Original article

Clinico-pathological study of 273 cases of rhinosporidiosis over a period of ten years in a tertiary care institute catering predominantly rural population of tribal origin

Sengupta S[1], Pal S[2], Biswas BK[3], Jana S[4], Biswas S[5], Minz RS[6]

Abstract:

Introduction: Rhinosporidiosis is a chronic granulomatous infection caused by Rhinosporidium seeberi, an organism whose taxonomy is still debated. The present study was aimed to document the clinico-pathological presentation of rhinosporidiosis in different parts in reference to caste, age and gender. Evaluation of diagnostic role of cytology in the diagnosis of rhinosporidiosis was also explored. ***Materials and Methods:*** All histology confirmed rhinosporidial cases were included in the study. Detailed clinical history and examination findings including previous hematological and cytological reports, if available, were collected and tabulated. Periodic Acid Schiff (PAS) and Mucicarmine stains were used over cyto- and histological slides, if necessary. ***Observations:*** Male cases were more frequent in these series though this sex difference is less pronounced among tribal population. Majority of the cases belonged to 21-40 years age group. Nose and nasopharynx was the commonest site of infection and polypoid mass lesions were the commonest presentation. Both scrape and aspiration cytology could successfully detect rhinosporidiosis in 100% cases. ***Discussion:*** Most of the cases are among poor-socioeconomic status and probably out-door activities and pond bathing habit. Haematological data correlation did not revealed any significant association. Histology is the preferred method for confirmed diagnosis of rhinosporidiosis. Rare cases of misdiagnosis can be avoided by use of special stains. ***Conclusion:*** Rhinosporidiosis commonly presents as polypoidal lesions in nose and extra-nasal sites. Histopathology is the standard method for confirmation of diagnosis. Cytology can be used as an adjunct for pre-operative diagnosis of extra-nasal rhinosporidiosis. We recommended use of special stains for diagnosis of difficult cases.

Keywords: rhinosporidiosis; nasal and extra-nasal; cytology; haematological profile; rural and tribal population; clinico-pathological study

DOI: http://dx.doi.org/10.3329/bjms.v14i2.16151
Bangladesh Journal of Medical Science Vol.14(2) 2015 p.159-164

Introduction

Rhinosporidiosis is a chronic granulomatous infective disorder producing polypoidal, pedunculated, soft tissue mass. More than 70% cases involve nose and nasopharynx. Ocular lesions, particularly of conjunctiva and lachrymal sac, account for 15% cases. Rhinosporidial polyps are also reported from different rare sites like lips, palate, uvula, maxillary antrum, epiglottis, larynx, trachea, bronchus, ear, scalp, vulva, penis, rectum or skin. Bone involvement was also documented. Disseminating infections with simultaneous involvement of limbs, trunk and internal organs are rarely encountered. Disseminated rhinosporidiosis of limbs may result in destruction of limb bones. Brain lesions, though uncommonly occur even during systemic spread of disease, are usually associated with high rate of fatality. Spontaneous regression of nasal polypoidal lesions

1. Sengupta Sanjay, Associate Professor, Dept of Pathology, B S Medical College.

2. Pal Subrata, Resident, Dept of Pathology, B S Medical College.

3. Biswas Biplab Kr, Associate Professor, Dept of Pathology, B S Medical College.

4. Jana Sritanu, Assistant professor, Dept of Pathology, B S Medical College.

5. Biswas Sudhanya, Post Graduate trainee, Dept of Pathology, B S Medical College.

6. Minz R S, Demonstrator, Dept of Pathology, B S Medical College.

Department and institution: Dept of Pathology, Bankura Sammilani Medical College.

Corresponds to: Dr Subrata Pal, Resident, Dept of Pathology, B S Medical College. Gobindanagar, PO-Kenduadihi, Bankura, West Bengal. PIN- 722102, E-mail address: subratapal1985@gmail.com.

are infrequently reported during study of natural progression of rhinosporidiosis[1].

