraping(Locatcher-Khorazo and Seegal 1972).

Antibiotic susceptibility

Some of the strains of Haemophilus isolated were tested. The Bauer-Kirby standardized, single disc susceptibility test was used (Bauer-Kirby, 1966).

The antibiotic tested are set out in the following table:

Antibiotic or chemo-therapeutic agent	Disc Potency	Inhibition zone diameter mm		
		Resistant	Intermediate	Sensitive
Ampicillin	10 ug	20 or less	21-28	29 or more
Cephalothin	30 ug	14 or less	15-17	18 or more
Chloramphenicol	30 ug	12 "	13-17	18 "
Erythromycin	15 ug	13 "	14-17	18 "
Gantrisin (sulfisoxazole)	300 ug	12 "	13-16	17 "
Gentamycin	10 ug	- "	-	13 "
Kanamycin	30 ug	13 "	14-17	18 "
Neomycin	30 ug	12 "	13-16	17 "
Penicillin G	10 units	20 "	21-28	29 "
Polymyxin B	300 "	8 "	9-11	12 "
Streptomycin	10 ug	11 "	12-14	15 "
Tetracycline	30 ug	14 "	15-18	19 "
Troleandemycin	5 units			
Vancomycin	30 ug	9 "	10-11	12 "

Zone-size interpretative chart (Bauer-kirby, 1966).

CHAPTER IV

RESULTS

Results

This study comprised 184 cases clinically diagnosed by ophthalmological examination as severe or moderate intensity conjunctivitis. Children below 10 years of age attending the Ministry of Health Ophthalmic clinic and the out-patient clinic of Ophthalmology Department of Alexandria University were examined.

The age distribution of the cases of conjunctivitis included in the study is illutrated in table 1, where 42,9% of the cases fell in the age group below 2 years of age, 28.8% in age group 2-5 years, and 28.2% in the age group 5-10 years.

The severity of conjunctivitis in these age groups shows that an average of 45.3% of the cases were moderate intensity conjunctivitis and an average of 44.5% were severe intensity conjunctivitis. The age group below two years represented a larger number in both clinical entities.

MICROBIAL PATHOGENS RECOVERED FROM THE CONJUNCTIVA

1. Bacterial cultures

Bacteria were isolated by inoculating the conjunctival swabs directly on to blood agar and VCN chocolate agar plates. In 134 cases (72.8%) of the 184 cases ocular pathogens were isolated, in 20 cases (10.8%) only normal flora were found, and in 30 cases (16.3%) the culture yielded no growth. The different species of bacteria isolated are represented in table 2, which shows that the most frequently isolated organism was Haemophilus (59.7%), next one was Staphylococcus aureus (10.3%), then Pneumococcus was isolated from 8.1% of cases. Coliform bacilli were recovered from only one case (0.54%) of a female aged 4 years, in pure culture. Moraxella was also isolated from one case (0.54%) of a male patient aged 4 years.

Concerning the organisms considered as normal flora, those isolated were diphtheroids from (47.2%) of the 184 cases, Streptococcus viridans (18.4%) and Staphylococcus epidermidis (11.4%).

Ocular pathogens were either found as a single infective agent in pure culture or as a combined infection with more than one pathogenic organism. These findings are illustrated in table 3 which shows that, Haemophilus species were isolated in pure culture in 26 cases (14.1%), Pneumococcus also were isolated in pure culture in only one case (0.5%), and Staphylococcus aureus in 4 cases (2.1%). We found that 14 of the 184 cases (7.6%) yielded more than one ocular pathogen. Haemophilus in addition to Pneumococcus was found in (3.2%), and Haemophilus and Staphylococcus aureus were found together in 8 cases (4.3%).

2. Giemsa stained conjunctival smears

Of the 184 cases, 173 were examined both cytologically and bacteriologically, since 11 cases refused cytological examination, 77 of the 173 cases (44.5%) were of severe intensity conjunctivitis and 96 (55.4%) of moderate intensity.

Intracytoplasmic chlamydial inclusions were observed in 24 of the 173 cases examined (13.8%), 8.6% from cases of moderate intensity conjunctivitis, and 5.2% from the severe intensity cases (Table 4). Children below two years of age respresented 5.8%, those below 5 years represented 4%, and 4% fell in the age group 5 to 10 years (Table 5).

