DOI: https://doi.org/10.53350/pjmhs2023173797

ORIGINAL ARTICLE

Biochemical and Pathological Evaluation of Antibiotic Resistance Patterns and Histopathological Changes in Patients with Chronic Pharyngitis

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ABSTRACT

Background: The persistence of the inflammation of the pharyngeal tissues is called chronic pharyngitis and is often complicated by developing antibiotic resistance. Systemic inflammation and adverse tissue remodeling are in part due to the development of resistant bacterial strains that have resulted from the misuse of antibiotics. Optimization of therapeutic strategies in chronic pharyngitis requires an understanding of the biochemical and pathological implications of antibiotic resistance.

Methods: In a prospective one-year study, n=70 patients with clinically diagnosed chronic pharyngitis were enrolled and divided into two groups antibiotic sensitive (n = 32) and antibiotic resistant (n = 38). Detailed clinical evaluation was performed on all patients, blood samples were collected for assessing inflammatory markers (CRP, ESR), cytokines (IL6, TNfα), and oxidative stress parameters (MDA, SOD). Microbial identification and antibiotic susceptibility testing were performed on pharyngeal swabs cultured. Furthermore, a subset of patients are permitted to conduct pharyngeal tissue biopsies for histopathological examination. Univariate and multivariate regression models were used to analyze data in SPSS version 26 for determining predictors of elevated inflammatory markers.

Results: Compared to the sensitive group ($p < 0.005$), CRP, ESR, IL6, and TNFα levels were significantly higher in patients with antibiotic-resistant isolates. MDA levels and SOD activity levels were higher in the resistant group ($p < 0.01$) compared to that of the susceptible group. Results of regression analysis showed that antibiotic resistance was an independent predictor of increased CRP ($\beta = 0.38$, $p = 0.001$) and IL-6 ($\beta = 0.35$, $p = 0.003$). There were more pronounced epithelial hyperplasia, inflammatory infiltrates, and submucosal fibrosis in resistant cases ($p = 0.002$).

Conclusion: Increased inflammation, oxidative stress, and severe tissue alterations are correlated with antibiotic resistance in chronic pharyngitis. These results underscore the necessity of targeted treatments and good antibiotic stewardship.

Keywords: Chronic Pharyngitis, Antibiotic Resistance, Inflammation, Oxidative Stress, Histopathology

INTRODUCTION

Chronic pharyngitis, i.e. prolonged inflammation of the pharyngeal tissues, is a persistent, recalcitrant clinical problem characterized by recurrent sore throat, irritation, and occasional dysphagia. The acute form of pharyngitis is most commonly associated with viral pathogens, but the chronic form of pharyngitis is increasingly related to bacterial infections, particularly for patients with a history of unresponsiveness to standard treatment regimens[1]. The vicious cycle of tissue injury and repair is compounded by relentless inflammation seen in patients with these diseases, contributed by repeated bacterial colonization and an aberrant host immune response. Over the last few years, the increasing development of antibiotic resistance in clinical settings further complicates this scenario, reducing the efficacy of conventional therapeutic regimens and causing a reconsideration of the diagnostic and treatment strategies[2].

Chronic pharyngitis is a major public health problem that is prevalent worldwide with a variable epidemiological pattern in different regions. Rigorous antibiotic stewardship programs and sophisticated diagnostic tools have significantly reduced the impact of the disease in many high-income countries, but misuse and overprescription of antibiotics fuel the development of multidrug-resistant strains[3]. However, developing regions have a dual challenge to overcome: lack of healthcare resources, and endemic culture of self-medication with antibiotics. Such has been the rise in resistant bacterial strains that the efficacy of available treatments is diminished, and the inflammatory process aggravated, inducing more severe histopathological changes in the pharyngeal mucosa[4].

The situation is especially bad in Pakistan. Chronic pharyngitis is highly prevalent in the country due to environmental factors a heavy burden of infectious diseases and suboptimal antibiotic regulation[5]. Empirical antibiotic therapy with or without proper microbial sensitivity testing is still the norm in many local healthcare settings and speeds up the emergence of resistant pathogens. Consequently, these patients are frequently symptomatic and the biochemical and histopathologic alterations are inadequately addressed. Thus, a detailed inflammatory biomarker evaluation, in conjunction with histopathological analysis of pharyngeal tissues, can elucidate the intricate interplay between chronic infection, antibiotic resistance, and tissue remodeling in this population[6].

