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BACTERIAL RESISTANCE AGAINST ANTIMICROBIAL AGENTS IN DIABETICS

Periodontics

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ABSTRACT

Purpose: To determine the resistance & sensitivity of the subgingival microflora against antimicrobial agents in untreated adult healthy & diabetic patients with chronic periodontitis.

Materials and Method: A comparative study comprises of the two population healthy & diabetics, diagnosed as chronic periodontitis. Total 60 patients with chronic periodontitis were selected for the study & divided in 2 groups – Healthy & Type II diabetes. Subgingival plaque samples were collected from all the patients & culture & sensitivity test was done for various antibiotics- Amoxicillin, Amoxiclav, Metronidazole, Cefixime, Tetracycline& Ciprofloxacin.

Results: Statistical analysis was done. In diabetic patients results revealed more resistance for Metronidazole (83.3%) & least for Tetracycline (16.7%) while healthy patients showed more resistance for Metronidazole (60%) & least for Tetracycline (3.3%) & Ciprofloxacin (3.3%). Significant results were found for Metronidazole & Ciprofloxacin resistance between two groups.

Conclusion: The current results showed alarmingly high resistance rate against Metronidazole. However, misuse of these agents in our city is huge. The emergence of antibiotic resistant bacteria within the oral flora will have an impact on the prescribing of antibiotic in dentistry.

KEYWORDS

Antibiotic Resistance; Chronic Periodontitis; Diabetes; Sensitivity

INTRODUCTION

Antimicrobial agents are substances produced by various species of microorganisms that suppress the growth of other microorganisms and may eventually destroy them. The "golden age" of antibiotics began with the production of penicillin in 1941, when this compound, discovered by Alexander Fleming in 1928. [1]

Microbial resistance is a natural biological response of microbes to a selective pressure, such as weather conditions, food, oxygen or water availability, or the presence of an antimicrobial drug. However, there is a scarcity of information on the susceptibility to antibiotics of bacterial species isolated from patients with periodontitis, since microbial sensitivity tests are not normally performed in daily practice either in dentistry or in medicine, especially in countries where there is high antibiotic consumption. [2]

At present, most systemic periodontal antibiotic treatment regimens appear to be empirically prescribed by clinicians without guidance from a microbiological analysis of subgingival bacterial biofilm populations, even though patients with periodontitis frequently yield multiple species of periodontal pathogens that potentially vary in their antibiotic drug resistance.[3]

Antibiotic resistance poses a threat to everyone, but people with diabetes are at particular risk. People with diabetes develop common infections at different rates than non-diabetics, and these infections are often more serious, because diabetes can limit blood flow and the body's ability to fight infection. As a result, diabetic patients may require weeks or months of broad-spectrum antibiotics. The more often bacteria are exposed to antibiotics, the more resistant they become.[4]

Immuno- compromised state and frequent antibiotic use are associated with antibiotic resistance of the bacterial pathogens.[5]The overuse of systemic antimicrobial therapies may not only alter the composition of the periodontal microflora but may also select for periodontal pathogens with a low susceptibility for a number of antimicrobial agents. Moreover, the misuse of broad spectrum antibiotics in the treatment of periodontitis may enhance the development of bacterial resistance, which will diminish their therapeutic potential and may cause health problems in the treatment of serious infectious diseases.[6]

There are limited studies reported the antibiotic resistance among the diabetic population & hence this study was conducted with the aim to determine the resistance & sensitivity of the subgingival microflora of untreated adult patients with periodontitis in healthy & type II diabetic patients.

MATERIALS AND METHOD

A comparative study was done in chronic periodontitis patients reporting to outpatient in the Department of Periodontics, Rama Dental College, Kanpur, India. Total 60 chronic periodontitis patients were selected for the study & were divided equally into two groups – healthy (n=30) and diabetic (n=30).

Inclusion criteria for healthy patients: age 25-70 years, patients diagnosed as generalized chronic periodontitis, site/quadrant with a probing pocket depth >5 mm for sample collection with no periodontal treatment history within 6 month. For diabetic patients: patients with Type 2 Diabetes mellitus, Duration of diabetes mellitus ≥ 10 years, Fasting blood sugar ≤150 mg/dl & $HbA_1C \leq 8$. Exclusion criteria: Patients with systemic or topical antimicrobial therapy 4 weeks prior to the study, Pregnancy & Lactation, Smokers and medically compromised patients other than Type 2 DM.

