

Prognostic Significance of Intraoperative KOH Wet Mount in COVID-19-Associated Rhinomaxillary Mucormycosis

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Abstract

Background: Fungal skin infections have been diagnosed with the help of the potassium hydroxide (KOH) wet mount technique for decades. Scrapings on a slide mixed with a drop of KOH are heated to dissolve cells other than fungi to visualize the fungal contents. Our study applied this principle with a modification as an intraoperative debridement guide in maxillary mucormycosis cases.

Materials and Methods: From our institute, 20 osteomyelitis cases admitted for surgical debridement with COVID-19 history were taken for study. Intraoperatively, fresh tissues from the borders of the debrided maxillectomy site were collected in test tubes with 20% KOH. The tissues were dissolved by boiling until turbidity cleared and left undisturbed. The sublimate was visualized and scanned under the light microscope with and without staining for fungus. For each site, the presence of fungal hyphae was noted as positive. Then, the number of positive borders, histopathological diagnosis, and recurrence after surgery are compared.

Results: Eight of 20 cases were negative in KOH, among which two cases had no demonstrable fungi in histopathology. Two of 20 cases had one positive border, four of 20 cases had two positive borders, and four of 20 cases had three positive borders. Two of the 20 cases were positive in all borders, of which one had a recurrence.

Conclusion: Applying this technique as an intraoperative guide can guide the surgeons to selectively debride the area of fungal invasion, make fungi-negative surgical margins for better prognosis, and conserve the remaining tissue.

Key words: COVID-19, Fungal osteomyelitis, Margins of excision, Mucormycosis, Prognosis

INTRODUCTION

During the COVID-19 pandemic, several opportunistic infections started resurfacing due to alterations in the immune system. According to a meta-analysis, the pooled prevalence of co-infections was 19%, and the pooled prevalence of superinfections was 24% in SARS-CoV-2 patients, among which 4% were fungal coinfections and 8% were fungal superinfections.^[1] In a study comparing bacterial and fungal osteomyelitis of the skull base, the

fungal osteomyelitis was the one that had an earlier presentation, faster spread, and higher morbidity and mortality.^[2] We know that fungal and bacterial osteomyelitis can masquerade as each other,^[3] this can be a reason for higher mortality by the delay of appropriate treatment.

Decades before, the diagnosis and treatment of osteomyelitis focused primarily on bacterial etiology during the empirical treatment phase. The fungal etiology was considered only after non-responsive antibacterial therapy. This delay is a disadvantage in this pandemic situation as the fungal osteomyelitis cases, especially in mucormycosis (zygomycosis). Mucormycosis has an overall case fatality of 50%.^[4] Hence, the diagnosis at the earliest possible time is essential for a good prognosis. Fungal infections, including oral mucormycosis (8.6%), aspergillosis, and invasive candidiasis (64%), have been reported in patients with severe COVID-19 or those

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recovering from the disease. Patients with diabetes got infected predominantly.^[5] According to the World Health Organization, mucormycosis has an incidence range of 0.005–1.7/million population. In India, the prevalence of mucormycosis is estimated at 140/million, about 80 times higher than that in Western countries.^[4] During the COVID-19 pandemic period, diabetes, along with the usage of steroids, these rare fungal infections, showed up their increasing chances and caused a sudden surge in the incidence rate; India contributed 45% of the world's new mucormycosis cases and 34% of deaths due to mucormycosis in 2021.^[6] More than 47,000 new cases have been reported in India in 3 months, around May 2021.^[7]

Due to its angioinvasive potential, the pathogenic invasion seems much wider than the visible necrotic bone area in an intraoperative situation. A recent case report article states the author's onerous problem of getting the mucormycosis in maxillae controlled only after two or more surgical interventions.^[8] It is still a dilemma whether the unresolved infection is a recurrent or residual one.^[9] However, studies show that debridement plays an important role. A study shows that 100% of rhinomaxillary mucormycosis cases needed surgical debridement to control the disease.^[2] Thus, the quality of surgical debridement seems to be an essential factor in disease recurrence, especially bone involvement.

Universally surgical debridement of osteomyelitis is concluded when fresh bleeding tissue is noted. However, mucormycosis being angioinvasive can spread more expansive in the tissue dissecting in and around the blood vessels [Figure 3], and initial invasion shows minimal to no signs. This phenomenon makes the complete removal of the pathogen difficult by judging the tissue with the help of bleeding spots. In literature, we have seen that many recurrent cases needed a complete resection of the involved bone after unsuccessful debridement efforts to control the disease. This shows a need for an intraoperative guide for enhancing surgical debridement. Necessity is the mother, and thus, a baby-like procedure that still needs experimentation, adaptation, and application came into existence in our institute.

