Isolation and Identification of Bacteria from Patients with Eye Infection and Study of Some Inflammatory Cytokines in Patients

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Abstract

Background: The eye is one of the important sensation members in our life. Health guidelines that are given to eye soundness and neatness are important because of many factors where dust, high temperature, microorganisms and other agents can cause many diseases of the eye which may lead to blindness. This study included the investigation of the bacterial species causing inflammation of the eye which include conjunctivitis, eye lid infection and cornea at different age groups. **Method**: One hundred and forty three swabs were collected from eyes of infected people in addition to 110 blood samples of people who visited Al-Hilla teaching hospital and Alnoor hospital for Children from December 2017 to Jun 2018. Culture investigations showed 137 positive bacterial growth and 6 without growth. *Staphylococcus aureus* was the most common isolate 48(35.0%) of the total samples followed by *E.coli* 20(14.59%) followed by *S. pneumoniae* 18(13.13%) followed by *S. epidermis* 17(12.4%) followed by Enterococcus 10(7.299%) followed by *K. pneumoniae* 8(5.8 3%) followed by *H. influenzae* 7(5.1%) followed by *P. aeruginosae* 6(4.37%) followed by *N. gonorrhoeae* 2(1.45%). Also, the study transacted with determination of cytokines concentration. **Results and Conclusion**: IL-8 showed elevated concentrations particularly in age group (1-10) year as it reached 282.67±20.53pg/ml compared to controls 134±97.51pg/ml. Also, IL-1 concentration was higher in patients than in controls particularly in age group (51-60) years as it reached 352.67±46.49pg/ml compared to controls 270±9.54.

Keywords: *Eye infection, IL-1, 1L-8, conjunctivitis, bacterial infection.*

Introduction

The eye is one of the important sensation members in our life. Health guidelines that are given to eye soundness and neatness are important because of many factors where dust, high temperature, microorganisms and other agents can cause many diseases of the eye which may lead to blindness [1]. The eye is also exposed to several types of infections caused by bacteria, viruses or fungi. Bacterial infection of the eye is a major problem requiring treatment. Bacterial infection is the result of bacterial transmission of air or contact from one person to another. Eye infections include lid infection, conjunctivitis and dacryocystitis .The infection is caused by bacterial adhesion and invasion of the membranes and epithelial cells through the acquisition of factors that help adhesion and penetration of the host.

Bacteria cause eye sickness because of their effectiveness and combat host condensation of different factors such as socioeconomic level, personal cleanliness, way of life, feeding, entail, physiology and age [2].The areas in the eye that are frequently infected are conjunctiva, lid and cornea [3]. Conjunctiva is a fluffy transparent mucous membrane which lines the internal surface of the eyelids and covers the sclera (white fraction of the eye). Causes of Conjunctivitis are five types; bacterial conjunctivitis caused by *Staphylococci* and *Streptococci*. These organisms may arrive from the patient's own skin or upper respiratory tract. Conjunctivitis happens in various ages particularly in newborns which is defined as hyperemia and eye discharge in the neonates and is a prevalent contagion occurring in the neonates in the first month of life, the incidence of neonates' conjunctivitis varies in the world from 0.9-21% depending on socioeconomic conditions

