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Determination concentration of interleukin-1 in patients with keratitis caused by bacteria *Pseudomonas aeruginosa* and pharmaceutical treatment in Al-Diwaniya City, Iraq

Syoof Khoman Alwan Al-Ramahy*

Biology Department, College of Sciences, University of Al- Qadisiyah, Diwaniya, Iraq

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ABSTRACT

The study included the collection of 150 samples, 110 samples of them were keratitis and 42 samples were wounds.42 isolates were obtained and diagnosed as *P. aeruginos*a 28 isolates (%*25. 45*) from keratitis and 12 isolates (%28.57) from wound swabs. Biochemical tests diagnosed all bacterial isolates. The study also included the collection of 42 serum samples from patients infected with keratitis and wound 40 samples from healthy persons as a control group) the concentration of IL-1 in the control samples was 14.534pg/ml while it increased significantly in the experimental group to reach *28.424*pg/ml.

* Corresponding Author

Name: Syoof Showman Alramahy
Email: syoof.alramahi@qu.edu.iq

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INTRODUCTION

Pseudomonas aeruginosa is the most virulent bacterial species of the eye due to the use of contaminated contact lenses or eye injury or eye surgery. Bacteria cause diseases ranging from central keratitis to hypopyon and iris inflammation, dacryocystitis, endophthalmitis, panophthalmitis and neonatal ophthalmia. The spread of bacteria in the eye occurs quickly within 24-48 hours and the breakdown of the internal parts of the eye may lead to blindness (Qarah, 2004).

It was observed that virulent strains of these bacteria could cause damage to the cornea for their ability to produce a larger amount of hemolysin measured by non-virulent strains. It has been found that the inflammation of the cornea can be caused by the enzymes of the status of proteins

(Proteases), including the enzyme Elastase, which is released during the infection of these bacteria to the cornea of the eye (Kloevekorn *et al.,* 2004).

Bacteria can produce a number of Proteases, such as Basal Protease, Elastase, Staphylolytic Protease (LasA) and protease IV, in addition to their production of the blood-thin, heat-sensitive and heat-stabile Hemolysin enzyme, Phospholipase C, and several types of toxins, including external enzymes T, S, U (Exoenzymes, and Exotoxin A) (Cowell *et al.,* 2003), all of which are important factors in inflammation of the cornea, Or damaged corneal tissue when injected into the eye alone or through the bacteria produced (Mariencheck *et al.,* 2003).

LPS contributes to the pathogenesis of bacteria, where its presence as a bacterial product leads to an increased inflammatory response of the host in the cornea (Kbatri *et al.,* 2002).

Alginate is another associated agent that protects bacteria from the body's defences such as lymphocytes and phagocytes. And the ciliary movement of the respiratory tract and the antibodies and complement (Head & Yu, 2004)

The biological membrane is a developing layer of the microorganism on smooth surfaces and adhered to by the secretion of sugars and sugary proteins (Head & Yu, 2004). Studies have shown that lactate is a bacterial method that helps in adhesion,

where it can bind to glycosphingolipids (GSL) receptors in a similar way to ligation (Lyczak *et al.,* 2000). In corneal injury (Zolfaghar *et al.,* 2003).

Hemolysin is an important factor in the virulence of these bacteria. It is composed of several types: Rhamnolipid, Phospholipase and Lecithinase, which have been observed to act as refractory lipid crackers and Lecithin. The bacteria produce Phospholipase C, which most studies indicate is an insignificant ferocity factor in causing damage to the eye. This is due to the ability of the mutant bacterium isolates (not producing this enzyme) to cause corneal injury; (Zhu *et al.,* 2004).

Other external virulence factors of P. aeruginosa are exotoxin A. Pillar Hobden (2002). Another type of exogenous toxin is Exotoxin T, which is more virulent in the inflammation and development of the cornea because it has an anti-internalization effect from which the bacteria produced in the cornea can resist the phagocytic process by immune cells (Geiser *et al.,* 2001). One study indicates that bacterial isolates isolated from keratitis have an effective efficacy of external T lymphocytes of 49% (Lomholt *et al.,* 2001). This has a major role in the development of corneal disease (Ellen *et al.,* 2003).