Rhinosporidiosis was first reported by Guillermo Seeber as early as 1900 from the new world 'Buenos Aires of Argentina'. It presented as a nasal polyps and Seeber correctly assumed the infective etiology of the lesion. He concluded that the invading organism responsible for this chronic granulomatous infection producing polypoidal mass lesion was a fungus. In 1923, the responsible organism, still presumed to be a fungus, was isolated by Asworth. He also described the life cycle of the pathogen and established the nomenclature Rhinosporidium seeberi[2,3]. Later on, rhinosporidiosis was reported from both the new and old world. But 90% of the total case found to be present in Indian Subcontinent. Human beings are not the only definitive host for the organism. A wild range of domestic and wild animals including cows, buffaloes, dogs, cats, horses, mules, ducks and swans found to be affected[4].

Bankura Sammilani Medical College is situated in Bankura district of Pachimbanga, erstwhile West Bengal, India. Being the tertiary care institute of an economically backward district, this hospital serves predominantly rural population. There is a good proportion of tribal population (10.36%) among the residents of Bankura district. Of these only a small fraction (0.76 of total urban population) lives in a 3 urban municipalities of Bankura District. Majority of tribal people are villagers and their percentage in different blocks of Bankura varies from as high as 47.28% (Ranibandh) to only 1.90% (Indus)[5]. So, a major portion of patients coming to Bankura Sammilani Medical College are rural people of tribal origin. As we have studied our cases with rhinosporidial lesions involving different parts of the body for a prolonged period in BS Medical College, typical distribution of cast pattern at the locality is expected to be reflected. However, we conducted the study to explore the clinical and haematological profiles of the cases in tribal and non-tribal populations and assess the role of cytology for diagnosis in available cases before final histological confirmation.

Materials and Methods

Present study was conducted in the Department Pathology, Bankura Sammilani Medical College, Bankura, West Bengal, for a period of April 2003 to March 2013. All histologically diagnosed cases of rhinosporidiosis during the period were included in this study.

Detail clinical data were collected in each case from the available records or after examination of the patients, if possible. Data regarding routine hematogical examination and ABO blood grouping were also recorded.

Cases reported to the laboratory for assessment of polypoid lesions prior to surgery underwent fine needle aspiration using 23-gauge needle. If rhinosporidiosis was clinically suspected, scrape cytology alone or in combination with FNAC were also utilized. Collected aspirates spread over slides and stained with May-Grünwald-Giemsa (MGG) or periodic acid Schiff (PAS) stains. Cytological confirmation of disease did depend on demonstration of endospores as reported by previous workers[6].

Histopathological samples were processed following standard recommendations. Specials stains like PAS and mucicarmine were used. Confirmation of rhinosporidial infection depended upon demonstration of thick walled sporangia containing numerous endospores in fibrovascular stroma infiltrated with chronic inflammatory cells, as recommended by experts[7].

The study was approved by ethical committee of Bankura Sammilani Medical College, Bankura, West Bengal

Result

During study period of 10 years, total 273 cases of rhinosporidiosis were detected histologically. Among them 159 were male and 114 female. Majority of the patients 79.5% (217 out of 273) were from tribal population. Interestingly, proportion of male and female cases for tribal people were almost equal (113 versus 104) with disproportionately higher number of male cases among the non-tribal population (46 vs. 10). Age-distribution of the cases were almost similar between tribes or non-tribes with maximum number of cases of both sexes occurring in the 21-40 year (138 cases) age group, followed by 0-20 year (77 cases) age group [Table 1].

Maximum proportion of patients had lesions nose and nasopharynx (197 cases, 72.2%) followed by eye (45 cases, 16.5%). Total 31 cases were reported from other uncommon sites. Among uncommon sites lips and palate were most frequently affected- 15 and 11cases, respectively. Polypoid lesions were also rarely reported from parotid duct (one case), naso-lacrimal duct (one case), urethra (two cases) and long bone (one case) [Table 2].