[4]. Cytokines play a part in preserving safety of natural cornea as they participate in the arrangement of the inflammatory and immunological reactions of the ocular surface [5]. IL8 is one of the most particular cytokines. Chemokine particularly activates polymorph nuclear neutrophils and causes chemotaxis of T-lymphocytes. It has also been referred as monocyte- derived neutrophil chemotactic factor, neutrophil- activating peptide-1 and neutrophil-activating factor. This substance has the capacity to cause chemotaxis of neutrophils, neutrophil form change, exbcytosis of neutrophilic granules, surface expression of adherence molecules, respiratory blast and a high in cytosolic Ca2+ concentrations in the neutrophil [6]. On the other hand, IL-8 causes an inflammation distinguished by the flow of neutrophils into different anterior ocular compartments of the injected eye. Peripheral blood monocytes, alveolar macrophages, endothelial cells, fibroblasts, epithelial cells, hepatoma cells and retinal pigment epithelial cells all are capable of elaborating IL-8. Elevated concentrations of IL-8 have been considered as a cause of disease for human and empirical inflammatory sickness and inflammation of the eye [7]. IL-1, the pro-inflammatory cytokine, is a substantial middleman for inflammation and immunity. IL-1 has been implicated in the pathogenesis of human inflammatory diseases such as septic shock, rheumatoid arthritis, periodontal disease, corneal diseases, pink eye surface diseases, pharyngeal keratopathy, conical cornea and sterile corneal ulceration. IL-1 is a potent promoter of other inflammatory cytokines such as IL-6, IL-8, TNF-α and GMCSF stimulating factor, and stimulates the production of MMP enzymes by epithelial and inflammatory cells [8,9]. IL-1 is closely involved in the contribution to inflammation of the surface of the eye. It promotes the activation and migration of leukocytes, the expansion of pathogenic T cell, and the manufacture of other pathogenic cytokines that act as an intermediary sickness [10]. This study was aimed to investigate bacterial species causing inflammation of the eye which include inflammation of Conjunctivitis, eye lid infection and cornea at different age groups

Sample collection

The present study included the collection of (143) clinical samples from patients at Hilla Teaching Hospital and AL-Noor Hospital for Children during the period from December 2017 to June 2018. Eye swabs were collected from patients by sterile swabs with transport media for bacteriological study. Microbiological analysis was done and the organisms were identified by direct Gram staining, culture methods on Nutrient Agar medium, Blood Agar medium, MaCConkey agar medium, Chocolate Agar Medium, Simmon Citrate Medium and Eosin Methylene Blue (EMB) Agar at 37°C for 42h**.** in addition, different biochemical tests like catalase test, oxidase, indole, Simmon Citrate, coagulase, voges-proskauer (VP), methyl red (MR),capsule test, motility test, Bacitracin sensitivity test and urease test were performed for the identification of various bacterial pathogens after their isolation. Also, 5ml of blood were collected by disposable syringe; blood was put in tube in the absence of anticoagulant. The serum was separated by centrifugation at 3000rpm for 5min. Serum levels of IL8 and IL-1were measured by enzyme linked immunosorbent assay (ELISA) applies a technique called a quantitative sandwich immunoassay using Peprotech (USA) kit antibiotics susceptibility.

Results

Bacteriological identification

One hindered and forty three (143) patients with different types of eye infection were studied including conjunctivitis 66(46.15%), eye lid infection 33(23.07%), cornea 44(30.76%). Bacterial causes of eye infection included *Staphylococcus aureus* was the most common isolate 48(35.0 36%) of the total samples followed *E. coli* 20(14.59%) followed by *S. pneumoniae* 18(13.13%) followed by *S.epidermis*17(12.4%) followed by Enterococcus 10(7.299%) followed by *K. pneumoniae* 8(5.83%) followed by *H. influenzae* 7(5.1%) followed by *P. aeruginosae* 6(4.37%) followed by *N. gonorrhoeae* 2(1.45%) and no growth 6(4.19%). In conjunctivitis patients *Staphylococcus aureus* 20(38.46%) and *Streptococcus pneumoniae* was 6(12.5%), *Staphylococcus epidermis* and *Escherichia coli* were 5(10.4 1%), *Klebsiella pneumoniae* was 4(8.33 %), *Pseudomonas aeruginosae* 3(6.25%), Entrococcus and *Hemophilus influenza* were 2(4.16%), *Neisseria gonorrhea* 1(2.08%), while in eye lid infection patients most common bacteria isolated were *Staphylococcus aureus* 18(32.72%), *Staphylococcus epidermis* 12(21.81%), *Escherichia coli* 10(18.18%), Enterococcus 6(10.90%), *Streptococcus pneumoniae* 4(7.27%), *Klebsiella pneumoniae* 3(5.45%), *Pseudomonas aeruginosae* and *Neisseria gonorrhea 1*(1.81%). In cornea infection *Staphylococcus aureus* formed the predominant bacteria with a rate of

10(29.41%), *Streptococcus pneumoniae* 8(23.52%), *Hemophillus influenza* 6(17.64%), *Escherichia coli* 5(14.70%), Enterococcus and *Pseudomonas aeruginosae* 2(5.88%), *Klesiella pneumoniae* 1(2.94%) as shown in Table (1).