Exotoxin S is one of the strains of P. aeruginosa that has the efficacy of ADP-ribosylating activity of various nucleotide proteins (Geiser *et al.,* 2001). Thirty-eight percent of isolated isolates from ulcerative keratitis was found to be the result of this toxin (Winstanley *et al.,* 2005).

The high-productivity bacterial isolates of Proteases can analyze mucus, while non-producing enzymes cannot analyze mucus, which is the primary barrier of the cornea, which protects the epithelial layers beneath the bacterial attack. Both elastase And Protease IV have a higher susceptibility to mucus analysis than baseline protease (Marquart *et al.,* 2005). Several studies suggest that protease enzymes such as Elastase and basal protease have the potential to inhibit the function of many cells that play a role in immune response such as TCLs, Natural Killer (NK) and PMN (Twining *et al.,* 1993). The inflammation of the cornea caused by the bacteria *P.aeruginosa* Inflammatory inflammatory cells with rapid tissue crash and that this injury can lead to the hole of the cornea, and injuries within the eye, the crash of the iris, and the darkness of the lenses, and finally cause inflammation of the soles of the eye (Endophthalmitis) (Twining *et al.,* 1993).

The corneal infection of the cornea is rapid and often results in vision damage due to scarring in the cornea. Liquefactive necrosis is associated with severe ulceration and corneal puncture (Ayelet *et al.,* 2006).

The mucous membrane is the first barrier between the external environment and epithelial cells of the cornea under the membrane. Thus, the mucosal decomposition (Mucin), which is the main component of the lacrimal membrane mediated by bacteria, is one of the virulent factors that should be possessed by the isolates that cause the eye injury. Of the cornea is the increased adhesion of the bacteria to the surface of the cornea. Proteases are not producing proteins that cannot analyze mucus, i.e., the ability of bacteria to consume mucus coupled with the susceptibility of bacterial isolates to the production of proteases (Aristoteli & Willcox, 2003).

The last stage of bacterial pathogenesis is spread by invading the bloodstream. The bacteria that help to spread are resistant to thrombocytopenia and polysaccharide. Proteases also inhibit the compliment, Immunoglobulin G (IgG), Interferon (IFN), Tumour necrosis factor (TNF) and other cytokines (Fleiszig *et al.,* 1997).

Of the other enzymes affected by the bacterial infection Hohal Cathepsin where it was found that the enzymatic efficiency of many types of it increases when the cornea is infected with bacteria, and the peak of the increase in effectiveness appear after 6 days of injury, in the cornea sound is expressed only in Cathepsin epithelial tissue, while in the cornea Infected Cathepsin is expressed throughout the cornea, and the increased effectiveness of cathepsin in the bacterial cornea is related to the inflammatory response, and the stimulation of enzymatic activity of cathepsin suggests a mechanism for the degradation of host proteins in the extracellular matrix, Tm cornea after injury bacteria (Dong *et al.,* 2001).

Due to the increase in the percentage of eye injuries in these bacteria and the lack of local studies that address eye injury, this study was conducted, which aimed at the following:

a) Isolation of P. aeruginosa bacteria from eye injuries and wounds
b) Determination of the concentration of Interleukine-1 in the origin of patients with corneal ulcers caused by these bacteria.

Methodology

Isolation and purification of P. aeruginosa bacteria

Samples of keratitis and wound lesions were planted on the center of blood agglutination, MacConkey agar and Agaromal agar. The dishes were incubated at 37 ° C for 24 h, allowing the development of bacteria on the center of the tri-sugars agar and the development of bacteria at 42 ° C.

Following the implantation of the samples on the Pseudomonal agar medium, the developing isolates were taken to the center and replanted on the center of King A using the sterile carrier until pure isolates were obtained, incubated at 37 ° C for 24 hours.

Diagnosis of bacterial isolates

Physiological and biochemical tests were conducted based on scientific sources for the diagnosis of bacterial isolates (Cruickshank *et al.,* 1975; Holt *et al.,* 1994; Colle *et al.,* 1996).

Phenotypic and microscopic characteristics

The size, colour, surface and quilts of the developing colonies were included on the center of Pseudomonal Agar and the center of King A as well as the response of the cells to the Gramm and its shape under the microscope.

