Assessment of Keratitis Damage in an Age Dependent Mouse Model Using Analytical Software

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Abstract

Background: *Streptococcus pneumoniae* (pneumococcus) is a gram-positive bacterium that is responsible for diseases such as, otitis media, conjunctivitis, bacterial keratitis, pneumonia, and meningitis. Bacterial keratitis is one of the most common after-effects of trauma to the eye. Some reports have shown the *S. pneumoniae* spreads through enzymes that are produced to digest the cornea, which in turn can causes blindness. There is a need for more improved measures that can reverse the detrimental effects of the bacteria. The long-term goal of this research is to better understand the complete role of *S. pneumoniae* and its components in bacterial keratitis to develop next generation therapies to prevent blindness. The purpose of this study is to develop alternative measures to evaluate damage associated with keratitis infection by use of computer applications. **Methods:** This study analyzed images of the established Keratitis pneumococcal mouse model. The eye images of mice 7-8-week-old and 9-month-old were collected. Additional images were taken on post-infection days one, three, five, and nine, revealing the progression of the infection. **Results:** The ImageJ Application provided more in depth review to determine the detrimental effects of *S. pneumoniae*. Through the software, a "Color Threshold" was created on every image to emphasize the area of damage caused by the bacteria. A scatter plot of every image created a map of the

particles, and the diameter created a scale demonstrating the impact of keratitis. Data revealed that the most significant increase in infection occurs between Day 1 and 3 post-infection. **Conclusions:** The study has created a computer model to establish a baseline for the infection process of *S. pneumoniae* in the traditional mouse model. ImageJ has proven to be a useful tool to analyze the impact of disease on the murine model. Results from this study also provide evidence of the importance of early intervention in ocular disease.

Keywords: ImageJ, *Streptococcus pneumoniae,* keratitis, aging

Introduction

Ocular infections such as keratitis, conjunctivitis, and endophthalmitis are caused by an opportunistic pathogen known as *Streptococcus pneumoniae*. *S. pneumoniae* is considered one of the leading bacterial causes of keratitis, which is a serious ocular infectious disease.[1,2] There is a poor clinical outcome of the patients suffering from bacterial keratitis that do not seek immediate medical care.[3] The incidence of ocular morbidity and blindness worldwide is related to infections of the cornea. Traditionally, pneumococcal infections affect elderly individuals or young children due to their weakened immune systems.[4] The elderly population is more susceptible to microbial keratitis than younger patients, because they are more likely to have had previous or co-existent ocular disease or surgery.[5] Consequently, visual outcome is significantly worse in older patients.[5] Aging is also strongly associated with alterations in the structure and function of the eye and with the development of ocular diseases.[6] Various *in vivo* models of aging have been described and developed in *Pseudomonas* and *Staphylococcus aureus* keratitis models. The studies demonstrated the inability of the immune cells to fight off infection and increased pathology in the aged mice.[7,8]

Wayne Rasband of the National Institute of Mental Health developed ImageJ (NIH, Bethesda, Maryland), which is a public domain Java-based image processing and analysis program.[9,10] ImageJ has been extensively used for image processing in immunohistochemistry,[10] tissue segmentation in microscopy

images,[11] and muscle morphometric measurements.[11] Use of this sophisticated software offers a refinement in the use of animal models in respect to determining the time-points for treatment of infection. The software offers the possibility for biomedical modeling of keratitis to predict outcome that will occur in the ocular environment.

The long-term goal of this research is to better understand the complete role of *S. pneumoniae* and its components in keratitis and to develop next generation therapies to prevent blindness. The purpose of this study is to develop alternative measures to evaluate damage associated with keratitis infection by computer application compared to the established keratitis pneumococcal mouse model. The study evaluates the progression of ocular damage with analytical software, ImageJ Application, which measures the diameter of the infection, from early to late progression of disease in both young and old murine models.