Clinical features of the patients as depicted in Table no-3 revealed that polypoidal mass lesions were the most common presentations among lesions of nose and nasopharynx 95.9% (189 out of 197 cases),

Table 1: Sex- Caste- Age distribution of the cases of rhinosporidiosis

Total cases	Sex distribution		Caste	No			Age- group											
				M	F	T	≤ 20			21-40			41-60			>60		
							M	F	T	M	F	T	M	F	T	M	F	T
273	M	F	Tribal	113	104	217 (79.5%)	31	33	64	68	57	125	11	12	23	3	2	5
	159 (58.2%)	114 (41.8%)	Non-tribal	46	10	56 (20.5%)	12	1	13	25	8	33	7	1	8	2	-	2
			Total	159	114	273	43	34	77 (28.2%)	93	65	158 (57.9%)	18	13	31	5	2	7 (2.6%)

(M- Male, F- Female, T- Total)

Table 2: Site Distribution of the cases of rhinosporidiosis

Total no of cases	**Site distribution of cases**							
	Nose & nasopharynx	**Eye**	**Other uncommon sites**					
			Lips	**Palate**	**Parotid ducts**	**Nasolacrimal ducts**	**Urethra**	**Long bones**
273 (100%)	197 (72.2%)	45 (16.5%)	15 (5.49%)	11 (4.29%)	1 (0.36%)	1 (0.36%)	2 (0.73%)	1 (0.36%)

Table 3: Clinical features of rhinosporidiosis cases

Groups	**Number**	**Symptoms**	**Number**	**Percentage (%)**
Lesion of nose & nasopharynx	197	Polypoidal lesions	189	95.9
		Nasal obstruction	46	23.3
		Epistaxis	148	75.1
		Rhinorrhoea	29	14.7
		Features of dissemination	----	----
Ocular lesion	45	Polypoidal lesions	37	82.2
		Watering of eyes	21	46.7
		Features of dissemination	------	---------
Other lesion	31	Polypoidal lesions	22	70.9
		Features of dissemination	------	--------

Table 4: Haematological investigation findings of rhinosporidiosis cases

Total no of cases	Total Leukocyte Count (10^3/cmm)		Eeosinophil (%)				ABO Blood Grouping			
	4-11	>11	1-5	6-10	10-20	>20	A	B	AB	O
273	254 (93.1%)	19 (6.9%)	177 (64.8%)	56 (20.5%)	31 (11.3%)	9 (3.4%)	29 (10.6%)	47 (17.2%)	71 (26%)	127 (46.2%)

Table 5: Cytodiagnosis of lesions

Total number of histology confirm cases	Number of cases evaluated by cytology	Types of evaluation method(s) used	No of cases	Cyto-histological correlation
273	67	FNAC	43	43/43 (100%)
		Scrape cytology	6	6/6 (100%)
		Both	18	18/18 (100%)

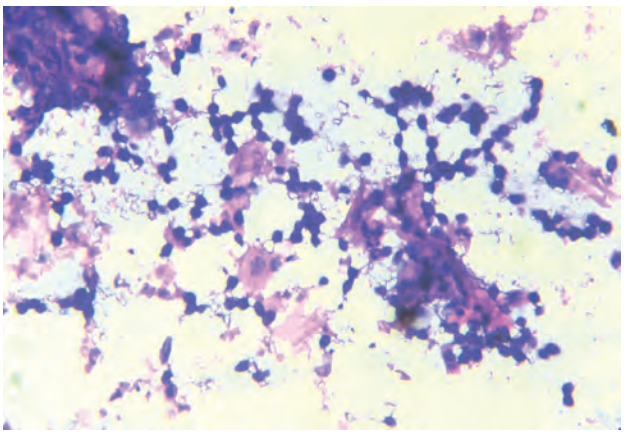

Figure 1: Cytology smear of rhinosporidiosis showing clusters of endospores and intact sporangium (Leishman-Giemsa stain, high power view)

