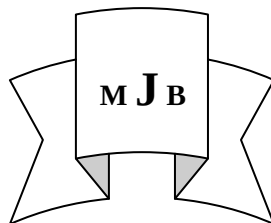


Etiology of Bacterial Eye Infections and Determination of Immune Response of Infected Patient

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Abstract

Aim of the study: The current study was conducted to investigate the causes of bacterial eye infections which included inflammation of conjunctivitis, eye lid infection (Blepharitis) and dacryocystitis and detection of *Chlamydia Trachomatis* at different age groups.

Method: study was conducted in laboratory of Microbiology-College of science for women, Babylon University, involve taken sample from Al Hilla teaching hospital, Al Noor child hospital and Babylon maternity and paediatric hospital from November 2011 to May 2012.

Results: Culture investigation showed 176 positive bacterial growth of 191, 119 isolate represent Gram positive and 57 isolate Gram negative, in Gram positive bacteria *Staphylococcus aureus* showed high percentage (38.06%), while in Gram negative *Escherichia Coli* showed high percentage (22.7%), were infection for *Chlamydia* only one sample positive for *Chlamydia Trachomatis* ELISA IgG and IgM.

Conclusion: The current study concluded there single case infection with *Chlamydia* and showed high level of IL-12 in infected with eye infection.

الخلاصة

الهدف: أجريت الدراسة الحالية للتحري عن مسببات البكتيرية لالتهاب العين والمتمثلة بالتهاب ملتحمة العين Conjunctivitis والتهاب الجفن (Blepharitis) والتهاب كيس الدمع Dacryocystitis وعند فئات عمرية مختلفة.

الطرق: أجريت الدراسة في مختبر الاحياء المجهرية-قسم علوم الحياة-كلية العلوم للبنات-جامعة بابل (تشرين الثاني 2011-أيار 2012) وتم جمع العينات من مستشفى الحلة التعليمي ومستشفى النور للأطفال ومستشفى بابل للولادة والاطفال، من المرضى ممن راجعوا المستشفيات وكانوا يعانون من التهاب في العين.

النتائج: تم جمع 191 مسحة من المصابين من موقع الإصابة من العين اضافة الى 191 عينة دم وكانت من اصل 191 فقط 176 موجبة للزرع وتوزعت 119 تمثل البكتريا الموجبة والتي اعطت نسبة اكبر فيها المكورات العنقودية (38.06%)، اما البكتريا السالبة فقد اعطيت نسبة 57 وكانت بكتريا الاشريشيا القولونية اعطت اكبر نسبة (22.7%).

الاستنتاج: استنتجت الدراسة الحالية وجود حالة إصابة واحدة بالكلاميديا، وارتفاع مستوى IL-12 في المصابين بالتهاب العيون.

Introduction

Infection of eye lead to Conjunctivitis, Keratitis, Blepharitis and other infection Which are responsible for increased incidence of Morbidity and blindness Worldwide [3,7]. Pathogenic Microorganisms cause diseases to the eyes due to their virulence and hosts reduced resistance from any factor such as personal hygiene, living, condition, nutrition, genetics, physiology, fever and age [10]. The

areas in the eye that are frequently infected are the Conjunctiva, lid and cornea [1]. Conjunctivitis is a thin, transparent mucous membrane which lines the inner surface of the eyelids and cover the sclera. (White part of the eye), the Conjunctiva contain glands which produce secretions that help to keep the eyes infection [2]. Conjunctivitis presents with an acute onset of redness, grittiness, burning and discharge on waking in the morning, the eyelids are frequently

stuck together and difficult to open as a result of the accumulation of exudates during the night both eyes are usually involved although one may become affected before the other [8]. Causes of Conjunctivitis. There are five types, Bacterial Conjunctivitis caused by Staphylococci and Streptococci. These organisms may come from the patient's own skin or upper respiratory tract. Viral Conjunctivitis is often associated with Common cold. This may be caused by a Virus called (Adenovirus). Chlamydia

