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Microbial etiology of chronic sinusitis

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Abstract:
Back ground and Objectives*: The purpose of this study is to analyze the microbial flora of paranasal sinuses of the patients with chronic sinusitis undergoing functional endoscopic sinus surgery.*
Materials & methods: *A prospective study done at Government ENT hospital, Koti, over a period of 6months. A total of 125 patients were included in the study those were clinically diagnosed to have chronic sinusitis and confirmed by CT-imaging of paranasal sinuses. Intra-operative sinus aspirates were collected during FESS and they were subjected to bacterial and fungal culture. Bacterial pathogens were isolated and identified according to the standard protocol. AST was performed on all bacterial isolates. Fungal cultures were identified by their rate of growth, colony morphology and microscopic characteristics in LCB mount and slide culture.*
Results: *over all 125patients were studied. The common presenting complaint was nasal discharge. Common associated factor was deviated nasal septum (51.2%), followed by nasal polyposis(32%). The most common sinus affected was maxillary sinus (69.6%). Culture positivity was seen in 115(92%) patients. Majority of aspirates (91%) yielded single organism. Bacterial isolates were 87.7% among them staphylococcus aureus (43.92) was common pathogens followed by CONS (24.29), E.coli (7.47%), Klebsiella species (11.21%), Pseudomonas aeruginosa (8.41%). Fungi isolated were 15(12.29%). Aspergillus flavus is the common fungus. The most sensitive antimicrobial agent against Gram positive organisms were Ciprofloxacin & Vancomycin, for Gram negative organism were Cefparezone + sulbactum & imipenem.*
Conclusion: *Chronic sinusitis is predominantly caused by bacteria.*
Key words: *antibiotic sensitivity; aspergillus flavus, Chronic sinusitis; Functional Endoscopic Sinus Surgery; Staphylococcus aureus.*

I. Introduction:

Sinusitis can be defined as any inflammation of the paranasal sinus mucosa. (1) Chronic paranasal sinusitis (chronic rhinosinusitis) is infection of the sinuses lasting for more than 3 months. (2) It is generally a mild disease. However it is important to realize that it afflicts a significant percentage of population and causes considerable long term morbidity. (3) It seems that almost 15% of population suffers from chronic paranasal sinusitis. (5) Microorganism play major role in the causation of chronic sinusitis. So antimicrobial agents are primary line of therapy. (7) But it is empirical not based on isolation and sensitivity of microorganism, which inturn leading to increasing incidence of resistance in many organism and making the management of these infections more complex. (8) Detailed microbiological data is essential in guiding the treatment of chronic sinusitis. (9)

The purpose of this study was to isolate and identify the causative organism of chronic sinusitis along with antibiotic suseptibility testing of of bacterial isolates in patients undergoing functional endoscopic sinus surgery.

II. Aims and objectives:

To conduct a prospective study to determine the spectrum of various microorganisms in chronic sinusitis and to determine sensitivity patterns of bacteria isolated, at Government E.N.T hospital, Koti, Hyderabad over a period of 6 months.

III. Materials and methods:

The present study was under taken on 125 patients who were undergoing Functional endoscopic sinus surgery for chronic sinusitis at government E.N.T hospital, Koti, Hyderabad over a period of 6 months from April 2013 to November 2013.

Selection of patients:

Patient inclusion criteria of chronic sinusitis were defined by Rhinosinusistis Task Force. (9) (Any of 2 major factors (post nasal drip, rhinorrhea, facial pain, nasal blockage, and nasal cavity purulence) or 1 major and 2 minor factor (halitosis, earache, headache, tooth pain, cough, fatigue, low grade fever) with confirmatory findings by CT scan or DNE examination. Patients of all ages and both sex were enrolled.

Patient with complaints less than 12 weeks and Patient undergoing Functional endoscopic sinus surgery for other causes like neoplasm were excluded from the study.

The specimens were collected intra- operatively during functional endoscopic sinus surgery which is usually done under local or general anaesthesia depending on the patient need.

specimen collection:

The procedure begins with decongestion of the nose and infiltration of lidocaine with epinephrine (1% lidocaine with 1 in 100,000 epinephrine is used for injection).