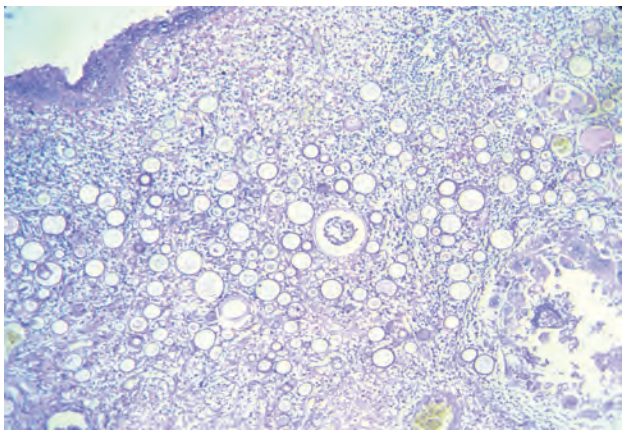

Figure 2: Section shows multiple variable sized sporangia with large number of microspores inside (H & E stain, low power view).

ocular lesions 82.2% (37 out of 45 cases) and lesions involving other uncommon sites 70.9% (22 out of 31 cases). Nasal obstruction, epistaxis and rhinorrhoea were the other reported symptoms of nasal lesions. Watering of the eyes was seen in 46.7% ocular lesions. Not a single case in our series presented with features of dissemination.

Rise in total leukocyte count was encountered in only 19 (6.9%) cases. Majority of the patients 64.8% (177 out of 273) had eosinophil count 5% or less. Mild (≤10%), moderate (≤ 20%) and significant (>20%) eosinophilia were demonstrable in 56 (20.5%), 31 (11.3%) and 9 (3.5%) cases respectively. Almost half of the patients 46.2% (126 out of 273) had blood group O, followed by AB 26% (71 out of 273), B 17.2% (47 out of 273) and A 10.6% (29 out of 273) blood groups. According to the unpublished data collected from blood bank of B S Medical College, the relative proportion of A, B AB and O bold groups of samples from various blood donation camps organized with in the vicinity of the Medical College for was 22.5%, 38%, 8.25% and 31.25% respectively.

Cytology samples were available in 67 cases. Only FNAC or scrape cytology or both modalities were utilized in 43, 6, and 18 cases respectively. In all the cases, cytology confirmed rhinosporidiosis (100% cyto-histological correlation) [Table 5].

Discussion

Rhinopsoridium seeberi, etiological agent of rhinosporidiosis, has little similarity with other pathogenic fungus. Herr et al[8] classified this organism as Mesomycetozoa which includes others fish and amphibian pathogens. Surface water is considered to be the natural habitant of R seeberi. Human infection probably occurs owing to its contact to traumatized epithelium present in contaminated water. For this reason, the highest incidence of infection is reported among river- sand workers[8]. Because of location B S Medical College is catering a large population of poor villagers, accustomed to take bath in surface water explaining larger case load, as experienced during study period.

Male cases outnumbered females in our series approximately at the ratio of 3:2. Eighty percent (80%) of the reported cases were of tribal origin. Higher proportion of tribal population in the area, relatively poor socio-economic status and more outdoor activities may be attributed to this higher incidence. As majority of tribal women take part in outdoor activities, proportion of male and female cases among tribal population as estimated in our study, was almost 1:1 quite distinctive from 4.6:1

seen among non-tribal population. Highest incidence of rhinosporidiosis, irrespective of sex or caste, observed in 21-40 years age group (57.9%) followed by 0-20 years (28.2%). These two age groups together accounted 86.1% (235 out of 273) of cases. This is again probably reflecting the fact that people exposed to more outdoor activities are susceptible to the R seeberi infection.

Nose and nasopharynx was the commonest site of infection followed by eye. Rhinosporidial lesions were also reported from uncommon sites like lips, palate, parotid duct, naso-lacrimal duct, urethra and bone. Similar experiences were also reported by previous workers[1,9]. Polypoid mass lesion found to be the commonest (90.8%, 248 out of 273 cases) clinical presentation at various locations in this study. Nasal obstruction and watering of the eyes was the next common complains in nasal and ocular lesions, respectively. This is in consistence with other reports[1,9].