Immunological study

This study included the collection of 42 serum samples from infected patients with keratitis and 40 serum samples from healthy individuals for comparison. The IL-1 concentration was measured and the work was performed according to the instructions of the manufacturing company.

RESULTS AND DISCUSSION

Isolation and diagnosis

Forty-two bacterial isolates were obtained from *P. seudomonas*. Twenty-eight bacterial isolates were obtained from corneal ocular swabs. The percentage of isolating bacteria from keratitis patients was 25.45%, which is higher than that of Levy and Cohen (1996). The percentage of isolating bacteria from patients with keratitis is 10%. In the United Kingdom, researcher Schaefer et al. (2001) points out that the rate of isolation of bacteria from patients with keratitis is 9%. It is an approach to what researchers in Australia have pointed to a high rate of isolation of these bacteria from cases of keratitis caused by the use of contact lenses, which is 70% (Kathleen & Helen, 2005). Numerous studies of patients with bacterial keratitis suggest that bacteria are the most common and frequent cause of isolation from disease (Rudner *et al.,* 2000).

Twelve bacterial isolates were obtained from wound swabs, with an isolation rate of 28.57%, lower than in Nigeria, where bacteria were isolated from surgical wounds by 33% and were the predominant bacteria in the microscopic lesions of wounds (Oguntibeju & Nwobu, 2004). It was also isolated by 30.5% (Chang *et al.,* 1994). The bacteria were isolated by an approximation of 8.5% (Karray *et al.,* 1993).

The percentages of isolation of these bacteria differ from one researcher to another, and the causes of variation are numerous at the time of collection of samples, size of samples taken, health and economic conditions, geographical location, variation in diagnostic methods and different sources of isolation.

2-IL-1

The study included the collection of 42 serum samples from patients infected with keratitis and wound 40 samples from healthy persons as a control group) the concentration of IL-1 in the control samples was 14.534pg/ml while it increased significantly in the experimental group to reach 28.424 pg/ml.

The following steps involve the colonization of the cornea, followed by the induction of cytokines such as TNF and Interleukin-1 (IL-1) and finally the migration of polymorphonuclear leukocytes (PMNs) to the cornea to get rid of the pathogen, The flow or entry of PMNs is due to the induction of cytokines that attract these cells. In other words, the damage caused by the inflammation of the cornea by bacteria is caused by factors associated with colony colonization and also results from the response of PMNs (Pillar & Hobden, 2002). The inflammatory response of the cornea to the bacteria is the response at the beginning of entry PMNs, which cause corneal tissue crash, although they mainly analyze the injury, where PMNs migrate from the tear membrane and iris and peripheral vessels to the corneal areas of the cornea (Thakur *et al.,* 2004). The infiltration of PMNs is central to the bacterial pathology of the eye, Although these cells are necessary to dispose of living bacteria and result in the final healing of the cornea, the survival of these cells in the cornea increases the severity of corneal damage (Thakur *et al.,* 2001).

During the infection, leukocyte cells attach to the inner lining (Endothelium). This adhesion is induced by cytokines such as TNF, IL-1 (Hazlett et al. al., 2000). The inflammation caused by an eye injury to *P.aeruginosa* starts with a series of host interactions such as edema, white blood cell spasms, angiogenesis. As a result of the disintegration of the corneal ulceration and puncture, this inflammatory response is produced by the products of the bacteria separated and associated with the cell Huang *et al.,* 2005). Primary cellular infiltration during injury consists of PMNs, followed by macrophages (Hazlet, 2002). One of the studies indicates that on the sixth day of the injury, corneal Teske is

Table 1: Percentages of Pseudomonas aeruginosa isolated from keratitis and eye injuries

Total	Percentage	Number of bacterial non-infections *P. aeruginosa*	Percentage	Number of bacterial Infections *P. aeruginosa*	Insulation source
110	74.54	82	25.45	28	Keratitis
42	71.42	30	28.57	12	Wounds

Table 2: concentration of IL-1 in the group of patients with keratitis and control group

Relationship	St.error	Average	Highest value	Less value	Number	Group
*	1.632	28.424	87	10	42	Infected
	1.211	14.534	20	6	40	Control

* significant difference

Observed due to edema and inflammation of the inflammatory cells. The presence of PMNs in the cornea, accompanied by capillaries in the cornea, is observed. On the ninth day of the infection, PMNs are decreased and the phagocytes grow (Dong *et al.,* 2000).