Table (1) Allocation and percentage of bacteria isolates from patients according to site of eye infection

Type of bacteria	Lid No.(%)	Conjunctiva No.(%)	Cornea No.(%)	Total No.(%)
S. aurus	18(32.72)	20(41.66)	10(29.41)	48(35.03)
S. Epidermis	12(21.81)	5(10.41)	0	17(12.4)
S. pnuemoniae	4(7.27)	6(12.5)	8(23.52)	18(13.13)
H. influenzae		2(4.16)	6(17. 64)	7(5.1)
Enterococcus	6(10.90)	2(4.16)	2(5.88)	10(7.29)
P. aeruginosae	1(1.81)	3(6.25)	2(5.88)	6(4.37)
E. coli	10(18.18)	5(10.41)	5(14.70)	20(14.59)
K. pneumoniae	3(5.45)	4(8.33)	1(2.94)	8(5.83)
N. gonorrhoeae	1(1. 81)	1(2.08)	0	2(1.45)
Total number	55	48	34	137

IL8

In ELISA assay using for detection of IL-8 concentration in eye-infected patients, the results showed that IL-8 had increased in patients compared to controls especially in age group (1-10) years as it reached 282.67±20.53pg/ml compared to controls 134±97.51and less in age group (61-70) years as it reached 7.50±5.16pg/ml (Table (2).

Table (2) IL-8 concentration in eye-infected patients and their healthy controls

Age group/yr	IL-8 (pg/ml)	
	Patient (M±SD)	Control (M±SD)
1-10	282.67±20.53	134±97.51
11-20	125.67±23.63	70.67±13.65
21-30	193±11.27	130.67±9.02
31-40	161.33±7.09	120.67±10.07
41-50	85±13.75	41±8.54
51-60	51.33±8.08	22.00±7.00
61-70	7.5000±5.15655	0.68±0.18

LSD=6.3.

Interleukin-1 (IL-1)

Using ELISA assay for detection of IL-1 concentration in eye-infected patient, the results showed that IL-1 concentration was higher in eye-infected patients compared to controls especially age group (51-60) years as it reached 352.67±46.50pg/ml compared to controls 270±9.54 and less in age group (1-10) years as it reached 0.39±0.06pg/ml (Table 3).

Table 3 IL- 1 values in eye-infected patients and their healthy controls

Age group/yr	IL-1 (pg/ml)	
	Patient (Mean±SD)	**Control (Mean±SD)**
1-10	0.9333±0.5508	0.6367±0.7767
11-20	0.386667±0.055076	0.2533±0.3512
21-30	38±4.58258	20.0000±5.000
31-40	80.6667±4.04145	61.333±7.09460
41-50	239.6667±7.02377	210.6667±10.06645
51-60	352.6667±46.49014	270±9.53939
61-70	127.000±4.35890	108.333±7.63763

LSD=9.55.