The lateral nasal wall near the uncinate process is injected. Using a 3-mL syringe while placing a slight bend to the 27-gauge needle facilitates the injection.

Next, the superior inlet and the anterior face of the middle turbinate are injected submucosally.

Then the middle turbinate is gently medialized, carefully using the curved portion of the freer elevator. Next uncinectomy is performed via an incision with either the sharp end of the freer elevator or a sickle knife.

Once the uncinate process was taken down, the true natural ostium of the maxillary sinus should be identified, and it was widened. Secretions from sinus were aspirated through the maxillary ostium with the help of a bent cannula which is connected to sterile syringe. (14)

Bacterial culture:

Specimen was inoculated on one chocolate agar, one blood agar and one Mac Conkey agar. The chocolate agar plates were incubated at 37C for 24 – 48hr in 5-10% co2 in a co2 incubator. The blood agar and Mac Conkey agar plates were incubated at 37c for 24hrs in bacteriological incubator.

The plates are checked for growth and colony morphology. Gram's staining was done to visualize the morphology of bacterium, hanging drop for motility. Subculture was made into nutrient broth for Gram positive cocci and peptone water for Gram negative bacteria. Bacteria are further processed and identified by standard methods.

Antibiotic sensitivity testing was performed on all bacterial isolates using Kirby Bauer disc diffusion method on Muller Hinton agar using Hi Media antibiotic disc.

Drugs used for Gram positive organisms

Penicillin, Augmentin (30mcg), Cefoxitine (30mcg), Gentamycin (10mcg), Ciprofloxacin (5mcg), Azythromycin, Clindamycin (10mcg)

Vancomycin (30mcg)

Drugs used for Gram negative organism cefuroxime (30mcg)**,** Ceftazidime (30mcg)**,** Piperacillin (100mcg)**,** Gentamicin (10mcg)**,** Ciprofloxacin (5mcg)**,** Cotrimoxazole **,** Cefparazone + sulbactum (75+30 mcg)**,** Imipenem (10mcg)

Bacterial species isolated are identified by morphology, culture characteristics and biochemical reactions according to standard techniques.

Fungal culture:

The specimen from sterile container was inoculated on Sabouraud dextrose agar with chloroamphenicol, incubated at 28C in a BOD incubator and examined for 7 to 10days.

On SDA agar if growth is present depending upon Colony morphology, Rate of growth, Texture, Obverse characteristics, Reverse pigment.

The colony is teased on a clean glass slide with a drop of LCB, cover slip is placed over it, and then the preparation is examined under low power & high power objectives. Depending upon colony morphology and microscopic appearance the isolate is identified. (61, 62)

IV. Results:

Total number of 125 samples were collected intraoperatively among them 72(57.6%) were males 53(42.4%) were females showing male preponderance. The age distribution of cases were 11-20yr age group 27(21.6%) cases, 21-30yr age group 43(34.4%) cases, 31-40yrage group 39 (31.2%), 41-50yr age group 13(10.4%), 51-60yr age group 3(2.4%). Maximum prevalence of disease is seen between 21- 30 yrs (34.4%) fallowed by 31-40 yrs (31.2%).

Distribution of symptoms among the cases were Nasal discharge 119(95.2%),Nasal blockage 118(94.4%), Facial pain 70(56%), Post nasaldrip 63(50.4%), Anosmia/hyposmia 8(6.4%). Distribution of associated factors seen in CT scan among the cases were Deviated nasal septum was present in 64(51.2%), Nasal polyposis in 40(32.0%), Choncabullosa in 10(8%) and none in 11(8.8%). Among 125 patients maxillary sinus was effected in 87(69.6%), Frontal sinus effected in 8(6.4%), Ethmoidal sinus effected in 6(4.8%),

Sphenoidal sinus effected in 5(4%) and pansinusitis seen in 19(15.2%). The most common sinus affected was maxillary sinus (69.6%).

Out of 125 cases culture was positive in 115(92%) patients, polymicrobial growth was seen in 10(8%) patients, and monomicrobial growth was seen in 105 (84%) patients. Among the 115 specimen 122 organism were isolated. Out of 122 isolates bacterial isolates were 107(87.70%) and fungal isolates were 15 (12.29).

