Journal Homepage: - www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)

Article DOI: 10.21474/IJAR01/17703
DOI URL: http://dx.doi.org/10.21474/IJAR01/17703

RESEARCH ARTICLE

ASSESSMENT OF SHIFT IN THE ANTIBIOTIC SUSCEPTIBILITY PATTERN OF AEROBIC BACTERIAL ISOLATES FROM MICROBIAL BIOFILMS IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMA AND PRECANCEROUS CONDITIONS

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Manuscript Info

Manuscript History
Received: 15 August 2023
Final Accepted: 18 September 2023
Published: October 2023

Key words:-
Antibiotic, Antimicrobial Resistance, Microbiome, Oral Cancer, Precancerous Lesions

Abstract

Background: Antimicrobial resistance is a concerning global health problem in reference to cancer patients as they are more susceptible to drug-resistant microorganisms because of their immunocompromised states.

Aim: The aim of this study was to study the changes in the pattern of aerobic bacterial flora in the patients of oral cancer and precancerous lesions and to identify the susceptibility to commonly used Antibiotics in clinical practice.

Methods: This prospective observational study was carried out in a randomly selected cohort of oral cancer and precancerous patients being treated at tertiary care centre in Rajasthan, for a period of 1 year. Oral swabs were collected from patients presenting with oral cancer and precancerous lesions and subjected to microbiological examination for their colony characters, morphology on Gram stain as well for Antibiotic sensitivity patterns for commonly used drugs.

Results: Of the 100 oral cancer patients, isolated bacterial colonies showed a mixture of Gram-Positive and Gram-Negative organisms. *Staphylococcus* species (n = 15) were seen in high numbers in the case of Gram-positive organisms, while in Gram-negative bacteria *Pseudomonas* species (n = 45) increased in number. Among the precancerous patient group normal commensal (*Micrococci*, n= 24) was the predominant species. In Gram-negative bacteria, all were susceptible to Piperacillin Tazobactam and Imipenem while in Gram-positive bacteria all were susceptible to Linezolid, Gentamicin, and Vancomycin.

Conclusion: This study showed 90% susceptibility to every class of commonly used antibiotics. Multidrug resistance (MDR) was seen in 9.52% of the bacterial isolates. Antimicrobial resistance is emerging among cancer patients. Studies on cultural sensitivity patterns will help clinicians to prescribe antibiotics judiciously and will further decrease the chances of antimicrobial resistance in these patients.

Introduction:-

Oral cancers (cancers arising from lips, tongue, floor of the oral cavity) account for 40% of all the cancers in India. [1],[2]There is also an alarming increase in their incidence globally.[3]Oral carcinogenesis is a complex process

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involving the exposome's impact on keratinocyte cytogenetics and epigenetics.The predominant etiological causes for this disease are often seen as being external exposures such the use of tobacco and alcohol, poor dental hygiene, and poor eating habits. [4]. It is extensively researched how oral microbiota (internal exposome) affects the development and spread of oral malignancies. [5] Dysbiosis which is defined as an imbalance in the collection of the oral microbes in humans, is often associated with an exposure to certain environmental factors, smoking of tobacco, diet with high sucrose content, and even injudicious use of antimicrobials [6], [7]. The normal microbiota of the oral cavity and oropharynx exhibit predominance of *Streptococcus, Bacteroides* species (excluding *Bacteroides fragilis), Peptostreptococcus* species, *Prevotella* species, *Fusobacterium* species, *Veillonella* species, Enterobacteriaceae, and *Staphylococci*. [8]

Empiric use of antibiotics in oral cancer patients to treat various diseases can lead to serious complications like antimicrobial drug resistance. This necessitates the need to study the shift in the microbial flora of the patients with the progress in their pre-malignant stage to oral carcinogenesis which will further help improper selection of appropriate antibiotics and better management of these patients.

Materials and Methods:-

This prospective observational study was carried out in a randomly selected cohort of oral cancer and precancerous patients at medical oncology department from January 2021 for one year. Ethical clearance was obtained from the Institutional Review Board (Ref. No. 286 MC/EC/2021) before the study. All patients were briefed about the study, counseled, and informed consent was taken.

Sample size:

Sample size of 98 patients of both the study groups will be required at 80% study power, confidence limit upto 95% and alpha error of 0.05, assuming 56.7% prevalence of pathological bacterial flora in oral squamous cell carcinoma patients and 13.3% in control subjects as found in the study of Milos et al [22].