Conjunctivitis type of Conjunctivitis caused by an organism called Chlamydia Trachomatis. Allergic Conjunctivitis is Common in people who have other signs of allergic disease, such as High fever, asthma. Chemical Conjunctivitis some people are susceptible to chemical in Swimming pools or to smog or fumes and this can cause an irritation of Conjunctivitis with discomfort, redness and watering [13]. Bacterial conjunctivitis divided to Acute Conjunctivitis and Chronic Conjunctivitis, acute due to bacterial differs from infection due to viruses, it is more likely to affect only one eye, and amount of this discharge and lid swelling is usually greater, the patient complains of irritation, foreign body sensation. Chronic bacterial Conjunctivitis, bacterial infection of the eyelid margins can lead to chronic Conjunctivitis, the patient complains of sore eyelids and sore eyes with little discharge [2]. Conjunctivitis occur in different ages especially in newborn Which defined as hyperemia and eye discharge in the neonates and is a common infection 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 the socioeconomic status.[18], Conjunctivitis caused by *Neisseria gonorrhoeae* referred to as ophthalmia neonatorum is primarily a

disease of neonates, caused by exposure of the neonatal conjunctivae to the cervicovaginal exudate of infected women during delivery[20]. Diagnosis The traditional criteria differentiating bacterial from other types of conjunctivitis have been: a yellow-white mucopurulent discharge; a papillary reaction (small bumps with fibrovascular cores on the palpebral conjunctiva, appearing grossly as a fine velvety surface); and bilateral infection. One systematic review was unable to find any quality research basis for these criteria [11]. Infection with *Chlamydia Trachomatis* in the world's most frequent and used different method to diagnostics of this disease include IgG, IgM test are quantitative and qualitative immunoassays for detection of human antibodies in serum[21], IgM; Occurrence of IgM antibodies without the presence of IgA/IgG antibodies is the evidence of primary infection, IgM are generally not produced during re-infections., IgG; Isolates occurrence of IgG antibodies without clinical manifestation of this disease is characteristic for the post-infectious stadium[22]. The ocular surface is constantly exposed to wide array of microorganisms [23]. The ability of outer ocular system to recognize pathogens as foreign, combination of mechanical, Immunological defense evolved to protect the outer eye [23] These host defense are classified as Innate immunity is the first line of defense against corneal infection [24]. Elements of innate immunity are present at birth, these elements include; tears, corneal nerves, cytokine [24]. While other host defense Acquired Immunity when innate immunity fails and the invading microorganisms, cells mediated immunity can help bring microbial replication under control [25]. IL-12 is interleukin that is naturally produced by dendritic cells, macrophage and human B-

lymphoblastoid cells in response to antigenic stimulation.[26], functions of IL-12 as immunoregulatory cytokine that bridges innate resistance and adaptive immunity and to be instrumental in resistance to many infection [27].

Material and Methods

The study was conducted in the Department of Microbiology, College of science for women Babylon University, involve samples taken from different hospitals, Hilla Teaching Hospital , AlNoor Child Hospital, Babylon maternity and paediatric hospital. A total number of 191 cases of eye infections were studied and 191 Translate swabs for all patients were taken for bacteriological study. 5 ml of blood were collected from all patients (level age 1 hour-70 years) and divided into two parts, One part for hematological study and other for serological investigation. All swabs were cultured in 7 different culture media ,Nutrient Agar, Blood Agar, MacConkey, Chocolate, Mannitol salt Agar, Simmon citrate, and Eosine methylene blue, These were chosen as a selective media for differential of bacteria isolate. Bacteria were identified by using routine Morphological and biochemical test [9], In addition to culture media. White blood cell count and differential count measured by Handley Method [6,4]. Detection of *Chlamydia Trachomatis* infection in infants and newborn were carried out using specific Kit (Viracell) *Chlamydia Trachoma* ELISA IgG and IgM. Immunological investigations for eye infected patients were done using ELISA assay for detection of IL-12 Concentration in patient and control group according to (eBioscience), Static analysis used for data were carried out a single completely randomized design.