Chlamydial infection was found to be associated with affection by other ocular pathogens namely, Pneumococci in 25% of inclusion positive cases, Haemophilus in 29.1% of these cases and Moraxella in 12.5% (Table 6).

The bacteria were detected in this study both by cultures and in Giemsa stained conjunctival smears. The rates of recovery using these two methods can be illustrated in table 7. In cases of Haemophilus, it can be seen that cultivation was found to be a more sensitive method for recovery of the organism which was detected in 22.5% and 59.8% by smears and cultures respectively. Staphylococci and Alpha-haemolytic streptococci were identified by cultural methods rather than Giemsa stained smears. Moraxella however were observed in Giemsa stained conjunctival scrapings in 16.7% of conjunctivitis cases, while they were isolated by bacterial culture from only one case (0.54%). Pneumococci were observed in 32.5% of the Giemsa stained smears, and isolated from 8% of cultures. Diphtheroids showed a higher recovery rate by culture being 45% and 8.6% from smears.

Non pathogenic bacteria

The non pathogenic bacteria recovered from cases of conjuctivitis were Diphtheroids, isolated from 84 (45.6%) of the 184 cases "viridans streptococci" (optochin negative), from 34 cases (18.4%) and Staphylococcus epidermidis (coagulase negative) from 21 cases (11.3%, table 2).

Antibiotic sensitivity of Haemophilus

Sensitivity to antimicrobial agents of Haemophilus species isolated was tested for in some of the strains (10 strains) by the Bauer-Kirby method (1966). All organisms tested showed the same sensitivity pattern namely to Ampicillin, Cephalothin, Chloramphenicol, Erythromycin, Sulfisoxazole, Gentamycin, Kanamycin, Neomycin, Penicillin, Polymixin B, Streptomycin and Tetracycline. They were resistant to Troleandomycin and Vancomycin.

Table 1: Distribution of intensity of conjunctivitis by age intensity of conjunctivitis

Age group	Moderate		Severe		Total	
	No.	%	No.	%	No.	%
Below 2 years	44	55.6	35	44.3	79	42.9
2-5 years	30	56.6	23	43.3	53	28.8
5-10 years	28	53.8	24	46.1	52	28.2

Non-pathogens						No. growth	
Diphth-eroids		Staph-epider-midis		St. vinidans			
No.	%	No.	%	No.	%	No.	%
41	22.3	12	6.5	8	4.3	12	6.5
43	23.3	9	4.8	26	14.1	18	9.8
84	45.5	21	11.3	34	18.4	30	16.3

Table 3: Ocular pathogens recovered from cases of conjunctivitis in urban children below 10 years

Pathogens	Haemophilus		Staph. aureus		Pneumococcus		Moraxella		Coliform	
	No.	%	No.	%	No.	%	No.	%	No.	%
Single infection	26	14.1	4	2.1	1	0.54	1	0.54	1	0.54
Combined infection	14	7.6	7	3.8	7	3.8	-	-	-	-

Table 4: Distribution of inclusion positive cases by intensity of conjunctivitis

Intensity	No.of exam.*	inclusion positive		inclusion negative	
		No.	%	No.	%
Severe	77	9	5.2	68	39.3
Moderate	96	15	8.6	81	46.8
Total	173	24	13.8	149	86.1

*11 cases refused cytological examination

Table 5: Distribution of inclusion positive cases by age

Age group	No. Examined	Inclusion positive No.	%
Below 2 years	71	10	5.8
2-5 years	50	7	4.0
5-10 years	52	7	4.0
Total	173	24	13.8

Table 6: Inclusion positive cases* associated with other bacteria by smear and/or culture

Pneumococci		Haemophilus		Diphobacillus		No other bacteria	
No.	%	No.	%	No.	%	No.	%
6	25	7	29.1	3	12.5	9	37.5

*24 cases are inclusion positive

Table 7: Comparison of smears and cultures in detection of organisms

Intensity	No. of cases examined*	Giemsa smears			Cultures		
		Haemophilus like %	Pneumococcus like %	Diplobalillus %	Haemophilus %	Pneumococcus %	Moraxella %
Severe	77	32.4	5.2	22	67.5	9	-
Moderate	96	14.5	16.6	12.5	80	9	1.04

*11 cases refused cytological examination

CHAPTER V

DISCUSSION

Discussion

Conjunctivitis is the commonest eye disease in Egypt, where it represents a major problem of public health (Nakhla et al., 1970). It has long been known that trachoma and associated bacterial infections of the outer eye are extremely widespread in Egypt and in the pat accounted for a major part of the visual disability (Thygeson and Dawson, 1959, Hanna, et al., 1959).