The objective of this study was to fill the gap between clinical observation and laboratory results through a full biochemical and pathological study of chronic pharyngitis. This current study examines key inflammatory markers including C-reactive protein, cytokine profiles, and oxidative stress parameters as well as histopathological features including epithelial hyperplasia, inflammatory cell infiltration, and submucosal fibrosis in an attempt to explain mechanisms of persistent pharyngeal inflammation [7]. At the same time, antibiotic resistance patterns of bacterial isolates will be assessed to provide key insight into the therapeutic difficulties that are faced both globally and within the Pakistani context. The study aimed to use this integrative approach to broaden our understanding of chronic pharyngitis and to help develop target, effective treatment strategies that overcome microbial resistance and underlying pathological changes[8].

METHODOLOGY

Study Design and Population: This is a prospective observational investigation spanning over a one-year period from January 2022 till January 2023 involving a total sample of n=70 patients clinically diagnosed with chronic pharyngitis. The study protocol has been approved by the institutional ethics committee and patients are being recruited from Aziz Bhatti Shaheed Teaching Hospital, Gujrat, Pakistan and Pir Abdul Qadir Shah Jeelani Institute of Medical Sciences, Sindh, Pakistan. Every participant is enrolled after obtaining their informed consent in writing, and it follows ethical standards.

Inclusion and Exclusion Criteria: Patients who are included in this study are those who have chronic pharyngitis (a persistent sore throat, irritation, or dysphagia lasting at least 3 months) and

are aged 18 years or older. All participants must be willing to undergo all the necessary investigations, which include blood sample collection, pharyngeal swabbing, and if applicable, pharyngeal tissue biopsy. In contrast, the study is excluded from those who had acute pharyngitis or else an upper respiratory tract infection within the past four weeks. Patients with immunodeficiency disorders, those on immunosuppressive therapy, those with a history of head and neck malignancies or prior pharyngeal surgery, and pregnant or lactating women, are also excluded.

Clinical Evaluation: For each participant, a comprehensive clinical evaluation is done including a detailed medical history with regard to duration and severity of symptoms, previous antibiotic use and comorbidity. A standardized assessment form is used to document the clinical evaluation so that it is consistent in recording all relevant clinical findings and symptomatology for a detailed understanding of the patient's clinical status.

Biochemical Analysis: All 70 patients are given a systemic inflammation and oxidative stress check, by blood sampling. The biochemical analysis includes measurement of the inflammatory markers such as C-reactive protein and erythrocyte sedimentation rate to quantify systemic inflammatory response. Furthermore, interleukin 6 and tumor necrosis factor-alpha levels are quantified through enzyme-linked immunosorbent assay techniques, and cytokine profiling is done. The analysis is also extended to evaluating oxidative stress parameters such as the measurement of serum malondialdehyde levels and the assessment of superoxide dismutase activity as complementary parameters reflecting lipid peroxidation and antioxidant defense balance.

Microbiological Assessment and Antibiotic Resistance Testing: Bacterial pathogens are identified in the patient's microbial culture and pharyngeal swabs from each patient. Standard microbiological techniques are used to isolate and identify the usual bacterial agents of chronic pharyngitis, focussing on streptococcal and Haemophilus influenzae. The disk diffusion method by Clinical and Laboratory Standards Institute guidelines is then used to test the antibiotic susceptibility testing. Antibiotics tested include penicillins, cephalosporins, macrolides, fluoroquinolones, and tetracyclines, which allow for a broad study of the resistance patterns of isolated organisms.

Histopathological Examination: Pharyngeal tissue biopsy is done on a subset of about 30 disease patients who give additional consent for more invasive diagnostic procedures. The tissue samples are fixed in 10% formalin, embedded in paraffin, sectioned into 5 µm thick slices then stained with hematoxylin and eosin. The histopathological evaluation is performed to evaluate epithelium integrity, including signs of hyperplasia and dysplasia, and to describe the extent and nature of inflammatory cell infiltration (mostly lymphocytes, plasma cells, and neutrophils). It is directed further to the analysis of submucosal fibrosis, vascular proliferation, interstitial edema, etc., as indicators of tissue remodeling, i.e. the presence of myofibroblasts.

Data Analysis and Statistical Methods: SPSS version 26.0 was used to analyze all data collected from the biochemical assays, microscope cultures, and histopathological examinations. The data are organized in thematic groups which are the same as the different study variables. Computing descriptive statistics to summarize patient demographics, clinical characteristics, and laboratory findings and doing inferential statistics to compare continuous variables using parametric tests like t-tests or nonparametric alternatives (Mann-Whitney U test) based on the distribution of the data. Chi-square tests are used to examine categorical variables in patient groups harboring antibiotic-resistant bacterial isolates versus those with sensitive strains and in comparison, to measures of inflammatory and oxidative stress markers at varying levels. All the results are reported with corresponding confidence intervals whenever applicable and a significance threshold is set at $p < 0.05$.

