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Clinical appearance, microbiological findings and antimicrobials susceptibility pattern of orofacial infections of odontogenic origin in relation to cytokine analysis.

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ABSTRACT

The human oral cavity harbors a diverse consortium of microorganisms which has a complex relationship with host health and disease. Oral maxillofacial infections have the tendency to spread rapidly along facial planes and lead to highly morbid clinical conditions if left untreated with severe complications. These infections can range in their severity from those that either require only antibiotic therapy or aggressive surgical intervention. The victory of treatment depends upon the virulence of pathogen involved, the resistance of the host and strict observance to follow medical, pharmacological and surgical principles. In our results we found 78 samples with maxillofacial odontogenic infection fulfilling the inclusion and exclusion criteria and 50 samples were taken of control (healthy individuals). All the 78 patients presented with pain and swelling. Most common cause of odontogenic infection was dental caries i.e 62(79.4%), followed by gingivitis 44(56.4%), periodontitis 30(38.4%), periapical 5(6.4%) and pericoronitis 3(3.8%). The findings reports that the maxillofacial spaces frequently involved in infection are buccal space infection with 42(54%) in overall population. We found 40(51%) of *Staphylococcus aureus* isolates, 65(83%) of *Streptococcus mutans*, 23(29%) of *Steptococcus salivarius*, 30(38%) of *Streptococcus sanguis*, 21(27%) of *Streptococcus mitis*, 17(22%) of *Pseudomonas aeruginosa* and 14(18%) are of *Klebsiella pneumoniae*. The average sensitivity of antimicrobials against all isolated organisms was studied and it was found that common sensitive antimicrobials were clindamycin (88%), metronidazole (79%), cefotaxime (72%), linezoid (72%), erythromycin (72%), amoxclave (71%), ornidazole (67%), ciprofloxacin (67%), vancomycin (65%), imipenum (64%), cefadroxil (59%), ceftazidine (59%), azithromycin (58%), cefoperazone sulbactum (56%). The isolates which had shown significant values for TNF-α level and IL-10 were *Streptococcus mutans*, *Streptococcus salivarius*, *Streptococcus mitis*, *Staphylococcus aureus* and *Klebsiella*. Therefore we can conclude by our results that presence of these isolates was associated with odontogenic abscess and their increased pro-inflammatory level of TNF- α and decreased anti-inflammatory level of IL-10 indicates that the patients in our study suffered from severe systemic pro-inflammatory state in odontogenic infection.

Keywords: Maxillofacial, Odontogenic infection, antimicrobials, cytokines, orofacial infection

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INTRODUCTION

The oral cavity has various fundamental functions. Besides playing an important role in ingesting, speaking and breathing it is an entry from the external environment to the gastrointestinal tract and the human immune system. In health, oral microorganism and the host immune system are in ecological equilibrium, which is a premise for sustaining a barrier against ingested pathogens[1,5]. Odontogenic infections are among the most common infections of the oral cavity. They can be caused by dental caries, deep restorations, pulpitis, periapical abscess, periodontitis, periodontal abscess and pericoronitis.

Odontogenic abscess are polymicrobial infections comprising aerobic and anaerobic bacteria. Viridans group *Streptococci* are predominant species in pus samples, in addition *Staphylococci* are frequently isolated[5,2,7]. As the main focus of this study was towards microbiological findings, clinical parameters, cytokine profile, antimicrobial activity in

patients associated with odontogenic infection. In this context, maintaining good oral health remains the key approach to prevent odontogenic orofacial infection and other distant site infections.

The aim of the present study is to determine the anatomic and microbiologic considerations of odontogenic infections of maxilla and mandible, their clinical manifestations and discuss their response to medical as well as surgical treatment. It is interesting to demonstrate whether the odontogen would be able to induce the expression of inflammatory cytokines in patients which plays an important role in pathogenesis of infection.