Several studies have been conducted to compare the production of interleukin-1 (IL-1) after infection of the cornea in susceptible mice (leading to corneal puncture) and other resistant mice (corneal healing). IL-1 was observed in both groups, The IL-1 production peak was observed within one day of injury. It was observed that IL-1 production remained high in the susceptible group while its productivity was lower in the injury-resistant group. For IL- 1, which is used to treat sensitive mice, where antibodies are associated with IL-1, resulting in a reduction of injury Cornea, as the reduction of the severity of injury in the group of sensitive mice was associated with reduced number of PMNs of the cornea (Szliter *et al.,* 2006).

Drug therapy for keratitis caused by *P. aeruginosa* bacteria

Most antiseptics are ineffective in the eradication of bacteria. In general, bacteria are resistant to many commonly used antimicrobials, and resistance forms develop in these bacteria (Thakur *et al.,* 2004). The incidence of *P.aeruginosa*, which causes keratitis, is more difficult to treat than other microbiological lesions (Levey & Cohen, 1996). The bacteria can be treated with anti-life drugs Amikacin, Gentamicin, Carbenicillin, Tobramycin and Azithromycin (Thakur *et al.,* 2004). Studies indicate that most of the isolates isolated from the eye in European countries are sensitive to Ciprofloxacin, where this antibiotic is used as a single and effective treatment against the disease of keratitis and is widely used as an initial treatment in many countries, while the cases of resistance to antimicrobial isolate isolated from Corneal inflammation in India and America, as well as high cases of resistance in both Italy and Japan and the same disease, where the resistance rate reached 90% (Lomholt & Kiliam, 2003). Numerous studies are indicating that there is no point in using antipsychotics that remove *P.aeruginosa* bacteria that do not affect Proteases, which break down tissue. Antibiotics should be replaced with an inhibitory drug for the effectiveness of Proteases to avoid damage caused by injury (Nagano *et al.,* 2001).

REFERENCES

AL-NASHI, A.P.Ali Abed Raheem; AL-AOSI, Ghaidaa Raheem Lateef (2013). Isolate and diagnose the bacteria present in the hospital in the city of Diwaniyah and the statement of the mechanisms to control the use of antibiotics and antiseptics. Al-Qadisiyah Journal Of Pure Science, V. 18 (3): 11-20.

Aristoteli, L.P. and Willcox, D.P. (2003). Mucin degradation mechanisms by distinct *Pseudomonas aeruginosa* isolates in vitro. Infect. Immun.71 (10): 5565-5575.

Ayelet, P.; Aaron, G. and Irina, B.S. (2006). Severe *Pseudomonas aeruginosa* keratitis shortly after initiation of corneal refractive therapy. Eye & Cont. Lens. Sci.Clin. Prac. 32 (1): 1-2.

Chang, L.L.; Sun, H.H. and Chang, C.Y.; Kuo, C.S.; Wang, H.L. and Chang, S.F. (1994). Drug resistance and plasmid analysis in *Pseudomonas aeruginosa*. J. Pharmacol. 10 (9): 508- 517.

Cohen,E.J.; Fulton, J.C.; Hoffman, C.J.; Rapuano, C.J. and Laibson, P.R. (1996). Trendsin contact lens-associated corneal ulcers. Cornea. 15 (6): 566-570.

Collee, J.G.; Miles, R.S. and Watt, B. (1996). Test for the identification of bacteria in Practical Medical Microbiology, by Collee, G.; Fraser, A.G.;

Cowell, B.A.; Twining, S.S.; Hobden, J.A.; Kwong, M.S. and Fleiszig, M.J. (2003). Mutation of *las*A and *las*B reduces *Pseudomonas aeruginosa* invasion of epithelial cells. Microbiol. 149: 2291-2299.