Said et al.(1969a) showed that 4.7% of the rural population and 1.5% of the urban population in Northern Egypt had significant visual disability. At least half the loss in the rural area and one-third of the urban area was due to purulent ophthalmias and infectious diseases.

In this work, moderate and severe conjunctivitis were diagnosed in 184 cases, 102 cases (55.4%) were diagnosed as moderate intensity, and 82 cases (44.5%) as severe infection. Haemophilus was the most frequently encountered organism isolated from 59.7% of our series of cases, 30.4% from severe, and 29.3% from moderate cases. This is a higher percentage than that obtained by other workers as 28% in Egypt by Said et al.(1980), 40% in Tunisia by Vastine et al.(1974), 34% in Denmark by Locatcher-Khorazo and Seegal (1972), 40% in Assiout by Nakhla et al.(1970), 35.7% in Lebanon Haddad et al.(1969), 36% by Haddad et al.(1968) and 1.6% in USA by Wood et al. (1967).

Antimicrobial sensitivity pattern was tested for some of the Haemophilus strains and was found to coincide with the pattern of strains isolated from a rural community (Mohamed, 1976).

Staphylococcus aureus (coagulase positive) were found in 10.2% in our series of cases. Nakhla et al.(1970) found the same organism in 5.1%; our result is low as compared to 29.1% (Wood, et al.,1967), 72% (Haddad, et al., 1969) and 34% (Locatcher, Khorazo and Seegal, 1972).

Pneumococci were isolated from 8.1% of conjunctivitis cases. It was isolated from 7.5% by Nakhla et al.,1970), and from 2.2% by Wood, et al.(1967) and 5% by Vastive et al.(1974). It is lower than 14% obtained by Said, et al.(1980), and is low as compared to 46% recovered by Locatcher, Khorazo and Seegal (1972), it is found in healthy conjunctivae in varying proportions: 3.2% (Smith, 1954) and 10 to 12% (Lundsgaard, 1927). It is difficult to determine how far it should be regarded as a pathogen but it was found alone in 1 of 8 cases.

Moraxella was recovered in only one case (0.54%) in pure culture. This organism, which is known to produce subacute angular blepharoconjunctivitis rather than acute diffuse inflammation, was found by Smith (1954) in a small proportion of healthy conjunctivae, but has also been reported in 4.7% of cases of acute conjunctivitis (Said, et al.,1980) and 16% of the Tunisian children (Vastine et al.,1974).

Coliform bacilli were isolated from only one case (0.54%), in pure culture. It was isolated from 0.5% of cases of acute conjunctivitis by Nakhla et al.(1970), and from 1.7% by Wood et al.(1967).

The data represented in table (2) show that, in cases of acute conjunctival inflammation, the local flora changes considerably, since isolations of Diphtheroids (84%) more than doubled those of Staph. epidermidis (11.3%), and those of Strept. viridans (18.4%), it has been isolated in varying proportions: 86.4% (Wood et al.,1967), 65.3% (Nakhla et al., 1970) from cases of conjunctivitis. It is known that in cases of conjunctival inflammation the local flora changes considerably (Duke-Elder, 1965).

Streptococcus viridans was isolated from 18.4% of our cases. Although it is frequently isolated from cases of conjunctivitis, it does not appear to be a cause of purulent conjunctivitis (Vastine et al., 1974). It has been isolated from 38.9% by Wood et al.(1967), 65% by Vastine et al.(1974) and from 13% (Locatcher-Khorazo and Seegal, 1972).

Staphylococcus epidermidis was isolated from 11.3% of cases. Other workers have isolated it in varying proportions from cases of conjunctivitis: 72.5% (Nakhla et al.,1970), 99.3% (Wood et al., 1967). Other workers showed that this organism is more prevalent in the conjunctiva in hot climates 62% in Queensland, (Gibson, 1951); 95% in Egypt(Kamel,1949) and less prevalent in cold climates (e.g. Great Britain: 34% in healthy conjunctivae (Smith, 1954), 11.3% of cases of conjunctivitis (Jones *et al.*, 1957). Our relatively low results can be attributed to the change in flora during inflammation.