RESULTS

Demographic and Clinical Characteristics: The study included 70 patients with chronic pharyngitis. The mean age of the study population 42.3 ± 10.2 years was balanced with equal gender distribution 35 males (50%) and 35 females (50%). Symptoms lasted from 3 to 24 months and a median of 8 months. Based on the antibiotic sensitivity testing, patients were further divided into two groups namely, antibiotic resistant group (n = 38) and antibiotic sensitive group (n = 32). The demographic and clinical characteristics of the entire study population and by group are presented in Table 1.

Table 1: Demographic and Clinical Characteristics of Study Participants

Characteristic	Total (n=70)	Resistant Group (n=38)	Sensitive Group (n=32)
Mean age (years)	42.3 ± 10.2	43.1 ± 9.8	41.2 ± 10.7
Gender (Male/Female)	35/35 (50%/50%)	20/18 (52.6%/47.4%)	15/17 (46.9%/53.1%)
Median symptom duration (mo.)	8 (range: 3–24)	9 (range: 3–24)	7 (range: 3–18)

The two groups were comparable in terms of age and gender distribution, which supports the validity of subsequent comparisons regarding biochemical and histopathological markers.

Biochemical Analysis: C reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were measured to assess systemic inflammation. The CRP mean overall was 18.5 ± 6.2 mg/L and ESR was 35.4 ± 12.7 mm/hr. The mean CRP for the antibiotic-resistant group (21.2 ± 5.8 mg/L) was significantly higher than in the sensitive group (15.3 ± 4.9 mg/L, $p = 0.002$) in comparing the groups. In the same way, the mean ESR was higher in the resistant group (41.7 ± 11.3 mm/hr) than in the sensitive group (29.8 ± 10.4 mm/hr; $p = 0.001$). These results suggest that people with resistant bacteria have a more powerful inflammatory response.

Additionally, cytokine profiling also revealed large differences between groups. Different between the resistant (12.8 ± 3.6 pg/mL) and the sensitive (9.1 ± 2.8 pg/mL) groups, the mean interleukin-6 (IL-6) concentration was significantly lower in the sensitive group than in the resistant group ($p = 0.004$). Resistant and sensitive groups had elevated tumor necrosis factor-alpha (TNF-α) levels of 15.5 ± 4.2 pg/mL versus 11.7 ± 3.9 pg/mL ($p = 0.005$). Summary of inflammatory and cytokine marker comparisons are summarized in Table 2.

Table 2: Comparison of Inflammatory and Cytokine Markers

Parameter	Resistant Group	Sensitive Group	p-value
CRP (mg/L)	21.2 ± 5.8	15.3 ± 4.9	0.002
ESR (mm/hr)	41.7 ± 11.3	29.8 ± 10.4	0.001
IL-6 (pg/mL)	12.8 ± 3.6	9.1 ± 2.8	0.004
TNF-α (pg/mL)	15.5 ± 4.2	11.7 ± 3.9	0.005

Serum malondialdehyde (MDA) and superoxide dismutase (SOD) activity were also measured to evaluate oxidative stress, in addition to these markers. In the resistant group, the MDA levels were significantly higher (4.2 ± 1.1 µmol/L) than in the sensitive group (3.1 ± 0.9 µmol/L, $p = 0.003$), and SOD activity was lower (1.8 ± 0.4 U/mL) than in the sensitive group (2.3 ± 0.5 U/mL, $p = 0.006$). The oxidative stress parameters are presented in Table 3.

Table 3: Oxidative Stress Marker Comparison

Parameter	Resistant Group	Sensitive Group	p-value
MDA (µmol/L)	4.2 ± 1.1	3.1 ± 0.9	0.003
SOD (U/mL)	1.8 ± 0.4	2.3 ± 0.5	0.006

Regression Analysis: Through the use of multiple linear regression models in SPSS version 26, it was determined the independent predictors of elevated inflammatory markers. Potential confounders like age, gender, and symptom duration were adjusted for using these models. Antibiotic resistance was a significant independent predictor of CRP levels ($\beta = 0.38$, $p =$

0.001) and IL-6 levels (β = 0.35, p = 0.003). These results suggest that antibiotic resistance, even after adjustment for demographic and clinical variables, adds to levels of inflammation independently.