For several years in dermatology, potassium hydroxide (KOH) wet mounts have been used to diagnose superficial fungal infections successfully and reliably. In the COVID-19 pandemic, most case reports show the usage of KOH wet mount from the nasal swabs of suspected mucormycosis cases preoperatively. This study proposes a new variation of the KOH wet mount technique to use intraoperatively as a guide for the debridement of the orofacial mycoses, which could enhance the debridement and near-complete removal of the pathogen from the affected as well as infected areas.

MATERIALS AND METHODS

From RUHS College of Dental Sciences, Jaipur, 20 newly diagnosed osteomyelitis cases admitted for surgical debridement with COVID-19 history were taken for study. Intraoperatively, fresh tissues from the debrided borders of the maxillectomy site were collected with a clean and sterile bone file (from hard tissue) or a scalpel/scissor (from soft tissue) in separate labeled test tubes with 20% KOH [Figure 1]. Before adding them to KOH, soft tissues were minced on a sterile glass slide. The tissues were dissolved by boiling until the solution cleared and left undisturbed. The sublimate from each test tube was taken in three wet mounts, one without any stain and one with saffranine O and one with India ink to visualize fungus, and was scanned by a pathologist thoroughly in a pattern similar to that used in WBC counting on a peripheral blood smear. On finding fungal hyphae [Figure 2], the border is considered positive, and the absence of hyphae is considered negative. The number of positive borders was noted for each case. Histopathological diagnosis is made with regular hematoxylin and eosin staining and periodic acid Schiff staining. A minimum follow-up data of 6 months were collected from the surgeons and surgical residents for each case. It was cross-checked that there was no variation in the pharmacological treatment protocols among the cases included in the study. The histopathological diagnosis, number of positive borders in KOH, and recurrence were tabulated and analyzed [Table 1].

RESULTS

In our study, only two patients showed positive KOH mounts from all the sites sampled, and among them, one patient had a recurrence and needed surgical reintervention. The rest of the cases had a variable number of positive sites, as shown in table. The most necrotic area from the debrided tissue was used for diagnostic purposes, and even among those, 50% showed no fungal hyphae in KOH mount. 5/10 cases that were negative for fungi in KOH had a histopathologically demonstrable fungal infection. About 60% of cases have demonstrated fungi in histopathology, among which two cases were not positive for fungi in KOH. Standardization of the collection site and centrifugation of the dissolved liquid can improve the results of KOH.

DISCUSSION

Our study evolved from the regular KOH wet mount when we started in the initial days of resurfacing mucormycosis. Most of the KOH mounts taken from the nasal swabs were negative; however, the histopathology had demonstrable

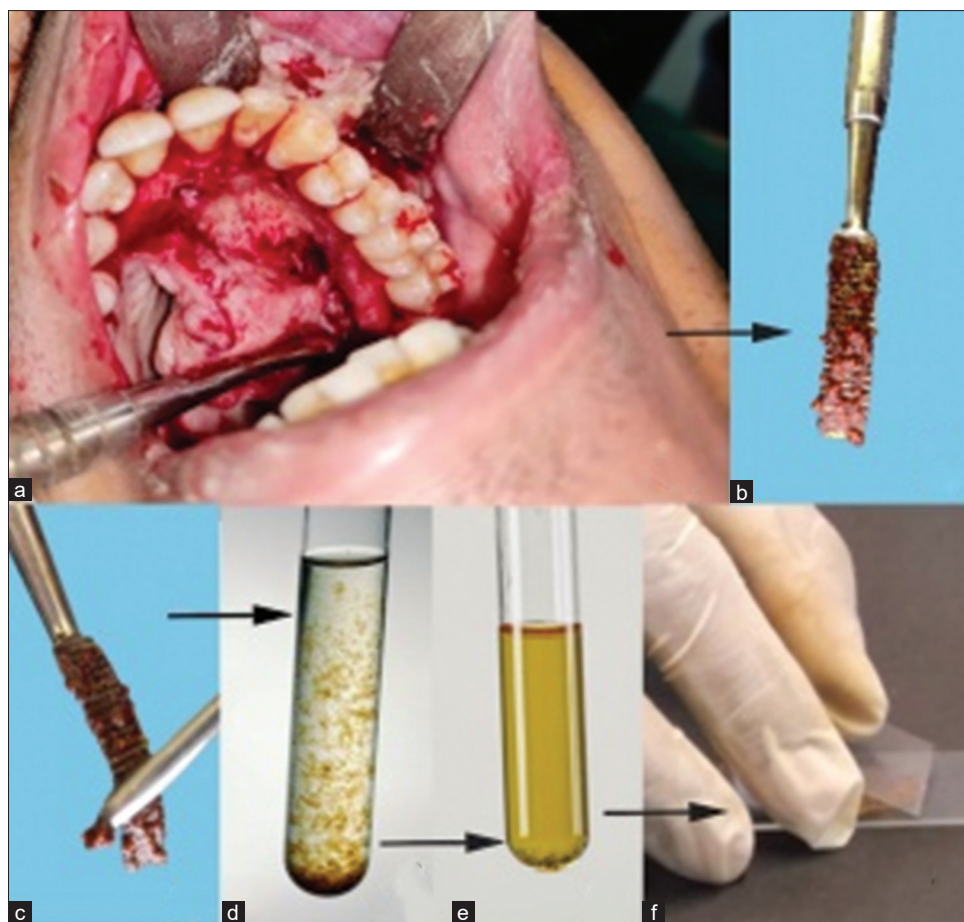


Figure 1: (a) Collection of bone fragments from the surgical site with a bone file, (b) bone file with the osseous coagulum, (c) collecting the sample with a periosteal elevator, (d) sample in potassium hydroxide solution, (e) after heating, dissolving and settling down, (f) and the sublimate on the wet mount