Discussion

Bacterial causes of eye infection include *Staphylococcus aureus* which was the most common isolate 48(35.03%) of the total sample. This was due to the fact that about 20% of healthy people are carrying them continuously through the nasal stream and the presence of bacteria in conjunctiva by 3.8-6.3 in healthy adults [11]. Staphylococci also have the ability to survive in harmful environments for very long periods. Some strains can withstand 60°C temperatures for 30 minutes. They can also remain in dust and dirt for weeks and they have high resistance to antimicrobial agents [12]. This study agreed with [13,14] who found that the percentage of bacteria in eye 37% and 32%, respectively. *Staphylococcus aureus* bacteria are mainly concentrated in conjunctivitis as they reach 20(41.66%). The first cause of conjunctivitis is part of the natural flora of the upper respiratory tract, especially nasal area as bacteria travel from the nose to the eye through the tear ducts, inflammation of the oocytes, the eyelid region and conjunctivitis [15]. Although there are many studies that are consistent with the current study rates of infection, there are other studies of different proportions [16]. They found that the isolation rate of staphylococcus within the conjunctiva only was 10%. The study showed that *Escherichia coli* were the second cause and percentage isolated 20(14.59%). The percentage of infection was differentiated by area of infection as the highest percentage was in eyelid as it reached 10(20.83%). This high percentage may be related to the ability of bacteria to colonize and cause infection, and their prevalence in children is higher due to lack of health care which facilitates transmission of bacteria to the eye from polluted sources [17]. This finding agreed with [18] who found 20% isolation rate. Bacteria are part of the natural flora of the intestinal canal for humans and animals they are responsible for the visual damage such as cornea, conjunctiva, eyelids and inflammation of the ocular tissue. Bacteria are abundant during the spring because they are present in the soil and are transmitted by fertilization with organic compounds of soil and plants [17]. The study found that *S. pneumoniae* isolates were 18(13.13%) the highest percentage of insulation was recorded in cornea was 8(23.52%). Many virulence factors participate in the risk of pneumococcal infection including pneumolysin, pneumococcal surface protein A, neuraminidase (Nan) and capsule. The capsule allows the bacterium to evade the host immune system by inhibiting interaction of complement components with their receptors on phagocytes, thus preventing its killing inside the macrophage which plays a major role in inflammation and infection [19]. This finding agreed with [20] who found that the percentage isolates of *S.pneumonia* in cornea was (8.69%). The study revealed that *S. epidermis* was isolated by 17(12.4%).The highest percentage of isolation in the eyelid area was 12(21.81%) these bacteria are part of the natural flora of the skin, especially the eyelids as they move from hands to eyelashes. This result agreed with [21] who

found the percentages of isolates was 19.3%. This study showed that Enterococcus was 10(7.99%) the highest percentages in eyelid. This study agreed with [13]. Also, the study showed that *K. pneumonia* was 8(5.8 3%). The highest percentage of isolation in the conjunctiva was 4(7.69%).

Interleukin-8 (IL-8)

The results of this study showed the role of interleukin in eye infections as it recorded a rise for most age groups and recorded the highest rise in the age group (1-10) years as it reached 352.6±46.50pg/ml compared to controls 134±97.51pg/ml. This was related to the effectiveness and activity of Th2 cells, which activate certain immune components when certain infections and diseases occur in children. The use of drugs, especially allergy drugs, increases the level of these interleukins, especially if we know that this group of children is more susceptible to allergies [21]. High concentration of IL8 has been detected in closed eye tears and in the tears of patients during contact lens wear, in allergic conjunctivitis and in sojourn syndrome keratoconjunctivitis. IL-8 has strong attractive chemical properties on T cells and neutrophil as observed by high concentrations in tears of dry eye patients compared with healthy subjects. Expansion of IL-8 by occupant tissue is a crucial mechanism for directing leukocytes to migrate, particularly through non-vascular tissues like cornea [22].

Interleukin-1 (IL-1)

The results of this study showed the role of IL-1 in eye infections as it recorded a rise for most age groups and recorded the highest rise in the age group (41-50) years as it reached 352.67±46.50pg/ml compared to controls 270±9.54pg/ml. The results also showed significant differences in the level of IL-1 between different age groups. High tear concentrations of IL-1 have been detected in rosacea and conjunctivochalasis. IL-1is the product of two polypeptide genes; IL-1α and IL-1β. IL1-β has been regarded as a pro-inflammatory cytokine capable of initiating the inflammatory cascade. IL-1induces the centripetal movement of the Langerhans cells and mediates keratocytes apoptosis. This cytokine is important mediator for corneal sore, iris and corneal transplant repudiation [24].

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of Interest: The authors declare that they have no conflict of interest.

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