Inclusion criteria

a) Newlydiagnosed cases of oral cancers (Group A) and precancerous lesions (Group B) of age above 18 years, b) patients willing to participate in the study .

Exclusion Criteria

a) Patients receiving chemotherapy, radiotherapy or surgical treatment, b) Patients receiving antibiotic treatment currently or who had received antibiotic treatment within the last 4 weeks, and c) Patients diagnosed with HIV, HBV, HCV and tuberculosis infection.

Microbiological Analysis-

1. Sample collection: In the study group, swabs will be taken from all the patients under standardized aseptic conditions. In oral cancer patients, the swabs will be taken from the tumour site and from precancerous patients swabs will be taken from lesion site. All specimens will be collected and transported within a maximum of two hours to the microbiology laboratory of the institute and inoculated immediately.

2.Isolation and identification of bacterial species: For possible isolation of bacterial pathogens,each swab sample will be inoculated onto Blood agar ,MacConkey agar and Thioglycollate broth then these plates will be cultured aerobically at 37*C for 24 hr.Each aerobic bacterial isolate will then be identified on the basis of morphology, Gram staining and biochemical tests that is conventional and by advanced VITEK II system as per standard lab protocol.

3.Antibiotic Susceptibility Testing: Antimicrobial susceptibility testing will be performed by Kirby –Bauer disc diffusion technique on Mueller Hinton agar media as recommended by the clinical and laboratory standards institute (CLSI 2021). The Antibiotic used for susceptibility testing were selected on the criteria of being regularly used in the hospital for controlling the infection and prophylaxis purpose.

Results:-

In Group A (N=100) 79% were males and 21% were females while in Group B (N=100)72% were males and 28% were females. The mean age of the patients in both the groups was 46.72+19.61 years. There was a statistically significant difference in tobacco smokers among the two groups (P = 0.008). [Table 1] Microscopic examination of the samples revealed that in Group A; the Gram Negative Bacilli were observed in majority (69.30%), followed by the Gram Positive cocci (22.81%) while in Group B the GPC were in majority (31.37%)

followed by Gram Positive Bacilli (22.55%). According to Gram staining, the difference was statistically significant (P = 0.001) in both the groups as depicted in table 2.

Majority of oral cancer patients (69.3%) in the present study showed Gram Negative bacterial growth compared to Gram Positive bacterial growth which was recorded in majority of the Precancerous patients (82.35%).Most common Gram-Positive bacteria found among oral cancer patients were *Staphylococcus* and *Streptococcus* while in precancerous patients it was *Micrococci* and *Diphtheroids*. These patients were administered with Vancomycin, Teicoplanin, Linezolide, Piperacillin and Tazobactam combination, Ampicillin, Cefepime, Cefoxitin, Ciprofloxacin, Gentamicin, Doxycycline, Clindamycin, and Erythromycin. It was found that most microorganisms were resistant to Clindamycin, Erythromycin, and Ampicillin while Linezolid, Vancomycin and Teicoplanin Antibiotic showed 100%susceptibility as shown in Table:3.

Among the Gram Negative Bacilli ,*Pseudomonas* was the most common species found in the oral cancer patients. In this study, *P.aeruginosa* showed highest susceptibility to Colistin (100%), Piperacillin and Tazobactam (93.3%) as shown in Table 4.

Multidrug resistance (MDR) was seen in 9.52% of the bacterial isolates. MDR in Gram-negative and Gram-positive bacteria were 8.64% and 12.5%, respectively. [Table 5] Maximum MDR (25%) was noted with gram negative bacteria A. baumannii different antibiotic groups which were Penicillins, Cephalosporins, Aminoglycosides, Carbapenem, Fluoroquinolones as depicted in table 5.

Table 1:- Socio-demographic data of the study participants.

Independent Variables	**Frequency (N)**		**P-value**
Gender	**Group A (Oral Cancer)**	**Group B (Precancerous lesion)**	
Male	79	72	
Female	21	28	0.324
Age in years			
<20	0	1	
20-30	9	11	
31-40	26	31	
41-50	33	32	0.926
51-60	27	20	
>60	5	5	
Tobacco History			
1) Smoking	26	21	0.505
2) Chewing	84	72	0.606
3) Smoking and Chewing	24	9	0.008

Table 2:- Comparison of the organisms with Gram staining in both the groups.