Active infectious trachoma is said to have practically disappeared in urban areas, since Egyptian ophthalmogists rarely see active trachoma, and "acute ophthalmia" is thought to be the major cause of infectious blindness in Egypt (Kamel, 1973). Studies in the last decade showed that the prevalence of visual disability is much higher in rural (4.7%) than in urban (1.5%) in northren Egypt. This is due for the most part to infectious diseases which eventually cause corneal scarring. In rural areas trachoma and associated infections is still the most common cause of blindness (Said *et al.*, 1969a, 1969b).

In communities where trachoma is endemic, the highest rate of infection is found among children under 10 years of age. That is why we chose this age group for our study. In one community in Tunisia with severe endemic trachoma, it was found that all children were infected by the age 2 years, the prevalence of the disease declined steadily until the age of 15 (Dawson et al.,1976).

Vastine et al.(1974) found that in one Tunisian village, all children had evidence of trachoma by age 2 years, and in another village 85% of children between 6 to 9 years were found to have active trachoma; in these populations only 17% showed inclusion bodies in Giemsa stained conjunctival scrapings, a higher rate than the 8% found in another part of Tunisia by Tarizzo et al.(1968).

In a trachoma endemic rural area outside Alexandria, most children had signs of trachom by the age of two years, and the highest rates of active disease occured between the ages of 1 to 5 years, and declined dramatically after the age of 9 years. Active trachoma was diagnosed in 62% of the children under 10 years, while inclusion positive smears were found in 14.6% of these cases (Said et al.,1980).

In our study we found that 13.8% of 173 cases of moderate and severe intensity conjunctivitis were positive for Chlamydial inclusions by cytological examination. The highest rate of inclusion positive cases was in children of age group below 5 years (9.8%), while the rate was 4% only in the age group 5-10 years.

Conjunctival smears and bacterial cultures were collected simultaneously from 173 children. Organisms presumed to be Haemophilus species were identified in 22.5% by their morphological characteristic on microscopic examination of direct Giemsa stained smears, and in 57% by culture. Vastine et al.(1974) detected Haemophilus species in 68% in Giemsa stained conjunctival smears and in 46% by culture. They were also identified in 21% in conjunctival scrapings and were isolated in culture from 28% of Egyptian children with moderate and severe intensity conjunctivitis (Said et al.,1980). Pneumococcus-like organisms were found in 32.5% of smears and 8% by culture also Diplobacilli (Moraxella) were detected in 16.7% of cytological examinations, while in culture they were isolated from only one case (0.54%).

The difference in results between smears and culture can be attributed to the difficulty in isolation of some organisms on blood agar, and the difficulty in observation of other organisms by direct smears. For example Haemophilus was detected less in smears than by culture owing to the difficulty in staining in direct smears, especially if the infection was not a heavy one. On the other hand, Moraxella were observed more in smears than by culture, this may be attributed to the fact that they require a selective medium for their isolation especially in cases of mixed infection. Mokhless (1980) found a higher rate of isolation of Moraxella on a selective medium (20%) than on blood agar (8%).

Chlamydial infection was found either as a single infection or combined with other ocular pathogens. The multifactorial nature of eye infection has been observed by other investigators (Vastine et al., 1974); Said et al.(1980).

It has been shown that bacterial infection does not enhance the growth of Chlamydia agent, and the joint effect appears to be on the disease process in the eye not in the interaction of the agents (Dawson et al.,1977).

The general concept of ophthalmologists in Egypt is that the leading cause of blindness is acute ophthalmia (Kamel, 1973). Studies in rural Egypt however have clearly documented the endemcity of trachoma, a potential cause for visually disabling lesion (Said et al.,1980). Hence it was assumed that the disease, although still present, is by and large restricted to rural communities, leaving the urban areas affected mainly by corneal ulcers due to bacteria and viruses as the main cause of blindness.

This study however shows that ocular Chlamydial infection has far from disappeared from our urban community and thus warrants preventive measures to control this potentially blinding disease by the public health authorities.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary and Conclusion

Conjunctivitis is the commonest eye disease in Egypt where it presents a major public health problem (Nakhla, et al., 1970). The leading cause of blindness in our country has been attributed to acute ophthalmias (Kamel, 1973), until Said et al. (1980) found rural communities to be endemic with trachoma. Such endemicity has been reported by other workers studying rural communities in many parts of the world. North Africa and Egypt (Tarizzo et al., 1968) Tunisia (Vastine et al.,1974 and Dawson et al.,1976) Iran (Jones, 1975) Egypt (Said et al.,1980).