Dong, Z.; Katar, M.; Linebaugh, B.E.; Sloane, B.F. and Berk, R.S. (2001). Expression of cathepsins B, D and L in mouse corneas infected with *Pseudomonas aeruginosa*. Eur. J. Biochem. 268: 6408-6416.

Ellen, J.L.; Cowell, B.A.; Evans, D.J. and Fleiszig, M.J. (2003). The contribution of *exs* A-regulated factors to corneal infection by cytotoxic and invasive *Pseudomonas aeruginosa* in a murine scarification model. Invest. Ophthalmol. Vis. Sci.44: 3892-3898.

Eqbal Dohan Chalap Al- Grawi and Ghaidaa Raheem Lateef Al-Awsi (2018). Expression of CDKN2A (p16/Ink4a) among Colorectal Cancer Patients: A cohort study. Journal of Pharmaceutical Sciences and Research. Vol. 10 (5), 2018, 1145-1147.

Fleiszig, S.M.; Kronish, J.P.; Vallas, M.V.; Mostov, K.E.; Kanada, D.; Yen, T.S. and Frank, D.W. (1997). *Pseudomonas aeruginosa* – mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exoenzymes S. Infect. Immun. 65 (2): 579-586.

Geiser, T.K.; Kazmierczak, B.I.; Ryan, L.K.; Matthay, M.A. and Engel, J.N. (2001). *Pseudomonas aeruginosa* ExoT inhibits in-vitro lung epithelial wound repair. Cell Microbiol. 3 (4): 223-236.

Hazlett, L.D.; McClellan, S.; Kwon, B. and Barrett, R. (2000). Increased severity of *Pseudomonas aeruginosa* corneal infection in strains of mice designated as Th1 versus Th2 responsive. Invest. Ophthalmol. Vis. Sci. 41 (3): 805- 810.

Hazlett, L.D. (2002). Pathogenic mechanisms of *Pseudomonas aeruginosa* keratitis: a review of the role of T cells, Langerhans cells, PMN, and cytokines. DNA Cell Biol. 21 (6): 383-390.

Head, N.E. and Yu, H. (2004). Cross-sectional analysis of clinical and environmental isolates of *Pseudomonas aeruginosa*: biofilm formation, virulence, and genome diversity. Infect. Immun. 72 (2) : 133-144.

Huang, X.; Barrett, R.P.; McClellan, S.A. and Hazlett, L.D. (2005). Silencing toll-like receptor-9 in *Pseudomonas aeruginosa* keratitis. Invest. Ophthalmol. Vis. Sci. 46: 4209 – 4216.

Karray, H.A.; Hammami, F.; Mahjoubi, M.; Bouaziz, J.& Jeddi, H.M. (1993). Etude in vitro de la sensibilite aux antibiotigues de 213 souches de *Pseudomonas aeruginosa.*Isolees service de reanimation du CHV de sfax (tunisie). Pathol. Biol. 41 (4): 307-312.

Kathleen, W. & Helen, S. (2005). Microbial keratitis in overnight orthokeratology: a review of the first 50 cases. Eye Cont. Lens. Sci. Clin. Prac.31 (5): 201-208.

Kbatri, S.; Lass, J.H.; Heinzel, F.P.; Petroll, W.M.; Gomez, J.; Diaconu, E.; Kalsow, C.M. & Pearlman, E. (2002). Regulation of endotoxin-induced keratitis by PECAM-1, MIP-2, and Toll-like receptor4. Invest. Ophthalmol. Vis. Sci. 43 (7): 2278-2284.

Kloevekorn, C.G.; Wilhelm, F.; Duncker, I.W. & Hammer, T. (2004). Nosocomial *Pseudomonas aeruginosa* – associated keratitis in soft contact lens wearer.Klin. Mona. Ange.221: 52-55.

Levey, S.B. & Cohen, E.J. (1996). Perspectives in refraction: methods of disinfecting contact lenses to avoid corneal disorders. Surv. Ophthalmol. 41 (3): 245-251.

Lomholt, J.A.; Poulsen, K. & Kilian, M. (2001). Epidemic population structure of *Pseudomonas aeruginosa*: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. Infect. Immun. 69 (10): 6284-6295.