MATERIAL AND METHODS

In this study the total population studied was 78 patients which were suffering from odontogenic infection (figure 1) and 50 samples were of control (healthy individuals). All patients and control population were examined and evaluated for the presence of associated pathogens along with clinical parameters and cytokines levels.

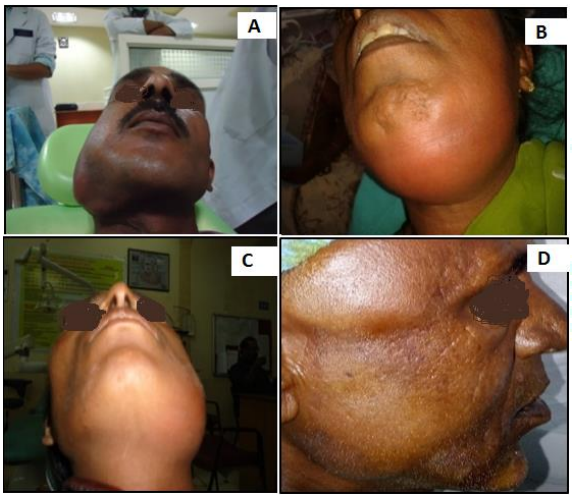

Figure 1: Patient's photographs

Study population

This study was conducted on patients who were screened from Maxillofacial Surgery Department, Peoples Dental Academy Bhopal. All patients presenting with oral infection with abscess in orofacial region was screened with proper case history, clinical symptoms and prior use of antimicrobials were recorded before specimen collection. The sepsis trial was explained to the patients and those agreed to be the part of study were signed consent form. Personal particulars of the patients, date of symptoms, vital signs, location of infection, treatment rendered with type of airway management was recorded. Medical history of each patient was taken and only those patients who appeared to be completely healthy except for the oral disease were included in the study. Exclusion criteria was pregnancy, lactation, antibiotic therapy in 1 week, use of mouth rinse containing antimicrobials in preceding 1 week, immunological disorders, diabetes, subjects on long term use of anti-inflammatory and immunosuppressive medications were also excluded.

Specimen collection and handling

For collection of specimens, swabs, syringes and container were used. Sample was collected before antimicrobial therapy has been administered. Collection of the pus sample from the patient presenting with single and multiple space abscess was done. Sample collection was done preferentially by closed aspiration using an 18 gauge needle and 10ml disposable syringe (figure 2,3). After careful examination the site of aspiration was chosen and cleansed with isopropyl alcohol. Intra-oral site was prepared using 0.2% chlorhexidine mouth rinse. Subsequently sterile dry gauze was used to wipe the area clean. Maximum sample was aspirated in a single attempt to avoid contamination of the aspirate. In cases where significant aspirate was not available, sterile culture swabs were introduced into the wound after incision and drainage (figure 4). Immediately upon aspiration residual air was evacuated from the syringe and the needle was capped with a sterile rubber cork. The sample was then transported to laboratory avoiding any delay.