Rhinosporidiosis of nasal passage usually appears as a polypoidal lesion, granular red in color with multiple, yellowish pin head sized spots representing underlying mature sporangia. This gross appearance, though distinctive, is not diagnostic[4,11]. Typical polypoid lesions wee also reported from skin, conjunctiva, urethral meatus, subcutaneous tissue and other locations. Nasopharyngeal lesions are usually multilobed and relatively less vascular often producing nasal obstruction. Both nasal as well as nasopharyngeal lesions also can present with epistaxis and rhinorrhoea[10,12]. Ocular rhinosporidiosis, in addition to producing polypoid mass lesions can produce following manifestation- watering of eyes, conjunctiva, and photophobia and itching[13].

Routine hematological investigations did not reveal any significant alteration owing to infection. Normal total lymphocyte and eosinophil count was observed in 93.1% and 64.8% cases respectively in the present study which is, however, supported by previous reports[4,10-12]. Of the total study subjects A, B, AB and O blood group were observed in 10.6%, 17.2%, 12.0% and 46.2% cases respectively. AB and O blood group belonged to 72.25% cases. The feature was also consistent with earlier report[4].

Absences of typical polypoidal appearance of rhinosporidial lesions may create confusion in pre-operative diagnosis. Fine needle aspiration cytology can produce fruitful results in these cases. But care must be exercised to reduce chances of bleeding[4]. Scrape cytology can also be an alternative or adjuvant method for assessment of superficial, easily accessible lesions. Microscopic demonstration of endospores (5-10µm) and sporangium (50-1000µm) in the cytological smears confirm the diagnosis. Epithelioid granulomatous infection also may be evident, but eosinophils are rarely encountered occasionally[6,13]. Epithelial cells of respiratory passage can create confusion during identification of endospores. Endospores are often enveloped by residual sporangial material and mucoid in nature. Beattie et al[14] coined the term 'comet' form for these covered endospores. Comet forms can simulate partly degenerated epithelial cells with large nuclei and residual cytoplasmic covering. PAS stain is particularly useful to differentiate PAS positive 'comet' forms from PAS negative epithelial cells[4].

Initial cytological evaluations were possible in 67 out of 273 cases (24.5%) in our series. FNAC or scrape cytology alone or in combination was utilized for cytodiagnosis. In each and every case, correct diagnosis was established, as later on confirmed by histopathological study,

Histopathological study done over biopsied or resected tissue specimens can identify various stages of the pathogen helping to confirm final diagnosis[4]. Sections demonstrate multiple sporangia in various stage of maturity enclosed in a thick chitinous wall. Overlying epithelium in most of the cases become hyperplasic with loose fibrovascular stroma infiltrated with lymphocytes, plasma cells and macrophages. Neutrophilic infiltration can also be evident. Giant cell reaction may occur due to rupture of sporangia[4,7]. Unlike invasive mycoses, prominent eosinophilic infiltration (Splendore-Hoeppli reaction), is not a feature of rhinosporidiosis[15]. Outer walls of the sporangia and endospores can be better visualized using special stains like PAS, mucicarmine, Gomori's methenamine-silver stain etc[4,7,16].

Mature forms of both R seeberi and Coccdioides immitis present with large thick walled spherical sporangium containing smaller round endospores. Cyto- or histological differential often become difficult. But distinction is usually made by large size of the endospores and presence of ensospores in a mature sporangium in a case of rhinosporidiosis. Mucicarmine staining can also be useful for differentiation of these two pathogens, as sporangium and endospores of C immitis do not take the stain[4].

Although hitopathological study is the gold standard for diagnosis of rhinosporidiosis and only in rare instances diagnosis may be missed. Commonest cause of diagnostic errors is faulty selection of the portion of the polypoidal mass for study, containing no or

only few rhinospodial tissue, where as other portions show typical picture. Other rare causes of false negative histopathological diagnosis include absence of well developed outer covering of sporangium, presences of only fragments of sporangium without endospores or absence of typical rhinosporidial bodies as a consequence of possible immunological reactions17.