Gram Staining	**Group A (Oral Cancer)**		**Group B (Precancerous lesions)**		
	Number	**%**	**Number**	**%**	**p- value**
Gram Negative Bacilli	79	69.3	18	17.65	<0.001
Gram Positive Bacilli	3	2.63	23	22.55	

Gram Positive Cocci	32	28.07	61	59.80	

Chi-square = 69.027 with 3 degrees of freedom;

Table 3:- Comparison of Susceptibility pattern of identified Gram Positive Bacterial isolates.

Antibiotic	**Group A**	**Group B**	**P-Value (Fisher's Exact test)**
***Staphylococcus aureus* (N=27)**			
Vancomycin	15	12	-
Teicoplanin	15	12	-
Linezolide	15	12	-
Piperacillin+Tazobactam	14	12	0.909
Ampicillin	14	12	0.909
Cefepime	13	12	0.565
Cefoxitin	13	12	0.565
Ciprofloxacin	12	5	0.099
Gentamicin	12	11	0.762
Doxycycline	11	10	0.877
Clindamycin	11	7	0.681
Erythromycin	7	8	0.516
***Streptococcus* (N=27)**			
Ciprofloxaxcin	9	12	0.141
Vancomycin	9	18	-
Teicoplanin	9	18	-
High dose Gentamicin	9	12	0.141
Linezolid	9	18	-
Ampicillin	7	10	0.481
Doxycycline	7	11	0.665
Clindamycin	5	0	0.003*
Erythromycin	4	9	0.892

Table 4:- Comparison of Susceptibility pattern of identified Gram Negative Bacterial isolates in both groups.

Antibiotic	**Group A**	**Group B**	**P-Value (Fisher's Exact test)**
***Klebsiella* (N=32)**			
Polymyxin B	26	6	-
Tigecycline	24	6	-
Imipenem	23	6	-
Ciprofloxaxcin	19	5	0.909
Piperacillin+ Tazobactam	19	6	0.909
Ceftazidime + Clavulanic acid			0.565
Cefepime	17	5	0.099
Ceftazidime	16	5	0.762
Amikacin	13	4	0.877
Gentamicin	10	4	0.681
Cotrimoxazole (Trimethoprim-sulphamethoxazole)	8	3	0.516

***Pseudomonas* (N=59)**			
Colistin	45	14	-
Piperacillin+ Tazobactam	42	12	0.730
Imipenem	37	12	0.917
Ciprofloxaxcin	36	12	0.931
Cefepime	35	12	0.792
Gentamycin	32	9	0.879
Tobramycin	32	10	0.753
Ceftazidime	27	8	0.903
Amikacin	35	9	0.509

Table 5:- Frequency of multidrug resistance pattern of bacterial isolates in Group A.

Bacterial Isolates	**MDR (%)**	**R**	**Antimicrobial Class**
S. aureus	1/15 (6.66%)	AM,P/TZ, GEN, CIP	Penicillins, Aminoglycosides, Fluoroquinolones
Streptococcus	2/9 (22.22%)	AMP, TET, ERY, CLI	Penicillins, Tetracyclines, Fluoroquinolones
K. pneumoniae	2/26 (7.69%)	AMP, CAZ, TOB,CIP, P/TZ, TRM/SXT, AMIK, GEN, TGC, ATM	Penicillins, Cephalosporins, Aminoglycosides, Fluoroquinolones, Tetracyclins, Monobactam, Folate pathway inhibitors.
P. aeruginosa	3/45 (6.67%)	P/TZ, CAZ, IMP, AMIK, GEN, CPM	Penicillins, Cephalosporins, Aminoglycosides, Carbapenem,
A. baumannii	1/4 (25%)	AMP, P/TZ, CAZ, CPM, IMP, AMIK, GEN, CIP, TGC	Penicillins, Cephalosporins, Aminoglycosides, Carbapenem, Fluoroquinolones
E. coli	1/6 (16.67%)	AMP, P/TZ, CAZ, CPM, AMIK, GEN, CIP	Penicillins, Cephalosporins, Aminoglycosides, Fluoroquinolones

AMIK- Amikacin, AMP- Ampicillin, CAZ- Ceftazidime, CIP- Ciprofloxacin, CLI- Clindamycin, CPM- CefepimeERY- Erythromycin, GEN- Gentamycin, IMP- Imipenem, P/TZ – Piperacillin/Tazobactam,, TET– Tetracycline, TGC- Tigecycline, TOB-Tobramycin, TRM/SXT-Trimethoprim-sulphamethoxazole

Note:
R- Resistance to tested antimicrobials.
MDR- The isolate is non-susceptible to at least one agent in >3 antimicrobial categories.