Materials and Methods

Sample Population and Analysis

Male and female C57BL/6 (Harlan, Houston, TX) were used in this study represented two age groups consisting of young mice (7-8-week-old) and old mice (9-month-old). The ocular images range from 1, 3, and 5 days post-infection. During experimentation, all animals were maintained according to institutional guidelines approved by the Institutional Animal Care and Use Committee (IACUC) approved protocol 2013-04010-101 (Prairie View A&M University) and the tenets of the Association for Research in Vision and Ophthalmology (ARVO) Resolution on the Use of Animals in Ophthalmic and Vision Research. Mouse eyes were infected using a protocol utilizing the established scratch mouse model.[12] The .jpeg images of the infected eyes from the aging study conducted were utilized for the analysis. The photos of the ocular images were visualized in ImageJ by cropping the photo to view the infection site. After the appropriate size was determined, an 8-bit photo filter was utilized to make the color black and white. Following the image color change, the image was adjusted using “Auto Threshold” and

the photo option that displays the proper black and white particles was chosen. The particles were analyzed with the "Analyze Particles" option. To continue the evaluation of the ocular image, the "Scatter Plot" option was utilized, and the tool for "Outlines" generates the data plotted as numbers. The diameter of the infection site was measured using the straight-line tool to draw a line. The infection site was visualized from a majority of particles that is surrounding the cornea to make a circle and using the "Analyze" tool the diameter was measured. The data collected was transferred to excel spreadsheet for analysis (figure 1).

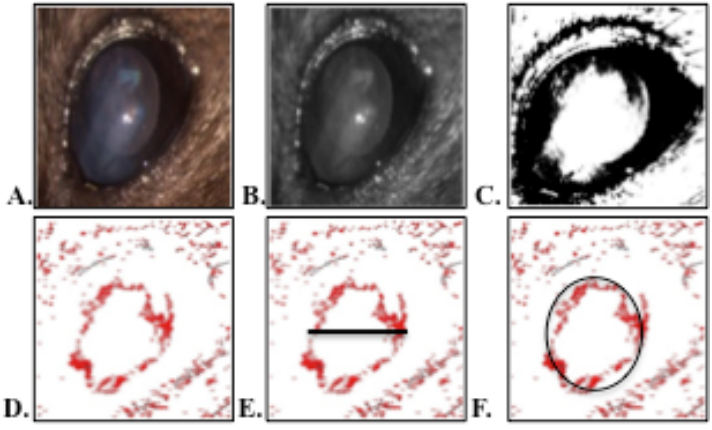

Figure 1 Sample Analysis. **A.** Selected ocular image for analysis. **B.** Image was converted to black and white using an 8-bit photo filter. **C.** Image adjusted using the "Auto Threshold" option displaying the black and white particles. **D.** Evaluation of the image using the "Scatter Plot" option. **E.** The diameter of the infection site is measured. **F.** The infection site was visualized from a majority of particles that is surrounding the cornea to make a circle and using the "Analyze" tool the diameter was measured.

Statistics

Scatterplot analysis data was analyzed using the Graph Pad InStat (GraphPad Software, San Diego, CA). Scatterplot diameters of the images were assessed using ANOVA, standard parametric analysis

of variance. Tukey's test was utilized to compare all pairs of columns. All experiments were with multiple eye samples. $P < 0.05$ was considered significant. Data were expressed as means ± the standard errors of the means (SEM).

Results and Discussion

Keratitis in mouse eyes is evident due to the opacity that is demonstrated in the cornea of the eye. The focus of the study was to determine if an assessment tool could be used to understand the spread of bacteria in a keratitis model. The study evaluates the use of ImageJ and its ability to analyze ocular images from mice infected with pneumococcal keratitis. ImageJ's image processing application was used to map the progression of keratitis infection in the aging mouse model from day 1 to day 5 post-infection. The analysis elucidated the damage associated with pneumococcal keratitis. The images in the study are from the experiments that followed the established scratch model mouse model.[12]

The ImageJ analysis was used to create a binary image, which demonstrates the area that is affected by the presence of the bacteria (opaque) versus health tissue (translucent). The outline of the keratitis infection illustrates the damage that occurs in the cornea (Figure 2). An eye trend map of infection was established with mice ocular images representing a variety of mice eyes of keratitis ranging from day 1 to day 5 post-infection (Figure 3). This tool can now be used in the future studies that want to analyze and compare infection progression in young and old mice.

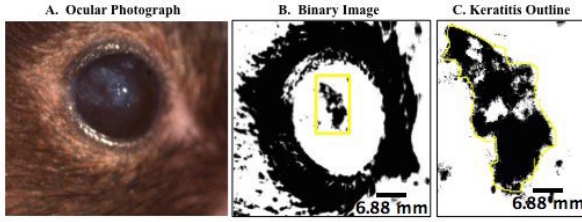

Figure 2 Analytical Exposure of Keratitis Image. **A.** Keratitis Image of infected mouse eye. **B.** Infected eye converted to binary image to reveal infection site. **C.** Enlarged view of Outline of Keratitis infection as seen in 1B.