Conclusion

Rhinosporidiosis is a chronic granulomatous infective disorder commonly presenting with polypoid lesions of nose and naso-pharynx or other organs.

In close to 10% of our cases, typical polypoid appearances of the lesions were absent making clinical diagnosis difficult, particularly when lesions were situated at uncommon sites. Cytology can be successfully utilized for pre-operative diagnosis in difficult cases, as evidenced in the present study. Histopathology is the standard method for confirmation of diagnosis though can be confusing at rare instances. We recommended use of special stains for diagnosis of difficult cases.

References

1. Arseculeratne SN. Recent advances in rhinosporidiosis and Rhinosporidium seeberi. *Indian J Med Microbiol* 2002; **20**: 119-31.
2. Ahluwalia KB. New interpretations in rhinosporidiosis, enigmatic disease of the last nine decades. *J Submicros Cytol Pathol* 1992; **24:** 109-14.
3. Karunaratne WA. Rhinosporidiosis in man. London: Athlone Press; 1964. p. 14-8.
4. Vukovic Z, Bobic-Radovanovic A, Latkovic Z, Radovanovic Z. An epidemiological investigation of the first outbreak of rhinosporidiosis in Europe. *J Trop Med Hyg* 1995; **98**: 333-7.
5. District Human Development Report: Bankura. Development & Planning Department; Government of West Bengal.
6. Pathak D, Neelaiah S. Disseminated cutaneous rhinosporidiosis: diagnosis by fine needle aspiration cytology. *Acta Cytol* 2006; **50:** 111-2.
7. Morelli L, Polce M, Piscioli F, Del Nonno F, Covello R, Brenna A, *et al*. Human nasal rhinosporidiosis: An Italian case report. *Diagn Pathol* 2006; **1**: 25.
8. Herr RA, Ajello L, Taylor JW, Arseculeratne SN, Mendoza L. Phylogenetic analysis of rhinosporidiumseeberi's18S small-subunit ribosomal DNA groups this pathogen among members of the protoctistan Mesomycetozoa clade. *J Clin Microbiol* 1999; **37**: 2750-4.
9. Karunaratne WAE. The pathology of rhinosporidiosis. J Path & Bact. 1936; 42: 193-202.
10. Sarker MM, Kibria AKMG, Haque MM. Disseminated subcutaneous rhinosporidiosis: a case report. *TAJ* 2006; **19**: 31-3.
11. Harissi-Dagher M, Robillard N, Corriveau C, Mabon M, Allaire GS. Histopathologically confirmed ocular rhinosporiodiosis in two Canadians. *Can J Ophthalmol* 2006; **41:** 226-9.
12. Kumari R, Laxmisha C, Thappa DM. Disseminated cutaneous rhinosporidiosis. *Dermatol Online J* 2005; **11**: 19.
13. Senaratne T, Edussuriya K, Dhanapal M, Bandara A, Arseculeratne S. Ocular rhinosporidiosis with staphyloma formation: a case with unusual features. *Eye and Brain* 2011; **3:** 1-4.
14. Beattie JM. Rhinosporidium kinealyi: a sporozoon of the nasal mucous membrane. *J Pathol* 1906; **11:** 270-5.
15. Arseculeratne SN, Atapattu DN, Rajapakse RPVJ, Balasooriya P, Fernando R, Wijewardena T. The humoral immune response in human rhinosporidiosis. *Proc Kandy Soc Med* 1999; **21**:9.
16. Hussein MR, Rashad UM. Rhinosporidiosis in Egypt: a case report and review of literature. *Mycopathologia* 2005; **159**: 205-7.
17. Arseculeratne SN, Panabokke RG, Atapattu DN. Lymphadenitis, trans-epidermal elimination and unusual histopathology in human rhinosporiodiosis. *Mycopathologia* 2002; **153**: 57-69.