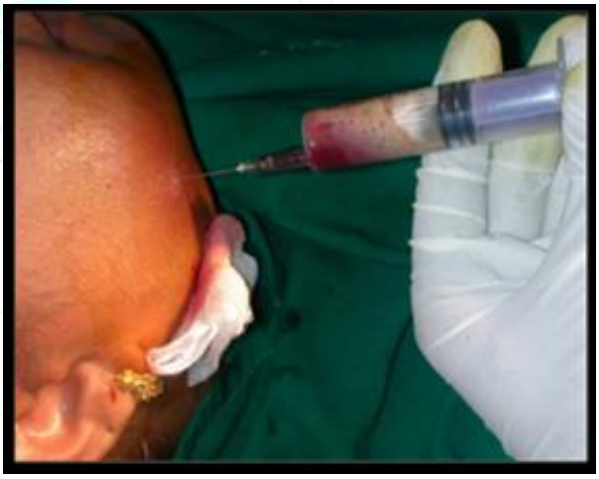

Figure 2: Aspiration of pus

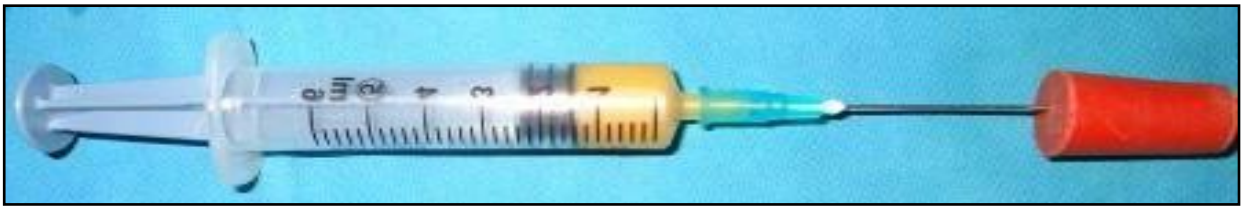

Figure 3: Aspirated pus sample

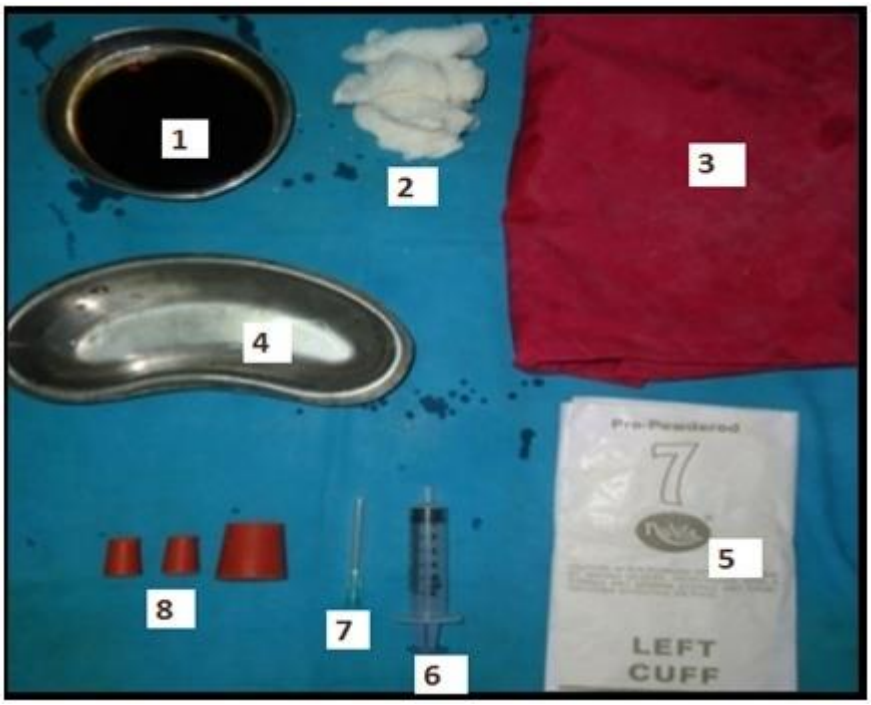

Figure 4: Instruments used for specimen collection

1. Betadine solution, 2. Sterile gauge, 3. Drape, 4. Sterile saline, 5. Gloves, 6. Syringe, 7. 18 gauge needle, 8. Rubber cork

Specimen culture

The inoculated samples were further studied on basis of culture characteristic[4]. Pus samples smear were observed by gram staining and further inoculated on blood agar, MacConkey agar and in Peptone water/Nutrient broth. All culture media used in the study were prepared by reconstituting the commercially available dehydrated media from Himedia, India. Mueller Hinton agar was employed to study the isolates for antimicrobial sensitivity assay. Interpretations were carried out based on the diameter of zone of inhibition as sensitive, moderate sensitive or resistant using manual provided by Himedia Pvt. Ltd., India. Antibiotics used were Amoxicillin-Clavulanic acid, Ampicillin, Ampicillin-Sulbactam, Cefadroxil, Clarithromycin, Clindamycin, Linezoid, Norfloxacin, Azithromycin, Vancomycin, Amikacin, Cefoparazone-Sulbactam, Cefotaxime, Ceftriaxone, Ceftazidine, Ciprofloxacin, Erythromycin, Gentamycin, Imipenen, Levofloxacin, Penicillin G, Metronidazole, Ornidazole and Ofloxacin.