Lomholt, J.A.; Poulsen, K. & Kilian, M. (2001). Epidemic population structure of *Pseudomonas aeruginosa*: evidence for a clone that is pathogenic to the eye and that has a distinct combination of virulence factors. Infect. Immun. 69 (10): 6284-6295.

Lyczak, J.B.; Cannon, C.L. & Pier, G.B. (2000). Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. Microbiol. Infect. 2: 1051- 1060.

Mariencheck, W.I.; Alcorn, J.F.; Palmer, S.M. & Wright, J.R. (2003). *Pseudomonas aeruginosa* elastase degrades surfactant proteins A and D. Am. J. Res. Cell. Mol. Biol. 28: 528-537.

Marmion, B.P. and Simmon, A. (eds), (14th ed). Churchill Living Stone New York. pp.131-146.

Marquart, M.E.; Caballero, A.R.; Chemnawang, M.; Thibodeaux, B.A.; Twining, S.S.& O'Callaghan, R.J. (2005). Identification of a novel secreted protease from *Pseudomonas aeruginosa* that causes corneal erosions. Invest. Ophthalmol. Vis. Sci.46: 3761-3768.

Nagano, T.; Long, H.J.; Nakamura, M.; Kumagai, N.; Abe, M.; Nakazawa, T. & Nishida, T. (2001). The stimulatory effect of pseudomonal elastase on collagen degradation by cultured keratocytes. Invest. Ophthalmol. Vis. Sci. 42: 1247-1253.

Oguntibeju, O.O. & Nwobu, R.A. (2004). The occurrence of *Pseudomonas aeruginosa* in post-operative wound infection. Pak. J. Med. Sci. 20 (3): 187-191.

Pillar, C.M. & Hobden, J.A. (2002). *Pseudomonas aeruginosa* exotoxin A and keratitis in mice. Invest. Ophthalmol. Vis. Sci. 43: 1437-1444.

Qarah, S. (2004). *Pseudomonas aeruginosa* infections Interanet explorer. eMedicin. Com.

Rudnex, X.L.; Kernacki, K.A.; Barrett, R.P. & Hazlett, L.D. (2000). Prolonged elevation of IL-1 in *Pseudomonas aeruginosa* ocular infection regulates macrophage-inflammatory protein-2 production and corneal perforation. J. Immun. 164: 6576-6582.

Schaefer, F.; Bruttin, O.; Zagrafas, L. & Crosier, Y. (2001). Bacterial keratitis: a prospective clinical and microbiological study. Br. J. Ophthalmol. 85: 842-847.

Szliter, E.A.; Ronald, B.; Manal, G.M.; Yunfan, Z. & Linda, H. (2006). *Pseudomonas aeruginosa* – induced inflammation in the rat extended- wear contact lens model. Eye Cont. Lens Sci. Clin. Prac. 32 (1): 12-18.

Thakur, A.; Kyd, J.; Xue, M.; Willcox, D.P. & Cripps, A. (2001). Effector mechanisms of protection against *Pseudomonas aeruginosa* keratitis in immunized rats. Infect. Immun. 69 (5): 3295-3304.

Twining, S.S.; Kirschner, S.E.; Mahnke, L.A. & Frank, D.W. (1993). Effect of *Pseudomonas aeruginosa* elastase, alkaline protease, and exotoxin A on corneal proteinase and proteins. Invest. Ophthalmol. Vis. Sci. 34 (9): 2699-2712.

Winstanley, C.; Kaye, B.; Neal, J.; Chilton, H.J.; Miksch, S. & Hart, A. (2005). Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis. J. Med. Microbiol. 54: 519-526.

Zhu, H.; Bandara, R.; Conibear, C.R.; Thuruthyil, S.J.; Rice, S.A.; Kjelleberg, S.; Givskov, M. & Willcox, D.P. (2004). *Pseudomonas aeruginosa* with LasI quorum- sensing deficiency during corneal infection. Invest. Ophthalmol. Vis. Sci. 45: 1897-1903.

Zolfaghar, I.; Evans, D.J. & Fleiszig, M.J. (2003). Twitching mortality contributes to the role of pili in corneal infection caused by *Pseudomonas aeruginosa*. Infect. Immun.71 (9): 5389-5393.