The presence and extent of chlamydial ocular infection has not been studied well in our urban community, thus it was the purpose of this study to assess the pattern of microbial infection in cases of conjunctivitis in Alexandria; children below 10 years were selected since the highest rate of affection by trachoma in endemic areas was found to be in children of that age group. The patients attending clinics of the Ministry of Health Ophthalmic Hospital and Alexandria University Hospital were examined ophthalmologically and cases diagnosed as moderate or severe conjunctivitis were selected for the study. Specimens for bacterial cultures were taken by cotton swabs and conjunctival scrapings were also taken and stained by Giemsa's method for cytological examination and detection of chlamydial inclusions. Bacteriological media used were blood agar and VCN chocolate agar. Bacteria isolated were examined for colonial appearance, morphology and further identified by special tests.

By clinical examination of children below 10 years of age 184 cases diagnosed as moderate and severe conjunctivitis were selected. 102 had moderate intensity conjunctivitis and 82 had severe conjunctivitis. As regards age distribution 42.9% of these cases were below 2 years, 28.8% 2-5 years and 38.2% 5-10 years of age.

Bacteriological results showed that the most frequently encountered bacterial pathogens were Haemophilus species recovered from 59.7% of cases. This was followed in frequency by Staphylococcus aureus found in 10.3% of cases, then Pneumococci from 8.1% of cases. Moraxella and Coliform bacilli were isolated each from only 0.54% of cases.

Ocular pathogens were either found as a single infective agent in pure culture or as combined infection with more than one pathogenic organism (14%). Haemophilus species were isolated in pure culture in 14.1%, Pneumococci in 0.5%, and Staphylococcus aureus in 2.1% of cases.

As regards the normal ocular flora, Diphtheroids were isolated from 45.5% of cases, Staphylococcus epidermidis from 11.3% and Streptococcus viridans from 18.4% of cases.

Intracytoplasmic chlamydial inclusions were observed in 13.8% of cases. Chlamydial infection was found to be associated with other ocular pathogens in 67% of inclusion positive cases.

It appears from our study that the commonest bacterial pathogen encountered in conjunctivitis in children is Haemophilus, a result that is expected and clearly documented by many investigations. Chlamydial infection in this urban community, however has not been reported previously and the general concept in Egypt is that the leading cause of blindness is the "acute ophthalmia". This finding therefore emphasies the fact that further search for trachoma, the leading cause of blindness in most developing countries, should be carried out in our urban population.

CHAPTER VII

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CHAPTER VIII

ARABIC SUMMARY

بسم الله الرحمن الرحيم

الملخص العربي للرسالة

مقدمة

التهابات الملتحمة والرمد الحبيبي (التراكوما) من أمراض العيون ذات الطابع المنتشر بين الأطفال في ج.م.ع. مما يسبب مشكلة صحية عامة، والاعتقاد السائد الآن أن الرمد الحبيبي (التراكوما) مقتصر على المجتمع الريفي فقط بينما الرمد الصديدي والتهابات العين الأخرى سائدة في المجتمع الحضري.

الهدف من البحث

١ - معرفة أنواع الميكروبات المسببة في التهابات الملتحمة الحادة والمتوسطة في الأطفال حتى سن العاشرة في مدينة الاسكندرية.

٢ - معرفة مدى انتشار الرمد الحبيبي (التراكوما) بين الاطفال.

خطوات البحث

استهدفت الدراسة بحث حالة ١٨٤ طفل أقل من ١٠ سنوات مصاب بالتهاب حاد أو متوسط بالملتحمة من بين المترددين على العيادة الخارجية لمستشفى الرمد العام بالاسكندرية وأيضا المترددين على العيادة الخارجية لقسم الرمد - جامعة الاسكندرية وقد تم اجراء ٣ فحوص لكل حالة الاول اكلينيكي - وتم بمعرفة أخصائي أمراض العيون وذلك لتحديد درجة الاصابة حادة أو متوسطة.