Cytokine TNF-α and IL-10 analysis

After clinical and microbiological examinations, peripheral blood samples were taken from each of the subjects for cytokines analysis. 5ml sample whole clotted blood was collected and sent to the laboratory where serum will routinely separate from blood samples by spinning in a centrifuge (Hettich; universal 16A, Tuttlingen, Germany) at a speed of 5000 rpm. The tests were performed on separated serum and cytokine levels were measured. The quantity/levels of TNF-α and IL-10 in the serum of patients were measured.

Statistical analysis of data

Immunology from serum samples, microbiology and antimicrobial sensitivity test results from pus samples were compared between the two groups using two sample t-tests. P-values of < equal to 0.05% was considered significant.

RESULTS AND DISCUSSION

In our study we screened and included 78 patients with odontogenic infection which were fulfilling all inclusion and exclusion criteria's as well as 50 samples were taken as control (healthy individual). Signs and symptoms of study population revealed that all 78 (100%) patients were suffered with swelling and pain (table 1), 29(37.1%) patients were tender, 16(20.5%) were having fever and limitation to open mouth, 10(12.8%) were found to have difficulty in chewing, 04(5.1%) suffered from difficulty in speaking, in 3(3.8%) ulcers are found, 39(50%) and 44(56.4%) have found stain and calculus respectively. The typical signs and symptoms of maxillofacial odontogenic infection have remained unchanged over the course of time.

Mostly it was found that the odontogenic infections arise as a sequel to pulp necrosis caused by caries, periodontal infections, gingivitis, pericoronitis, trauma and surgery are other sources responsible for orofacial infections [4,6,7]. In our study also it was observed that the main origin/cause of infection (table 2) was dental caries i.e 62(79.4%), followed by gingivitis 44(56.4%), periodontitis 30(38.4%), periapical 5(6.4%) and pericoronitis 3(3.8%).

Table 1. Patient's sign and symptoms

S.No	Patient signs and symptoms	No. of Patients (%)	Control Group
1	Swelling	78 (100%)	00
2	Pain	78 (100%)	00
3	Tender	29 (37.1%)	00
4	Fever	16 (20.5%)	00
5	Limitation to open the mouth	16 (20.5%)	00
6	Difficulty in chewing	10 (12.8%)	00
7	Difficulty in speaking	04 (5.1%)	00
8	Ulcer	03 (3.8%)	00
9	Stain	39 (50%)	00
10	Calculus	44 (56.4%)	00

Table 2. Origin/Cause of infection

S.No	Origin of infection	No. of patients (%)	Control Group
1	Periodontal	30(38.4%)	00
2	Pericoronitis	03(03.8%)	00
3	Periapical	05(06.4%)	00
4	Dental caries	62(79.4%)	00
5	Gingivitis	44(56.4%)	00

In our study the findings reports that the maxillofacial spaces which are frequently involved in infection are buccal space infection with 42(54%) in overall studied population followed by 30(39%) of submandibular space infection, 09(12%) of infra-orbital space infection, 08(10%) were found of submental space, 07(09%) were of canine space infection, 03(04%) of sublingual, submasseteric and palatal space infection, 02(03%) of pterygomandibular, temporal and parapharyngeal space infection and 01(01%) were of pretracheal and dentoalveolar space infection (table 3).