Results

One hundred ninety one patients with different types of eye infection were studied including Conjunctivitis 60(34.09%), eye lid infection (Blepharitis) 58 (32.95%), Dacryocystitis 58 (32.95%). Bacterial causes of eye infection include gram positive and gram negative, Gram positive include *Staphylococcus aureus* 67 (38.06%), *Staphylococcus epidermidis* 18(22.7%), *Streptococcus pneumoniae* 34(19.31%), while gram negative *Escherichia Coli* 40 (22.7%), *Neisseria gonorrhoeae* 5 (2.84%), *Klebsiella pneumoniae* 9 (5.12%), *Pseudomonas aeruginosa* 3(1.70%). Table (1). number of cases varies according to type and site of infection, percentage of bacteria isolate from Conjunctivitis patient, In Conjunctivitis patients *Staphylococcus aureus* 28(46.6%) & *Staphylococcus epidermidis* 6(10%) and *Klebsiella pneumoniae* 6(10%), *E. Coli* 8(13.3%), *Pseudomonas aeruginosa* 2(3.33%), *Streptococcus pneumoniae* 8 (13.3%), *Neisseria gonorrhoeae* 2(3.33%). Table (1). Percentage of bacteria isolate from eye lid infection, While in eye lid infection patients most common bacteria isolate were, *Staphylococcus aureus* 27(46.5%), *Streptococcus pneumoniae* 3 (5.17%), *Staphylococcus epidermidis* 12 (20.6%), *E. Coli* 10 (17.24%), *Klebsiella pneumoniae* 3(5.17%), *Pseudomonas aeruginosa* 1(1.72%) and *Neisseria gonorrhoea* 2(3.44%). Table (1). Percentage of bacteria isolate from Dacryocystitis, In Dacryocystitis infection *Streptococcus pneumoniae* for the predominant bacteria with a rate of 23(39.6%), *Staphylococcus aureus* 12 (20.6%), *E. Coli* 22(37.93%) and *Neisseria gonorrhoea* 1(1.72%). Table(1). Prevalence of bacteria that causes eye infection distributed as following; *Staphylococcus aureus* 67(38.06%) was the most commonly isolate

Streptococcus pneumoniae 34(19.31%), *Staphylococcus epidermidis* 18 (10.22%), *Pseudomonas aeruginosa* 3(1.70%), *E.coli* 40 (22.7%), *Klebsiella pneumoniae* 9 (5.12%), *Neisseria gonorrhoea* 5(2.84%). Table (2). Detection of *Chlamydia Trachomatis* was carried on eye infected patient using Chlamydia Trachoma ELISA IgG and IgM, one sample(33.33%) only show positive result, for this investigation. This positive result for infants aged (1 years), Table (3). ELISA assay using for detection of IL-12 concentration in eye infected patient, the result show enhancement in IL-12 increased in all patient compared with control group Table(4). White blood cell count and differential count show noticeable variation with age group (31-40) show high value of White blood cell count and D.C, While age group (21-30) have not a noticeable changes in all age period, Table(8).

Conclusion

The current study concluded there single case infection with *Chlamydia* and showed high level of IL-12 in infected with eye infection. Also showed most frequent causes to eye infection with bacterial include positive and negative it refers it eyes exposed to wide range of microorganism, must make to keep eyes from microbes, also showed high level of W.B.C. in infected patient it refers to infectious especially W.B.C increased in bacterial infection.

Discussion

This study shows that both Gram positive and Gram negative share in Eye infection. The most percentage Gram positive bacteria *S.aureus* 67(38.6%) and also Gram negative *E.coli* most percentage cause Eye infection. This agrees with [17], *S.aureus* 80(23.70%), *S.pneumoniae* 29(8.6%), *K.pneumoniae* 18(6.20%),

also agree with study in Finland(12) Show the *S.aureus* most pathogen. But disagrees with (14). it find, *S. aureus* (19.13%), less than in this study, *E.coli* less than in my study (1.10%), *S.epidermidis* (19.13%) agree, also agree with *P.aeruginosa* (4.92%), *K.pneumoniae* (2.74%). In this study most of infections especially in children come from mother through birth canal and when mothers have inflammation without treatment, it depended on history of patient, Nosocomial infection also play role in infection. In *Chlamydia Trachomatis* Conjunctivitis only one case appear positive for anti Chlamydia IgG and IgM in our subjects with a percentage of (33.33%) rare and low infection with Chlamydia trachoma may be due to rare infection of mother. Contiguous in the World varies infection rate in Tanzania [19] recorded infection 16(8.5%) positive from 189 samples, also in Egypt [5] high prevalence it detection IgG & IgM ELISA & found from 45 only 5 positive IgM (11.1%) & 20 positive IgG (44.4%). In IL-12 show increased concentration in level age (2-10), 32.00 ± 61 infectious While in control 4.75 ± 0.14 , also show decreased concentration in level (31-40) infection 4.11 ± 0.17 while control 3.72 ± 0.2 , eye diseases represent one of a major inflammatory disease caused by multi pathogenic agents, most of these agents induce host immune response, IL-12 play a major role in mediated cytotoxic activity of NK cell that share in attack the invader agents and contributed in lysis processes [15]. W.B.C showed high level and concentration in age group (31-40) while in control. Low level concentration show in age group (21-30) [8]. Also in control low level, W.B.C essential to the body. They help to fight infections and help to produce and transport antibodies. With the occurrence of an infection, white blood cells produced by the bone

marrow will approximately double in quantity to enable the body to fight the infection. Once the infection has been controlled by the white blood cells, they will die off. However, a few will remain alive as memory cells in order to fight the same bacteria if it ever re-enters the body. When this occurs they will multiply once more and fight the infection before any harm is done to the body, this is the manner in which immune systems function.[16]