Discussion:-

Antimicrobial resistance (AMR) has become a global menace which needs to be addressed as soon as possible. The range of infections caused by different microorganisms like the bacteria, parasites, viruses and fungi are now no longer susceptible to the common medicines used to treat them in the recent past. The problem of AMR in relation to bacterial drug resistance is particularly concerning. [9], [10] In cancer patient the second leading cause of death is infection. Loss of effectiveness of antibiotics brought on by bacterial antibiotic resistance is potential threat to the success of cancer treatment. [11]

Cultural sensitivity profile of oral cancer and precancerous patients can help the clinician in choosing an effective antimicrobial drug and to decrease the AMR. This is the first study that has compared bacterial sensitivity of precancerous and oral cancer patients. The six important bacterial phyla to which the oral microbiome belong includes *Firmicutes, Bacteroides, Proteobacteria, Actinobacteria, Spirochaetes,* and *Fusobacteria*. [12] Additionally, oral microbiome may contribute to the emergence of cancer. Patients with oral cancer typically have poor oral hygiene and harbors harmful bacteria, which causes persistent inflammation of the affected location. Large amounts of cytokines and growth factors released by immune and non-immune cells may also have an impact on carcinogenesis. [13],[14],[15]

The microbiome in the oral cavity appears to differ between healthy, precancerous and the cancerous patients.[16] Shiga et al. suggested that infection by *S. anginosus* might play an important role in carcinogenesis of squamous cell carcinoma of head and neck. [17] In this study were observed with majority of bacterial isolates belonging to *Staph. aureus* and *S.lentus* among oral cancer patients.

Mager et al. investigated the difference in the salivary counts of 40 common oral bacteria in patients with an oral squamous cell carcinoma (OSCC) lesion and their cancer free healthy controls andobserved that *Capnocytophaga gingivalis, Prevotella melaninogenica*, and *S. mitis* counts were significantly increased in the oral cancer patients.[18]In the present study, majority of *Pseudomonas* sp. (39.47%) were isolated in oral cancer patients followed by *Klebsiella*.(22.81%) while *Micrococci*(23.53%) and *Diptheroids* (22.55%) were seen among precancerous patients. In this study, Gram Positive cocci accounted for 28.07% of the total isolates from the swab samples of the oral cancer patients in comparison to 59.8 % in precancerous patients. This is contrary to a recent Chinese study which revealed an increased number of *Bacteroides* in the oral cancer and precancerous patients groups in comparison to healthy patients. [19] Schmidt reported an abundance of *Streptococcus* and *Actinomycetes* in oral squamous cell carcinoma. [20]

In this study, *P.aeruginosa* showed highest susceptibility to Colistin (100 %), Piperacillin and Tazobactam (93.3%). *Pseudomonas aeruginosa* is the common causative agent responsible for infections in cancer patients, having neutropenia. Even the novel Anti-Psudomonal antibiotics do not show activity against all strains of *Pseudomonas* so in these patients, preventive strategies such as minimizing resistant strain transmission, active surveillance screening for MDR (multidrug-resistant) strain colonisation, microbiological diagnostics, antimicrobial stewardship, and antibiotic prophylaxis becomes very important.As the resistant strains to pseudomonas are increasing so the need to explore newer antibioticsas first-line drugs in *Pseudomonas* infection in cancer patients becomes essential.

There are a few limitations to this study. In this study only the aerobic bacterial isolates were analyzed and studied for susceptibility pattern so the results cannot be generalized for all the microbial isolates from cancer patients. Also the duration for this study was one year so analysis of temporal trendsin the susceptibility patterns could not be studied. The strength of this study was that it provided a key information about the shift in the susceptibility patterns of aerobic bacterial isolates in oral cancers and precancerous patients.

Conclusion:-

In conclusion there is a shift from gram positive to gram negative bacteria in patients with precancerous condition and oral cancer respectively. Multidrug resistance was noted in both the Gram-negative and Gram-positive bacteria. We strongly recommend culture sensitivity testing in precancerous and cancer patients before starting antimicrobial therapy for these patients. This will decrease the antimicrobial drug resistance and the chances of infections in them.

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