Pathological Role of Biofilm in Fungal Rhinosinusitis

Thesis

Submitted for Partial Fulfillment
of Master Degree in **Otorhinolaryngology**

By

Hesham Yehia Falah
M.B.B.CH
Faculty of Medicine Misr University for Science & Technology

Supervised By

Prof. Dr. Waleed Farag Ezzat
Professor of Otorhinolaryngology
Faculty of Medicine, Ain Shams University

Dr. Anas Mohamed Askoura
Lecturer of Otorhinolaryngology
Faculty of Medicine, Ain Shams University

Dr. Ahmed Negm El-Shazly
Lecturer of Otorhinolaryngology
Faculty of Medicine Misr University for Science & Technology

Faculty of Medicine - Ain Shams University
2018

سورة البقرة الآية: ٣٢

Acknowledgments

First and foremost, I feel always indebted to Allah *the Most Beneficent and Merciful.*

I wish to express my deepest thanks, gratitude and appreciation to Prof. Dr. Waleed Farag Ezzat, *Professor of Otorhinolaryngology, Faculty of Medicine, Ain Shams University, for his meticulous supervision, kind guidance, valuable instructions and generous help.*

Special thanks are due to Dr. Anas Mohamed Askoura, *Lecturer of Otorhinolaryngology, Faculty of Medicine, Ain Shams University, for his sincere efforts, fruitful encouragement.*

I am deeply thankful to Dr. Ahmed Negm El-Shazly, *Lecturer of Otorhinolaryngology, Faculty of Medicine, Misr University for Science & Technology, for his great help, outstanding support, active participation and guidance.*

I wish to express my supreme gratitude to Dr. Yosef Shoukry Abdel All, *Assistant Professor of Anatomy, Ain shams university, for his kind supervision, indispensable advice and great help in this work.*

I would like to express my hearty thanks to all my family for their support till this work was completed.

Hesham Yehia Falah

List of Contents

List of Tables

List of Figures

List of Figures cont...

List of Abbreviations

Abb.	Full term
AFIFS	*Acute fulminant invasive fungal sinusitis*
APRS	*Allergic fungal rhinosinusitis*
BHI	*Brain heart infusion*
BMP	*Bilateral nasal polypi*
CLSM	*Confocallaser scanning microscopy*
CIFS	*Chronic invasive fungal sinusitis*
Cl	*Confedence interval*
CRS	*Chronic rhinosinusitis*
CT scan	*Computed topography*
EM	*Electron microscope*
ENT	*Ear, nose & throat*
EPS	*Extracellular polymeric substance*
FESS	*Functional endoscopic sinus surgery*
FISH	*Fluorescence in Situ Hybridization*
G-CSF	*Granulocyte colony-stimulating factor*
GERD	*Gastro -esophageal reflux disease*
GIFS	*Granulomatous invasive fungal rhinosinusitis*
GMS	*Modified Gomori Methenamine-Silver Nitrate Stain*
HSE	*Hematoxylin and eosin*
MRI	*Magnetic resonance imaging*
OCT	*Optical coherence tomography*
OSA	*Obstructive sleep apnea*

List of Abbreviations cont...

Abb.	Full term
PAS	*Periodic acid Schiff*
PIA	*Polysaccharide intracellular adhesion*
PNS	*Paranasal sinus*
ROC	*Rhino-orbito-cerebral*
S. aureus	*Staphylococcus aureus*
SD	*Standard deviation*
SE	*Standard error*
SEM	*Scanning Electron Microscope*
SFB	*Sinus fungus ball*
TEM	*Transmission electron microscopy*

ABSTRACT

Background: The first case of fungal sinusitis was reported in 1885. This fungal disease occurred rarely until the past decade, when a worldwide increase in its incidence occurred. Till one decade back bacteria was implicated as pathogen in most form of chronic rhinosinusitis (CRS). Fungi were thought to be responsible for few specific forms, sine 1999, when ponikau and associates claimed that fungi were responsible for nearly all cases of CRS. Their study demonstrated the presence of fungi & eosinophils from nose & paranasal sinuses from ~96 % cases of CRS. **Aim of the Work:** The aim of the work was to detect the presence of fungal biofilm, in patients with fungal sinusitis trying to find its role in recurrence of fungal sinus infection, resistance to medical treatment and occurrence of intra orbital and intracranial complications. **Patients and Methods:** The study consisted of 20 different cases of fungal sinusitis controlled by 10 cases of non fungal sinusitis. Control cases were divided into 5 cases of chronic sinusitis with nasal polyposis and 5 cases of deviated septum with no evidence of sinusitis undergoing nasal surgery. **Results:** Using statistical analysis, there was evidence of pressure of fungal biofilm in different cases of fungal sinusitis whether primary or recurrent, also whether invasive or non invasive. Comparing cases with controls, There was statistically significant difference between them as regard Biofilm (p-value: < 0.001) with incidence reaching (70 %) in cases while (0%) in controls. By applying risk analysis for biofilm, we used relative risk (p value = 0.0001) which is statistically significant indicating higher risk of positive biofilm in cases of fungal sinusitis, also by Using odds ratio (p value= 0.0002) which is statistically significant indicating higher risk of positive biofilm in cases of fungal sinusitis. **Conclusion:** The study showed the presence of fungal biofilm in different cases of fungal siunsitis whether primary or recurrent, also whether invasive or non invasive.