Among the bacterial isolates the commonest organism isolated was Staphylococcus aureus (43.92%) followed by CONS (24.49%) among Gram positive organism. Klebsiella species (11.21%) were common among Gram negative organism. (Table no :1) among the 15 fungal isolates Aspergillus flavus (6.4) is predominant (table no 2).

Table no 1: Distribution of bacterial isolates:

Organism	No of isolates	percentage
Staphylococcus aureus	47	43.92%
Coagulase negative staphylococcus	26	24.29%
Streptococcus pyogenes	3	2.80%
Klebsiella pneumoniae	10	9.34%
Klebsiella ozenae	2	1.87%
Escherichia coli	8	7.47%
Pseudomonas aeruginosa	9	8.41%
Citrobacter freundii	2	1.87%
Total	107	100%

TABLE No 2: Distribution of Fungal isolates:

Fungus isolated	No of isolates	Percentage
Aspergillus flavus	8	53.33%
Aspergillus fumigatus	4	26.66%
Aspergillus niger	1	6.66%
Rhizopus	1	6.66%
Chladophialophora	1	6.66%
Total	15	100%

The antibiotic suseptibility pattern of Gram positive organism showed more suseptibility to ciprofloxacin (90%) and clindamycin (90%) and all isolates are showing 100% sensitivity to vancomycin.

TABLE No 3: AST pattern in Gram positive organism

Antibiotic	Staphylococcus aureus		Coagulase negative staphylococcus		Streptococcus pyogenes	
	S%	R%	S%	R%	S%	R%
Penicillin	12.12	87.87	11.53	88.46	100	0
Augmentin	51.51	48.48	50	50	100	0
Cefoxitine	75.75	24.24	73.07	26.92	100	0
Azythromycin	60.00	39.39	42.30	57.69	33.3	66.6
Gentamicin	63.33	36.36	50	50	100	0
Ciprofloxacin	90.00	9.09	76.92	23.07	66.6	33.3
Clindamycin	90.00	9.09	84.61	15.38	100	0

TABLE No 4: AST pattern of Gram negative organism

Antimicrobial agent	Klebsiella spp		E.coli		Citrobacter spp		Pseudomonas aeruginosa	
	S	R	S	R	S	R	S	R
Cotrimoxazole	25	75	50	50	0	100	0	100
Cefuroxime	8.33	91.6	37.5	62.5	0	100	0	100
Ceftazidime	33.3	66.6	75	25	50	50	66.6	33.3
piparacillin	16.6	83.3	37.5	63.5	0	100	33.3	66.6
Cefaperazone+ sulbactam	75	25	87.5	12.5	100	0	66.6	33.3
ciprofloxacin	75	25	75	25	50	50	50	50

gentamicin	16.6	83.3	37.5	63.5	0	100	11.1	88.8
Imipenem	100	0	100	0	100	0	100	0

V. Discussion

Sinusitis is a disease faced by otorhinologists frequently. A large number of patients with chronic sinusitis are treated with out proper investigation and specialist opinion. Which will leads to persistence of symptoms under these circumstances further investigations may be required in guiding the direction of management. (3)

Role of bacterial infection is important in the pathogenesis of chronic sinusitis. Recent advances in endoscopic sinus surgery have helped to understand the microbiology of sinusitis without causing much discomfort patients as in antral puncture. This will also help to use proper antimicrobial agent based on the culture report. (38)

The present study was undertaken to describe the spectrum of microbial organism in causation of chronic sinusitis, antibiotic susceptibility patterns of bacterial isolates and associated factors in chronic sinusitis.

Out of 125 clinically diagnosed & radiologically (CT- imaging) confirmed cases of chronic sinusitis were included in the study. This study revealed male preponderance over female 72(57.6%) were males 53(42.4%) was females. These findings correlate with study done by others. D. Shrestha et al (2011) reported 56% in males 44% in females. Anuj Kumar Goel et al (2012) reported male preponderance and states that male are often exposed to inclemencies of the environment hence are more affected.