References

- (1).Alfonso,E and Miller,D.Detection of ocular infection:clinical application of the limulus amoebocyte lysate test,Boca Raton (1990).121pp.
- (2).Clave,G. and Tissa,S.primary eye care Conjunctivitis,Community eye health J.(2005) .18,53.
- (3).Chirambo,M.c.Tielsch,J.m.West,K. p and Kat,Z.y.Blindness and visual impairment in southern malawi,Bull WHO, (1986).64,567-572.
- (4).Dacie,J and Lewis,S. practical hematology.6th ed. Edinbuurgh, Churchi ll. (1984).pp.148-165.
- (5).Elham,R. A..Eman B. E. E.,Sahar m. A. and Amina m. A. Laboratory approach to Chlamydia Trachomatis Conjunctivitis.Life science,J. (2011). 8(1): 329-336
- (6). Hudson,L. Hay, F.practical immunology 3^{ed} ed,Black well scientific publication, (1989).pp287.
- (7).Juarez,V. M.A.Reyes,L. M.A. Cancino,D.M.E.Munoz,S..R.,Martinez s.Dela,S.F.J. and Hernandez,R. Isolation vancomycin resistance and biofilm production of *Staphylococcus epidermidis* from patient with Conjunctivitis.Revlatinoam microbial J.(2006).48(3-4):238-246
- (8).Kanski,J.Disorders of the Conjunctivitis, Clinical ophthalmology,3rd Edn, (1998).71-95.
- (9).MacFaddin,J .biochemical test for identification of medical bacteria.3rd ed.Lippincott Williams Wilkins, London , .(2000).pp57-88.
- (10).Prescott. L,M. Harley,J.P and Klein, D.A. Microbiology.5th Edn.Mc Graw Hill Education publisher (2002). 667-763.
- (11).Rietveld,R.p.Weert,H. and Bindels, P.J.E.Diagnostic impact of signs and symptoms in acute infectious Conjunctivitis,BMJ.(2003).(4):327:789
- (12).Sarvikivi,E. Karki,T and Lyyti.K. Repeated prevalence surveys of health care associated infection in neonatal intensive.Hosp J(2010). 76:156-160.
- (13).Sheikh,A.Hurwitz, B.and cave, JAntibiotic for acute bacterial Conjunctivitis (1999). Cochrane Library,3.
- (14).Sherwal, B.L.and Verma. A.K. Epidemiology of ocular infection due to bacteria and fungi-Aprospective study,JK science.(2008). 10(3):127-131
- (15).Temblay,J.N . Bertelli,E . Arques, J.L. Reyoli ,M and Nicoletti, C.production of IL-12 by peger patch-dendritic cells is critical for the resistance to food allergy,Allergy clinc Immunol J. (2007). 120,(3);659-665.
- (16).Terstappen,L.W.M.M,Grooth,B.G .de,Visscher,K.Vankouterik,F.A and Greve.JFour-parameter white blood cell differential counting based on light-scattering measurements, Cytometry J.(1998) .(9):39-43.
- (17).Ahanna,U.U. Common bacteial isolate from infected eyes.J NOA, (2009).15:40-47
- (18).Wadhwani,M. D.'souza,P. Jain,R. Duttar. Saili, A and Singh,A, .Conjunctivitis in newborn-comparative study. Pathol microbial J (2011). 54(2):254-257.
- (19).Wolle,MA. Munoz,BE. Mkocha, H. West,Sk.Constant ocular infection with *Chlamydia Trachomatis* predicts risk of scarring in chlidren in Tanzania.Ophthalmology J. (2009), 116: 243-247.
- (20).Woods.CR.Gonococcal infection in neonates and young children.pediatr infect Dis J.(2005).(16):258-270.