Keywords: Pathological Role, Fungal Rhinosinusitis, chronic rhinosinusitis

INTRODUCTION

The first case of fungal sinusitis was reported in 1885. This fungal disease occurred rarely until the past decade, when a worldwide increase in its incidence occurred ***(Lund et al., 2000).*** Till one decade back bacteria was implicated as pathogen in most form of chronic rhinosinusitis (CRS). Fungi were thought to be responsible for few specific forms. Since 1999, when ponikau and associates claimed that fungi were responsible for nearly all cases of CRS. Their study demonstrated the presence of fungi & eosinophils from nose & paranasal sinuses from ~96 % cases of CRS ***(Ponikau et al., 1999).***

The biofilm confers considerable protection for the organisms including resistance to host defenses and antifungal treatments. It acts as a physical barrier between the embedded fungal cells and clinically useful antifungal agents, thus leading to ongoing colonization of fungi in the sinuses despite maximal treatment. Biofilm may allow for chronic persistence of fungi in the nose and sinuses and make treatments more difficult and make the efficacy of antifungal treatments questionable ***(Ramage et al., 2012).***

Biofilm is a three-dimensionally structured specialized community of adherent microorganisms surrounded by an extracellular polymeric substance (EPS). Biofilm communities in most environments including human disease, tend to be

polymicrobial. By including multiple bacterial and/or fungal species in a single community; biofilms obtain numerous advantages, such a passive resistance, metabolic cooperation, by-product influence, quorum sensing systems, an enlarged gene pool with efficient DNA sharing, and many other synergies, the more robust the biofilm is in terms of its survivability ***(Wolcott et al., 2013).***

Even though only a few reports are available about the role of biofilms in chronic rhinosinusitis, the evidence seems to be convincing that such a role exists. Why do some patients form biofilms, what triggers biofilm formation and how can we prevent them from forming are questions that need to be answered ***(Ramadan, 2006).***

There is a growing body of evidence suggesting that fungal biofilm colonization of host surfaces may be an important factor in chronic disease in the absence of foreign bodies and fungal biofilms have been discovered on the sinonasal mucosa of CRS patients ***(Healy et al., 2008).***

Foreman and coworkers presented conclusive evidence that biofilms are present on the mucosa of chronic rhinosinusitis (CRS) patients. Less is known about the species constituting these biofilms. Staph.aureus was the most common biofilm-forming organism. Eleven out of 50 CRS patients had characteristic fungal biofilms present ***(Foreman et al., 2009).***

Fungi are increasingly recognized by their ability to adopt a biofilm phenotype both on live and abiotic surfaces. Much of the work in the fungal biofilm research has focused on Candida species involved in indwelling medical device infection ***(Ramage et al., 2006).*** Although vast ranges of fungal species are isolated from CRS patients, Candida species are rarely seen ***(Collins et al., 2003).***

However, Aspergillus fumigatus, is a frequent sinonasal pathogen and is known to form biofilms on bronchial epithelium ***(Seidler et al., 2008).*** Aspergilloma is a localized infection has been shown to consist of highly agglutinated hyphae encased in an extracellular matrix. A similar exopolysaccharide matrix is also produced at the surface of fungal hyphae during invasive pulmonary aspergillosis ***(Loussert et al., 2010).***

There is an apparent interaction and possible synergy between bacteria and fungi in biofilm development and survival. In a sheep model, bacteria appear to induce epithelial damage that promotes fungal biofilm formation by A.fumigatus. Co-inoculation of Staphylococcus aureus (S.aureus) and A. fumigatus into sheep sinuses resulted in 80% formation of biofilms versus 10% with A.fumigates inoculation alone ***(Boase et al., 2013).***

Fungi belonging to the class zygomycetes and the order Mucorales, including Rhizopus, Rhizomucor, Mucor,

Lichtheimia, Apophysomyces, Cunninghamella and Saksenaea often cause opportunistic infections which are similar to those caused by Aspergillus and are characterized by Angio-invasion and fungal-ball formation. Mucormycosis is categorized into rhino-orbito-cerebral (ROC), pulmonary, gastrointestinal, cutaneous and disseminated types depending upon the clinical presentation and the anatomical sites involved. The incidence of mucormycosis has increased globally over the last decades, with a phenomenal rise in the number of cases reported from India ***(Chakrabarti et al., 2009).***

Singh and his colleagues study results revealed the biofilm-forming potential of Rhizopus oryzae, Lichtheimia corymbifera and Rhizomucor pusillus, but not Apophysomyces elegans. At appropriate seeding densities, theses fungi produced robust, highly intertwined, filamentous, adherent structures that were encased in an extra cellular matrix ***(Singh et al., 2011).***

Fungal biofilms are poorly understood compared with their bacterial counterparts and represent and expanding area of research, both within otorhinolaryngology and the wider microbiological community. The more the understanding of bacterial and fungal biofilms the more the etiopathogenesis of CRS will improve ***(Foremen et al., 2009).***

AIM OF THE WORK

This study aims to detect the presence of fungal biofilm in cases of fungal sinusitis through scanning by electron microscopy and trial of detection its role in:

1) Recurrence of fungal infection in different cases of fungal sinusitis.

2) Resistance to medical treatment

3) Development of complications in postoperative period