The highest incidence of disease was observed in 21-40yrs (65%). These findings correlate with the work published by others. J. K. Kamau et al (2001) reported an increased incidence of chronic sinusitis 20-40yrs f age group (55%). M. Hashemi et al (2005) reported an increased incidence of chronic sinusitis in 21-40yr 54%. D. Shresta et al (2011) reported highest incidence in 21-40yrs that is 68%.

The most common symptom found in this study is nasal discharge (95.2%) followed by nasal blockage (94.4%). Others are Facial pain (56%), Post nasal drip (50.4%), Anosmia/ hyposmia (6.4%). These finding correlates with D. Shrestha et al (2011) who reported nasal discharge (96%) as common finding followed by nasal blockage (88%).

Deviated nasal septum is present in 64(51.2%) patients. D. Shrestha et al (2011) reported DNS in 28% of chronic sinusitis patients. Sanam Jindal et al (2013) reported DNS in 87% of CRS patients.

Nasal polyposis is seen in 40(32%) patients. This study correlates with Anuj Kumar Goel et al (2012) who reported (32.5%). Maxillary sinus affected in 87 (69.9%) patients. This study correlates with Anuj Kumar Goel et al (2012) who reported maxillary sinus affliction in 70% patients.

In this study 115(92%) were culture positive. This study correlates with others. Katriina Kostamo et al (2004) reported 93%, M. Hashemi et al (2005) reported 91.4%, Sanam Jindal et al (2013) reported 93.3%. In this study mono-microbial growth was observed in 105 (92%), poly microbial growth was observed in 10 (9%) patients. These findings correlate with others. D Shresta Et al (2011) reported mono-microbial growth in 70% and polymicrobial growth in 10%.

In this study bacterial isolates are predominant. The over all incidence of bacterial isolates was 87.70%. Of the isolates, Gram positive cocci accounted for 71.01%, the most common pathogen being Staphylococcus areus (43.92%%), followed by Coagulase negative Staphylococcus (24.29%). This correlated well with Katriina Kostamo et al (2004) reported Staphylococcus aureus (33%) most common isolate followed by Coagulase negative Staphylococcus (23%). Elisabeth Araujo et al (2007) reported Staphylococcus aureus (31%) and CONS (23%) as most common isolates. The other studies reported Staphylococcus aureus and Coagulase negative Staphylococcus as predominant isolates were P.W. Doyle et al (1991), Boo-Hwan Jee et al (1999), Ologe et al (2003), M. Hashemi et al (2004), Nicole Perez Blanc et al (2010), C.W.D. Chin et al (2010), D.Shrestha et al (2011), Sanam Jindal et al (2013).

Among the Gram negative aerobic organism Klebsiella species (11.21%) is predominant pathogen. This finding correlates with Ologe et al (2003) reported (20%), Katriina Kostamo et al (2004) reported (14%), M. Hashemi et al (2005) (10%). Among Klebsiella isolates 2 Klebsiella ozaenae were isolated. P.W Doyle et al (1991) reported Klebsiella ozaenae in his study. The percentage of Pseudomonas aeruginosa was 8.41%. This finding correlates well with D.Shrestha et al(2011) reported Pseudomonas aeruginosa in 8% of isolates. Other Gram negative organisms in this study are Escherichia coli (7.47%), Citrobacter freundii (1.87%). These findings correlate with Boo-Hwan Jee et al (1999), Ologe et al (2003), and M. Hashemi et al (2005). Katriina Kostamo et al (2004) reported 7% of Escherichia coli among the isolates.

All Gram positive cocci are sensitive to vancomycin (100%), followed by ciprofloxacin (90%), Clindamycin(90%), gentamicin (60%). These findings of sensitivity pattern are comparable with Boo-Hwan Jee et al (1999) and M. Hashemi et al (2005). Both reported 100% sensitivity to Vancomycin. The incidence of MRSA in present study is 24%. This finding correlates with Sanam Jindal et al (2013).