After obtaining clearance from the Ethical Committee, consent forms were signed by the patients ready for the participation in the study. Clinical parameter recorded for each patients were – Oral hygiene index (Greene & Vermillion), Sulcular Bleeding Index, Russells periodontal index & probing depth (taken by UNC 15 probe). After case history recording, sites were selected with periodontal pocket measuring more than 5 mm in a quadrant.

After removal of supra-gingival plaque & calculus, selected sites were isolated with cotton rolls, curette was inserted subgingivally as far as possible parallel to the long axis of the selected tooth to remove the subgingival plaque. Samples were then transferred to a test tube containing thioglycollate medium and was sent to microbiological laboratory for anaerobic culture.

Microbiological Procedure

After incubation of plaque samples for 24 hours at 37˚C the samples were transferred into the blood agar plate for culture. After preparation of culture plate, plate was kept into the anaerobic jar with anaerobic gas

pack for 5 days in the incubator. After 5 days blood agar plates were removed from the anaerobic jar & blood agar plates were examined for bacterial growth and total count was calculated. The colonies thus obtained were transferred to 1% peptone water for the pure culture at the incubation of 24 hours at 37˚C. After 24 hours the samples were checked for the turbidity and was transferred to the blood agar drug sensitivity plate by the pour plate method, which consisted of the following antibiotics (HIMEDIA Comp.): Amoxiclav (30 mcg), Amoxicillin (30 mcg), Cefixime (5 mcg), Metronidazole (5 mcg), Tetracycline (30 mcg) and Ciprofloxacin (5 mcg) and incubated for 72 hours at the temperature of 37 ˚C. After 72 hours agar plates were checked for the zone of inhibition or the zone free of the bacterial colonies. The zone of inhibition was measured against each antibiotic to know the drug sensitivity or the resistance according to the comparison chart supplied.

Statistical Analysis

The percentage of resistant bacteria, present in the subgingival plaque, was calculated for each antimicrobial drug & then comparison were made between healthy & diabetic group. All the analysis was done using SPSS version 14.A p-value of <0.05 was considered statistically significant. Comparison of antibiotic resistance & sensitivity was done using Chi-square test.

RESULTS

Total 30 healthy and 30 diabetic patients with chronic periodontitis were studied. Resistance and Sensitivity of bacteria was observed against 6 drugs commonly used in periodontal diseases are - Amoxiclav, Amoxicillin, Cefixime, Metronidazole, Tetracycline and Ciprofloxacin. Number of patients resistance and sensitive in each group is shown in Table 1. The comparison was done inter & intra group.

Intra-group Comparison

Diabetic Patients

Results revealed more resistant for metronidazole & least for tetracycline.

Our result revealed that diabetic patients were more sensitive for Tetracycline, Amoxiclav, Ciprofloxacin & Cefixime accordingly.

Healthy Patients

Results revealed more resistant for metronidazole & least for tetracycline & Ciprofloxacin.

Our result reveals that healthy patients are more sensitive for Tetracycline, Ciprofloxacin, Cefixime & Amoxiclav accordingly.

Inter-group Comparison

Both diabetic & healthy patients were more resistant to Metronidazole while diabetic patients were least resistant to Tetracycline & healthy patients were equally least resistant to Tetracycline & Ciprofloxacin. Significant results between both groups for resistance were seen for Metronidazole & Ciprofloxacin.

Diabetic patients were more sensitive to Tetracycline while healthy patients were more sensitive to both Tetracycline & Ciprofloxacin.

No significant difference in antibiotic sensitivity/resistance was seen between healthy and diabetics with respect to Amoxicillin, Amoxiclav, Tetra and Cefixime (p=0.118, 0.39, 0.085 and 0.152) respectively. However, with respect to Metronidazole, a significantly higher number of the diabetic individuals (83.3%) were resistant than controls (60%) (p=0.045) and with respect to Ciprofloxacin, a significantly higher number of the diabetic individuals (33.3%) were resistant than controls (3.3%) (p=0.003) as shown in table.

Table 1: showing the percentage & p-value for drugs used in study among healthy and diabetic chronic periodontitis patients.