Table 6: Regression Analysis for Predictors of Elevated Inflammatory Markers

Dependent Variable	Predictor	β	Standard Error	p-value
CRP	Antibiotic Resistance	0.38	0.11	0.001
IL-6	Antibiotic Resistance	0.35	0.10	0.003

The table above demonstrates that the levels of CRP and IL6 are significantly increased with antibiotic resistance. The standardized beta coefficients (β) represent this association and p values show these associations are statistically significant. This regression model supports that antibiotic resistance is an independent predictor of systemic inflammation in patients with chronic pharyngitis and should be clinically relevant.

Histopathological Analysis: A subset of 30 patients also had pharyngeal tissue biopsies obtained. Results of histopathological examination showed epithelial hyperplasia, inflammatory cell infiltration, submucosal fibrosis and vascular proliferation. The severity of these changes was rated with a semi quantitative scoring system, with more severe changes receiving higher scores. The mean histopathological score for the resistant group was 7 as opposed to 4 in the sensitive group, which was statistically significant (p = 0.002) by the Mann-Whitney U test. Table 4 details the histopathological findings.

Table 4: Histopathological Scores in Resistant vs. Sensitive Groups

Parameter	Resistant Group (Median Score)	Sensitive Group (Median Score)	p-value
Histopathological Score	7	4	0.002

The higher histopathological scores in patients with antibiotic-resistant isolates are due to more severe tissue inflammation and remodeling. These results are consistent with the biochemical findings that antibiotic resistance is related to increased systemic inflammation and more severe local alterations.

Overall, SPSS version 26 analysis of the results shows that patients with antibiotic-resistant bacteria have significantly higher systemic inflammatory measures (CRP, ESR, IL-6, TNF-α,) and oxidative stress (elevated MDA, low SOD) than patients with sensitive strains. It was demonstrated that antibiotic resistance is an independent predictor of these inflammatory markers, even after adjusting for demographic variables using the multiple regression analysis. In addition, this was corroborated by the histopathological analysis of pharyngeal tissue which showed more severe tissue damage and inflammatory infiltrates in the resistant group. Together, these findings emphasize the importance of targeting antibiotic resistance in chronic pharyngitis and the critical role of antibiotic stewardship in the management of this disease.

DISCUSSION

In the present study, we have undertaken a comprehensive study of biochemical markers, patterns of antibiotic resistance, and histopathological changes in patients with chronic pharyngitis. These findings show that patients with antibiotic-resistant bacteria have significantly hypervolemic systemic inflammatory markers such as CRP, ESR, IL-6, and TNF-α, as well as increased oxidative stress (higher MDA and lower SOD). These results emphasize that antibiotic resistance is not only a microbiological phenomenon but is closely tied to an enhanced inflammatory state[9]. The results of the multiple linear regression analysis were further confirmed in that antibiotic resistance independently predicts higher CRP and IL-6 levels independent of demographic and clinical variables, indicating that resistant pathogens directly implicate a more pronounced inflammatory response[10].

These biochemical observations are complemented by histopathological findings; patients who underwent pharyngeal tissue biopsies had more severe epithelial alterations, inflammatory infiltrates, and submucosal fibrosis in the presence of resistant bacterial strains[11]. These morphological changes are likely due to continued chronic inflammation and tissue remodeling caused by persistent infection together with inadequate therapeutic response[12, 13].

The results of the study are consistent with previous literature which shows that antibiotic resistance hurts disease progression. However, while these observations are extended to the biochemical and pathological data here, we integrate these observations to gain a more complete understanding of the pathogenesis of chronic pharyngitis[14]. The strong associations between antibiotic resistance and both systemic and local inflammatory responses highlight the essential importance of targeted therapeutic strategies and robust antibiotic stewardship[15, 16].

However, several limitations should be considered regarding the robust findings. Although the sample size of the study is adequate for detecting significant differences, generalization of results to other populations may be limited[17]. The study was also cross sectional, and therefore it cannot establish causal relationships. These findings should be validated in future longitudinal studies with larger sample sizes and they should also be investigated related to antibiotic resistance in the long term[18].

CONCLUSION

The Study revealed that systemic inflammation, oxidative stress, and more severe histopathological changes are higher in chronic pharyngitis patients with antibiotic-resistant bacterial isolates compared to those with antibiotic-sensitive infections. The clinical impact of microbial resistance in the pathogenesis and progression of chronic pharyngitis is shown by the independent predictive role of this resistance on inflammatory markers such as CRP and IL6. These results point toward the need for the creation of tailored treatment strategies and effective antibiotic stewardship programs. These observations should be further expanded in larger cohorts and potential interventions to reduce the inflammatory burden in patients with chronic pharyngitis should be studied in the future.

Funding: No funding was received.

Authors contribution: All authors contributed equally to the current study.

Acknowledgment: We acknowledge our colleagues and paramedical staff for supporting us and making the study possible.

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