All Gram negative organisms were sensitive to Imipenem (100%). Klebsiella species isolates were sensitive to) Cefaperazone+ sulbactam(75%), ciprofloxacin(75%. Escherichia coli isolates were sensitive to Cefaperazone+ sulbactam(87.5%), ciprofloxacin (75%), ceftazidime(75%). Citrobacter freundii isolates were most sensitive to Cefaperazone+ sulbactam. Pseudomonas aeruginosa isolates were sensitive to Cefaperazone+ sulbactam(66.6%), ceftazidime(66.6%). These findings correlate with Boo-Hwan Jee et al (1999) and M. Hashemi et al (2005).

In the present study incidence of fungal isolation is 12.29%.The number of fungal isolates are 15. The predominant fungus isolated was Aspergillus flavus, 53.3% among the fungal isolates. Katriina Kostamo et al (2004) reported 20% incidence the only fungus isolated was Aspergillus species. C. W. D. Chin et al (2010) reported fungi were isolated in 8% of patients. Sanam Jindal et al (2013) reported fungal isolation in 10.7% cases. Rajiv C.Michael et al (2008) reported Aspergillus flavus as common fungal isolates. Itzhak Brook et al (2011) reported Aspergillus species as common fungal agent in chronic sinusitis.

VI. Conclusion

In the present study 125 clinically diagnosed, radiologically confirmed cases of chronic sinusitis patients were included. Intra-operative sinus secretions were obtained during Functional endoscopic sinus surgery. Commonest symptom was nasal discharge (95.2%). The commonest finding was deviated nasal septum. Maxillary sinus was affected in 69.6% of cases

Bacteria were isolated in 115 out of 125 chronic sinusitis patients who were refractory to medical treatments and treated with Endoscopic sinus surgery. Mixed bacteria isolates of different species were observed in few cases. The most commonly observed organism were bacteria. Among the antibiotics included in the study cefperazone+ sulbactum, ciprofloxacin were higher sensitives for both Gram positive and Gram negative organism. A total number of 15(12.29%) were isolated. Among them Aspergillus flavus was common.

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Streptococcus pyogenes	3	2.80%
Klebsiella pneumoniae	10	9.34%
Klebsiella ozenae	2	1.87%
Escherichia coli	8	7.47%
Pseudomonas aeruginosa	9	8.41%
Citrobacter freundii	2	1.87%
Total	107	100%

TABLE No 2: Distribution of Fungal isolates:

Fungus isolated	No of isolates	Percentage
Aspergillus flavus	8	53.33%
Aspergillus fumigatus	4	26.66%
Aspergillus niger	1	6.66%
Rhizopus	1	6.66%
Chladophialophora	1	6.66%
Total	15	100%

TABLE No 3: AST pattern in Gram positive organism

Antibiotic	Staphylococcus aureus		Coagulase negative staphylococcus		Streptococcus pyogenes	
	S%	R%	S%	R%	S%	R%
Penicillin	12.12	87.87	11.53	88.46	100	0
Augmentin	51.51	48.48	50	50	100	0
Cefoxitine	75.75	24.24	73.07	26.92	100	0
Azythromycin	60.00	39.39	42.30	57.69	33.3	66.6
Gentamicin	63.33	36.36	50	50	100	0
Ciprofloxacin	90.00	9.09	76.92	23.07	66.6	33.3
Clindamycin	90.00	9.09	84.61	15.38	100	0

TABLE No 4: AST pattern of Gram negative organism

Antimicrobial agent	Klebsiella spp		E.coli		Citrobacter spp		Pseudomonas aeruginosa	
	S	R	S	R	S	R	S	R
Cotrimoxazole	25	75	50	50	0	100	0	100
Cefuroxime	8.33	91.6	37.5	62.5	0	100	0	100
Ceftazidime	33.3	66.6	75	25	50	50	66.6	33.3
piparacillin	16.6	83.3	37.5	63.5	0	100	33.3	66.6
Cefaperazone+ sulbactam	75	25	87.5	12.5	100	0	66.6	33.3
ciprofloxacin	75	25	75	25	50	50	50	50
gentamicin	16.6	83.3	37.5	63.5	0	100	11.1	88.8
Imipenem	100	0	100	0	100	0	100	0