		Group				p-value
		Diabetics		Healthy		
		N (30)	%	N (30)	%	
Amoxicillin	R	20	66.7%	14	46.7%	0.118; NS
	S	10	33.3%	16	53.3%	
Amoxiclav	R	10	33.3%	7	23.3%	0.39; NS
	S	20	66.7%	23	76.7%	
Tetracycline	R	5	16.7%	1	3.3%	0.085; NS
	S	25	83.3%	29	96.7%	
Metronidazole	R	25	83.3%	18	60.0%	0.045; Sig
	S	5	16.7%	12	40.0%	
Ciprofloxacin	R	10	33.3%	1	3.3%	0.003; Sig
	S	20	66.7%	29	96.7%	
Cefixime	R	11	36.7%	6	20.0%	0.152; NS
	S	19	63.3%	24	80.0%	

DISCUSSION

The present comparative study was investigated for antibiotic sensitivity & resistance of the subgingival microflora from untreated healthy & diabetic chronic periodontitis patients living in Kanpur, India. The huge misuse and easy access of antimicrobials were observed by many health workers before conducting this study. Increasing levels of resistance necessitate the use of broader and more potent antimicrobials to secure patients, but using these antimicrobials exacerbates the problem of bacterial resistance.

The present study investigated the antimicrobial efficacy of six antibiotic regimens commonly used as adjunctive modalities in the treatment of periodontitis. We used subgingival plaque samples to test for resistance levels of anaerobic periodontal pathogens because this method was proven effective and informative (Walker et al. 1983) & showed that the anaerobic periodontal bacteria in adult periodontitis patients in diabetics displayed a significant higher level of resistance towards a number of antibiotics.

Our study reported that diabetes patient were resistant to most of antibiotics when compared to healthy patients. This resistance could be due to the successful mutation as a result of increased use of antibiotic in our population. Due to common recurrent infections, diabetic patients have more antibiotic treatments compared with other subjects, which can increase the antibiotic resistance rates in the bacteria. Our study is in accordance with the study done by Abass & Omer 2011 [7] & Boyanova & Mitov 2013.[5]

Present study reported, resistance to Tetracycline by diabetic 16.7% & healthy 3.3% patients which is comparatively less than other antimicrobial drugs. However diabetic patients are more resistant than healthy groups. Both the diabetic & healthy group showed high percentage of sensitivity for tetracycline as 83.3% & 96.7% respectively. Our study is in accordance with the study done by Paul A. Mashimo et al (1983) [8] showed 93% diabetic patients (IDDM) were sensitive to tetracycline & most of the cultivable organisms were susceptible to tetracycline and its derivatives.

The popularity of tetracycline for treatment of non-dental infections has declined and has frequently been used for periodontal therapy. There is also evidence that tetracycline may cause alterations in bacterial cytoplasmic membrane, facilitating leakage of nucleotides and other compounds from the cell. This action would explain the rapid inhibition of DNA replication that occurs when cells are exposed to concentrations of tetracycline in excess of that needed for protein inhibition. Tetracycline are greatly effective in inhibition of gram-negative facultative anaerobes. Tetracycline administration may be an effective adjunct in treatment aimed at controlling periodontal breakdown in systemically healthy and diabetic adult periodontitis patients, because of Collagenase inhibition, Anti-proteolytic property & Inhibition of bone resorption. [9]

Present study showed resistance to Amoxicillin among diabetic & healthy chronic periodontitis patients as 66.7% & 46.7% & sensitivity as 33.3% & 53.3% respectively. Our study is in accordance with the Feres et al. [10] detected large transient increases in amoxicillin resistant subgingival populations secondary to systemic amoxicillin therapy in patients with chronic periodontitis, Van Winkelh off et al(2000) [6]study displayed a significant higher level of resistance towards amoxicillin while Rams TE (2013) [11] found one or more test species were resistant *in vitro* to, amoxicillin (43.3%) of the chronic periodontitis patients which elaborates beta-lactamase enzymes capable of hydrolyzing beta-lactam antibiotics such as amoxicillin.

Layton et al. have reported that Amoxicillin penetrates well into the gingival crevicular fluid, and both achieve and maintain levels inhibitory for the majority of the organisms cultivated from periodontal pockets. [12] Study done by Belibasakis and Thurnheer (2014) [13] showed that Amoxicillin alone caused a significant reduction in total bacterial numbers of the subgingival biofilm.