الثاني بكتريولوجي - وذلك بأخذ مسحة من الملتحمة المبطنة للجفن السفلى للعينين اليمنى واليسرى بواسطة مرود خشبي ملفوف أحد طرفيه بقطن ويكون معقما وتوزع العينة على طبقتين بتري الاول بيئة أجار الدم والثاني بيئة الشيكولاتة في ٥٠س.ن. ٥ يزرع ميكروب المكورات العنقودية البيضاء على الطبق الاول ويحتفظ به لمدة ٤٨ ساعة في حضانة عند ٣٧ درجة مئوية. الطبق الثاني يوضع في اناء خاص به نسبة عالية (١٠٪)

من غاز ثانى أكسيد الكربون ويحتفظ به أيضا فى حضانة عند ٣٧ درجة مئوية لمدة ٤٨ ساعة .

الثالث ميكروسكوبى وذلك بأخذ عينة من الملتحمة المبطنة للجفن السفلى لكل عين وفردها على شريحة زجاجية وتثبت بكحول ميثلى مطلق لمدة لا تقل عن ٥ دقائق وتصبغ بصبغة الجيمسا .

النتائج

- تم فحص ١٠٧ طفل بنسبة ٦ر٤٢٪ أقل من سنتين .
  ٥٣ طفل بنسبة ٨ر٢٨٪ من سنتين الى ٥ سنوات .
  ٥٢ طفل بنسبة ٦ر٢٨٪ من ٥ الى ١٠ سنوات .
- عدد الحالات المصابة بالتهاب الملتحمة المتوسط ١٠٢ بنسبة ٤ر٥٥٪ .
  عدد الحالات المصابة بالتهاب الملتحمة الشديد ٨٢ بنسبة ٥ر٤٤٪ .
- الحالات التى أعطت ميكروبات ١٥٤ بنسبة ٦ر٨٣٪ و ٣٠ حالة بنسبة ٣ر١٦٪ أعطت مزرعة سلبية .
- ميكروب الهيموفيلاس هو أكثر الميكروبات التى تم عزلها حيث أنها وجدت فى ١٠٠ حالة بنسبة ٧ر٥٩٪ .
- ميكروب المكورات العنقودية تم عزله من ١٩ حالة بنسبة ٣ر١٠٪ .
- ميكروبات الالتهاب الرئوى الثنائية تم عزلها من ١٥ حالة بنسبة ١ر٨٪ .
- ميكروب العصيات الثنائية (موراكسلا) تم عزلها من حالة واحدة بنسبة ٥٤ر٪ .
ميكروب عصيات الالتهاب المعوى (كوليفورم) تم عزلها من حالة واحدة بنسبة ٥٤ر٪ .
- بالنسبة للميكروبات الغير ضارة والتى تعيش على الملتحمة وجد الأتى
  ميكروب شبه الدفتريا ٨٤ حالة بنسبة ٥ر٤٥٪ .
  ميكروب المكورات العنقودية البيضاء ٢١ حالة بنسبة ٣ر١١٪ .
  الميكروبات المكورة السبحية ٣٤ حالة بنسبة ٤ر١٨٪ .
- تم فحص ١٧٣ حالة ميكروسكوبيا وبكتريولوجيا فى نفس الوقت حيث أن ١١ حالة

رفضت الفحص الميكروسكوبي ٠

- لوحظت أجسام الكلاميديا داخل سيتوبلازم خلايا الملتحمة في ٢٤ حالة بنسبة ٨ر٢٣٪ ووجد أن أكبر معدل لها يوجد في الاطفال أقل من ٥ سنوات حيث أن تم اكتشافها في ١٧ حالة بنسبة ٨ر٦٩٪ ٠
- أثناء اجراء الفحص الميكروسكوبي لوحظ أن بعض الميكروبات موجودة داخل سيتوبلازم خلايا الملتحمة منها الهيموفيلاس موجود في ٩ر٤٦٪ ، ميكروبات الالتهاب الرئوى الثنائية ٨ر١٢٪ والمكورات الثنائية (موراكسلا) بنسبة ٥ر٤٢٪ ٠

الاستنتاج

- مما سبق نجد أن ميكروب الهيموفيلاس هو أكثر الميكروبات المتسببة في التهابات الملتحمة في الاطفال أقل من ١٠ سنوات في مدينة الاسكندرية ٠
- مرض الرمد الحبيبي مازال موجودا في المدينة وليس مقتصرا على المناطق الريفية حيث وجدنا ٨ر٢٣٪ من الحالات مصابة بهذا المرض مما يدفع الجهات الصحية المسئولة بالنظر الى هذه المشكلة ومكافحتها في المدن ٠

تم بحمد الله