Table 3. Maxillofacial spaces involved in odontogenic infection

S.N	Spaces involved	No. of cases (%)	Control Group
1	Submandibular	30 (39%)	00
2	Buccal	42 (54%)	00
3	Submental	08 (10%)	00
4	Sublingual	03 (04%)	00
5	Submasseteric	03 (04%)	00
6	Canine	07 (09%)	00
7	Pterygomandibular	02 (03%)	00
8	Temporal	02 (03%)	00
9	Parapharyngeal	02 (03%)	00
10	Palatal space	03 (04%)	00
11	Infra-orbital space	09 (12%)	00
12	Pretracheal	01(01%)	00
	Total	112	

In our study it was observed that in total 78 patients (table 4) there were 40(51%) of *Staphylococcus aureus* isolates, 65(83%) of *Streptococcus mutans*,23(29%) of *Streptococcus salivarius*,30(38%) of *Streptococcus sanguis*,21(27%) of *streptococcus mitis*,17(22%) of *Pseudomonas aeruginosa* and 14(18%) are of *Klebsiella pneumoniae*. Total isolates in patients were 210 in which multiple isolates were found in single patient.

Table 4. Isolates from patients (n=78)

S No.	Isolates	No. of isolates (%)	Control (%)
1	*Staph. aureus*	40 (51%)	12
2	*Strep. mutans*	65 (83%)	28
3	*Strep. salivarius*	23 (29%)	25
4	*Strep. sanguis*	30 (38%)	07
5	*Strep mitis*	21 (27%)	15
6	*P aeruginosa*	17 (22%)	04
7	*K pneumonia*	14 (18%)	02
	Total Isolates	210	93

The average activity of implied antimicrobials on each isolates was measured. Total 27 antimicrobials were tested in present study (figure 5) in which sensitive antimicrobials were clindamycin (88%), metronidazole (79%), cefotaxime (72%), linezoid (72%), erythromycin (72%), amoxclave (71%), ornidazole (67%), ciprofloxacin (67%), vancomycin (65%), imipenum (64%), cefadroxil (59%), ceftazidine (59%), azithromycin (58%), cefoperazone sulbactam (56%). Evaluation and comparison of cytokines (TNF-α and IL-10) was done between patients and control group in which highly significant results were obtained (table 5). In patient group the level of TNF-α (figure 6) was higher than in control group that showed the presence of high level of inflammatory cytokines in patients whereas IL-10 (figure 7) level in patients was found lower. This result indicates that the severity of disease/infection is higher in abscess patient which may leads to systemic diseases and should be seriously treated and managed.