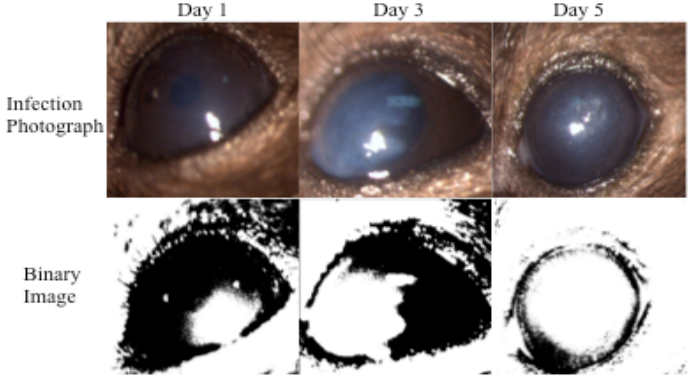

Figure 3 Binary Image analysis of 7-8 week C57BL/6 mice eyes on Days 1,3,and 5 post-infection. Binary image analysis comparison of keratitis infected mice eyes on day 1, day 3 and day 5-post-infection.

To further evaluate the impact of keratitis on the cornea of the mouse and to provide an alternate approach to studying keratitis, a scatter plot was performed (Figure 4). The scatterplot allowed for the spread of the infection to be measured. The data collected from the measurements was complied into a bar graph based on days post-infection starting at Day 1 and extending to Day 5; the results

indicate a significant increase occurs in the spread of the keratitis infection between Day 1 and Day 3 (Figure 5). Thus, the major impact of the keratitis infection occurs within the first 48 hours following infection. This data supports the need for early intervention to prevent irreversible damage.

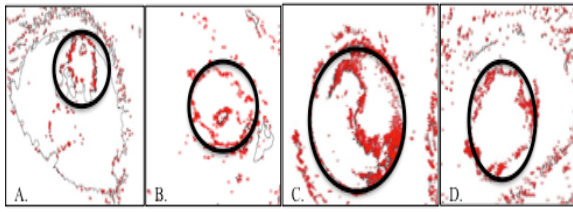

Figure 4 Scatter Plot Analysis of young and old eyes. **A.** Representative mild infection analysis for a young mouse cornea. **B.** Representative mild infection analysis of an old mouse cornea. **C.** Representative severe infection analysis of young mouse cornea. **D.** Representative severe infection analysis of old mouse cornea. The circle represents bacterial and bacterial products related damage of the cornea. The diameter of the scatterplot analysis was utilized for analysis.

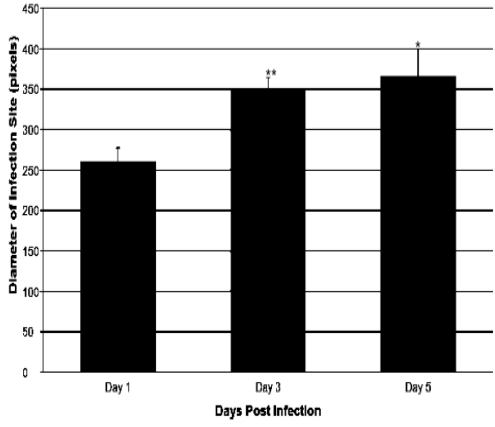

Figure 5 Diameter measurements of Scatter Plot analysis of infected corneas. The diameter scale demonstrated the spread of keratitis for day 1 vs. day 3; $p<0.05$, day 1 vs. day 5; $p<0.05$, day 3 vs. day 5; $p>0.05$.

This study has optimized and validated a standard protocol for analyzing keratitis in the murine model using the ImageJ Software. The S. pneumoniae bacteria progression towards the cornea as the days of the infection continued is now thoroughly documented and can be used as a baseline for future work. The study sets an alternative for studying the spread of infection for surface related infection models that could possibly reduce the number of animals utilized in an infection study. A disease computer model has been created to determine the results from the sequence of the events occurring post-infection. And timeline analysis has underscored the importance of early intervention in bacterial keratitis.

Acknowledgements

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