(21).Elham,R.Eman,B.Sahar,M.and Amina,M.Laboratory approach to Chlamydia *Trachomatis Conjunctivitis*. life science J. (2011).8;329-336.
 (22).Taylor,H.R.and Dax,E.M.New precision in measuring trachoma infection.Lancet J.(2003).362;198-204.
 (23).Akpek,E.K.and Gottsch,J.D.Immune defense at the ocular surface.Eye J.(2003).17;949-956.
 (24).Alberts,B.Johnson,A.Lewis,J.Raff ,M.Roberts, K.and Walter,P.The adaptive immune system in molecular

biology of the cell, 4th ed,garland science;New York(2002).
 (25).Mannis,M.J.and Smolin,G.Natural defense mechanisms of the ocular surface.ocular infection and immu J.(1996);185-190.
 (26).Kalinski,P.Hilkens,C.M.Snijders, A.Snijdewint,F.G.andKapsenberg,M.L. Immunol J.(1997).159;28-35.
 (27).Trinchieri,G.Interlukine-12 a cytokine at the interface of inflammation and immunity.Adv immunol J.(1998).70;83-243.

Table 1 Distribution and percentage of bacteria isolates from patient according to site of eye infection.

M.O	Conjunctiviti s	Blepharitis	Dacryocystiti s	Total %
	No %	No %	No %	
<i>S.auerus</i>	28(46.6)	27(46.5)	12(20.6)	67(38.06)
<i>S.epidermidi s</i>	6(10)	12(20.6)	0	18(10.22)
<i>S.pneumoniae</i>	8(13.3)	3(5.17)	23(39.6)	34(19.31)
<i>E.Coli</i>	8(13.3)	10(17.24)	22(37.93)	40(22.7)
<i>K.Pneumoniae</i>	6(10)	3(5.17)	0	9(5.12)
<i>P.aeruginosa</i>	2(3.33)	1(1.72)	0	3(1.70)
<i>N.gonorrhoeae</i>	2(3.33)	2(3.44)	1(1.72)	5(2.84)
	60	58	58	Total=176(99.95)

Table 2 Chlamydia Trachoma ELISA IgG,IgM in eye infected patients.

Age period	samples	Sex		True result	percentage
		male	female		
1hour-6month	27	19	8	-	0%
7month-1years	3	1	2	1(+)	33.33%
2years-5years	12	6	6	-	0%

Table 3 IL-12 concentration in eye infected patient.

Age period years	samples	IL-12 pg/ml M±SD
<1	Infectious Control	9.418 ±8.75 4.535 ±0.57
2-10	Infectious control	32.00 ±61 4.75±0.14
11-20	Infectious control	3.43 ±1.41 3.66 ±0.08
21-30	Infectious control	4.73 ±1.02 4.23 ±1.41
31-40	Infectious control	4.11 ±0.17 3.72 ±0.2
41-50	Infectious control	8.81±6.01 4.07 ±0.53
51-60	Infectious control	5.21 ±1.46 3.64±0
61-70	Infectious control	8.08 ±3.28 4.05±0.42

Table 4 Total numbers of W.B.C and D.C* in eye infected patient.

Age	Infectious				Controle			
	w.B.c M±SD	Neu M±SD	Eo M±SD	Lym M±SD	W.B.C M±SD	Neu M±SD	Eo M±SD	Lym M±SD
<1years	11 7.562	39.7 15.12	0.436 0.402	41.833 7.31	4.85 0.55	25.5 3	0.54 0.36	35.6 3.6
2-10	10.975 1.194	57.875 10.925	5.227 5.258	28.537 9.244	5.58 2.08	43.7 8.2	4.17 1.67	44.45 0.95
11-20	12.1 1.501	67.05 12.685	4.145 5.356	21.716 9.416	4 0.4	66.5 0.5	2.5 1.5	27 1
21-30	8.96 1.36	61.42 8.48	1.94 1.25	29.24 8.34	5.9 0.2	44.8 3.6	4.2 0.3	39.15 1.05
31-40	12.33 3.82	70.05 6.18	1.906 1.132	21.8 5.499	6.8 0.3	49.7 7.75	1 1	42.3 12.7
41-50	11.28 1.76	58.69 7.94	1.97 0.94	31.55 8.15	6.46 0.16	49.9 0.8	5.75 3.54	30.7 10.4
51-60	9.35 1.29	63.48 7.59	2.22 2.38	26.3 4.99	5.3 0.6	49.9 0.1	1.85 0.15	42.85 4.15
61-70	11.7 2.24	59.41 10.21	2.55 0.99	28.91 8.59	6 0.7	45.25 3.35	3.65 1.75	33.15 5.85

* D.C:Differential Count