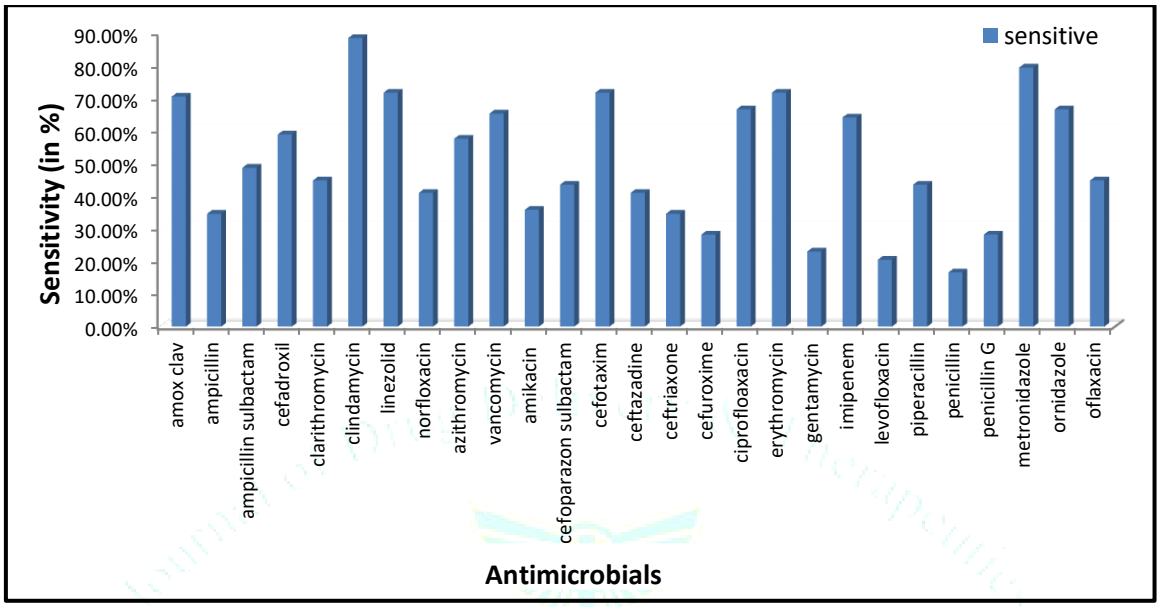

Figure 5: Antimicrobials profile (average) of all isolates

Table 5. Comparisons of cytokines (TNF- &IL-10) in patients and control group

S.N.	Cytokines	Cases No.	Mean±SD (pg/ml)	t-Test	p-Test	Significance
1	TNF-α (CASE)	78	0.1239±.03764	41.953	0.001	HS
2	TNF-α (CONTROL)	50	0.1207±.03397			
3	IL-10 (CASE)	78	0.1185±.02083	43.11	0.0001	HS
4	IL-10 (CONTROL)	50	0.4540±.03377			

HS- Highly significant

TNF-α and IL-10 was found highly significant

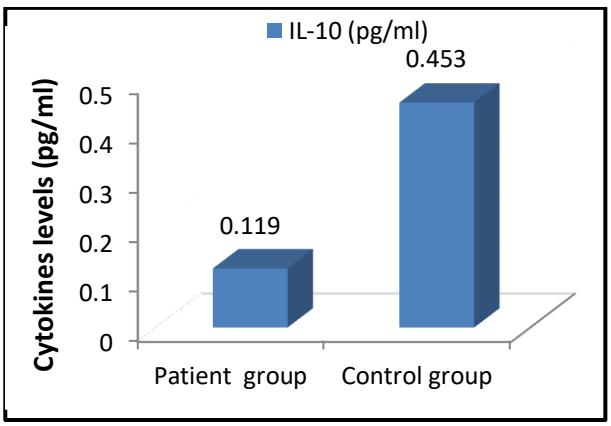

Figure 6: Comparison of TNF- α

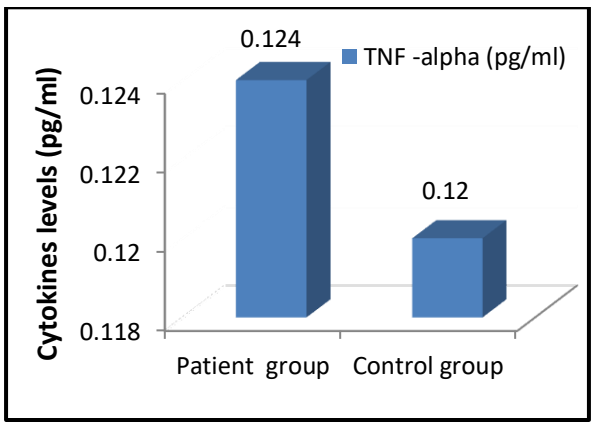

Figure 7: Comparison of IL-10

CONCLUSION

The severity of odontogenic infection demands swift detection of the infection followed by prompt and more aggressive treatment. Thus, failing to identify and treat these infections may result in disastrous outcomes. The purpose of this study was to compare the causative agents and the host response which contributes to understand the factors involved in the development of odontogenic abscess in oro-facial infection, about the pathogens with their susceptible antimicrobials to treat them.

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