Amoxicillin in combination with clavulanic acid is indicated in the

presence of oral bacteria capable of producing beta-lactamase. [14]Our study shows resistance to Amoxicillin-Clavulanate among diabetic & healthy patients as 33.3% & 23.3% & sensitivity as 66.7% & 76.7% respectively. Our study is nearly in accordance with the Fosse *et al.*(1999)demonstrated susceptibility of Gram-negative bacilli such as *Prevotella*to amoxicillin when combined with clavulanic acid**. Aziz Japoni (2011) found Amoxiclav 100% active against P. gingivalis isolated subgingivally from chronic periodontitis patients. [15]**

Dentists are the most frequent prescribers of Metronidazole. However, the emergence of resistance to this drug may be slower than if it were used alone, because in order to target both aerobic and anaerobic organisms, Metronidazole is used empirically in combination with one or more antibiotics, although resistance to the drug may be associated with mobile genetic elements, aiding spread.

Present study reported resistance to Metronidazole in diabetic & healthy patients were 83.3% & 60.0% & 16.7% & 40.0% were sensitive accordingly. In our results statistically significant resistance (p-value 0.045) was seen among diabetic patients when compared to healthy patients. According to Madinier*et al*. (1999) who studied the susceptibility of *Aggregatibacter actinomycetemcomitans* to various antimicrobials. This bacterium is an important factor in intractable periodontal disease, often leading to early loss of permanent teeth. Out of 50 strains tested, 72% were resistant to metronidazole. [16]Al-Jebouri et al. (2014)[17] demonstrated that 100% of anaerobic periodontal pathogens isolates were resistant to Metronidazole.

The development of resistance to Metronidazole leading to treatment failure during the course of Metronidazole therapy in a patient has been reported (Buta*et al*., 2010; Soki*et al*., 2013) [18]&Ardila et al 2010.[19] Numerous studies have demonstrated the incidence of Metronidazole resistance in recent years (Alauzetet al2010[20];Lynch et al., 2013; Abdollahiet al., 2011). [18]

Metronidazole resistance is when present, it is most likely to be the result of a lack of reducing potential, leading to impairment of pro-drug activation.[9]Alternative mechanism is involved in the resistance of anaerobic strains such as reduced uptake of metronidazole, reduced nitroreductase activity or decreased pyruvate: ferredox in oxid oreductase activity.[18] Metronidazole resistance could be a consequence of the activation of nim gene as a result of point mutation.[21] High resistance observed for Metronidazole could be due to the frequent use of the antibiotic in the empiric treatment of diarrhoea. The use of Metronidazole for dental infections may also add to selection pressure. In addition antibiotics self-medication is encouraged by free access and over the counter purchase and by ineffective drug control policy.[19]

Present study reported resistance to Ciprofloxacin in diabetic & healthy group was 33.3% & 3.3% while 66.7% & 96.7 were sensitive to this drug among diabetic & healthy patients. This study showed more sensitivity to ciprofloxacin which is in accordance with the study done by Kannan I et al. 2014.[22]Van Winkelhoff (2005) in an in vitro study reported that F. nucleatum and P. gingivalis exhibited resistance to ciprofloxacin. [23]

Clinical isolates of A. actinomycetemcomitans are highly sensitive to Ciprofloxacin .This antibiotic effectively penetrates the diseased periodontal tissues and can reach higher concentrations in the crevicular fluid than in the serum.[24]Quinolones act on DNA gyrase, the enzyme responsible for unwinding and supercoiling of bacterial DNA prior to its replication. Quinolones thus inhibit bacterial replication and transcription. It also inhibits topoisomerase IV in gram-positive bacteria and thus interferes with the separation of replicated chromosomal DNA into the respective daughter cells during cell division.[9]

Our present study highlights sensitivity to Cefixime in diabetic & healthy patients as 63.3% & 58.0%. Resistance was shown 36.7% & 20.0% accordingly, which is in accordance with the study conducted by Yi Xie (2014) found that out of16.7% of the obligate anaerobic bacterial strains were resistant to cefixime that were isolated from a periodontal abscess.[25] Shill et al 2010 showed resistance for cefixime as 51.2% in diabetic patients.[26]

CONCLUSION

The results of the present investigation show that the widespread use of antibiotics has an impact on the periodontal microflora in untreated adult patients with periodontitis. Results of our study support that Tetracycline, Ciprofloxacin, Cefixime and Amoxiclav could be the antibiotics of choice for the diabetic patients. The high level of resistance of the periodontal bacteria towards a number of antibiotics, susceptibility testing for a number of relevant antibiotics should be considered, although in vitro susceptibility does not necessarily indicate in vivo clinical efficacy.

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