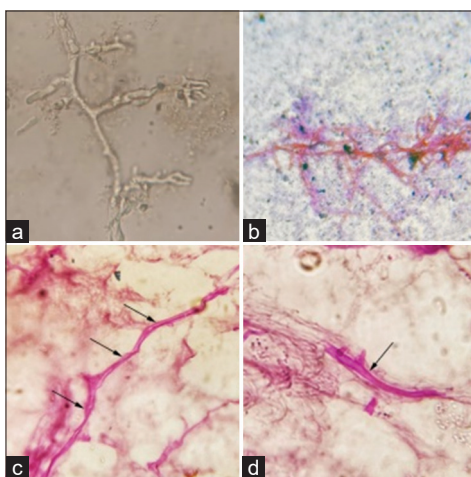


Figure 2: (a) Unstained potassium hydroxide (KOH) wet mount showing fungal hyphae with characteristics of mucor, (b) KOH + safranin O stain showing fungal hyphae, and (c and d) decalcified, paraffin-embedded, periodic acid Schiff-stained sections of the same patient's debrided hard tissue

fungi. Since mucor spreads deeply in osteomyelitis and does not show up in superficial tissues, we could not get fungal hyphae in superficial swabs and scrapings with a

considerable consistency compared to histopathology, which made us request the surgeons to send unfixed tissue in saline immediately after completion of the procedure. Those unfixed tissues yielded good diagnostic data despite being cumbersome to process on the slide. Subsequently, we started collecting and dissolving the tissues in 5 mL test tubes. Increasing the concentration to 20% KOH improved the clearance rate. This expedited diagnosis and evolved into collecting tissue from the debrided debris and the surgical borders after complete irrigation of the debrided area for better diagnostic purposes intraoperatively. During this evolution, we have undergone several changes in visualization also. Unstained wet mounts were challenging to scan and much more challenging to appreciate the fungal hyphae. Same time due to the situation, the demand for stains raised, fortunately, or unfortunately, which led us to use Safranin O and India ink for our KOH mounts. Even smearing and PAP staining of the sublimate of KOH solution with sample have been tried. After all these experimentations and the regular histopathological analysis, we standardized the KOH with India ink, KOH with Safranin O, and KOH plain for analysis. Safranin O produced a background precipitate;

Table 1: Comparison of intraoperative KOH preparations and recurrence

Serial number	Age	Gender	Intraoperative positive KOH wet mount preparations from different sites					Total	Demonstrable in H and E/PAS	Recurrence
			Lateral	Medial	Posterior	Superior	Visually most necrotic*			
1	50	Male	-	-	-	+	+	1	No	-
2	35	Female	-	-	-	-	-	0	No	-
3	24	Male	+	+	+	+	+	4	Yes	Yes
4	45	Female	+	+	-	-	+	2	Yes	-
5	32	Male	-	-	-	-	-	0	No	-
6	40	Female	+	+	-	+	+	3	Yes	-
7	30	Female	-	-	-	-	-	0	No	-
8	40	Male	-	-	-	-	-	0	No	-
9	29	Male	-	+	-	+	+	2	No	-
10	52	Female	+	+	+	+	+	4	Yes	-
11	60	Female	-	-	-	-	-	0	No	-
12	50	Male	+	-	-	-	+	1	Yes	-
13	50	Male	+	-	-	-	+	1	Yes	-
14	66	Male	-	+	-	+	+	2	Yes	-
15	45	Male	-	-	-	-	-	0	Yes	-
16	48	Female	-	-	-	+	-	1	Yes	-
17	51	Male	-	-	-	+	+	1	Yes	-
18	45	Male	-	-	-	-	-	0	No	-
19	35	Female	+	-	+	-	-	2	Yes	-
20	33	Male	-	-	-	-	-	0	Yes	-

*Not counted in the total positive sites, taken for diagnostic purposes only from most necrotic areas in the gross specimen. In KOH columns, "-": Negative for fungal hyphae, "+": Positive for fungal hyphae, No: Fungi nondemonstrable, Yes: Histopathologically demonstrable fungi in tissue. H and E: Haematoxylin and eosin, PAS: Periodic acid Schiff, KOH: Potassium hydroxide

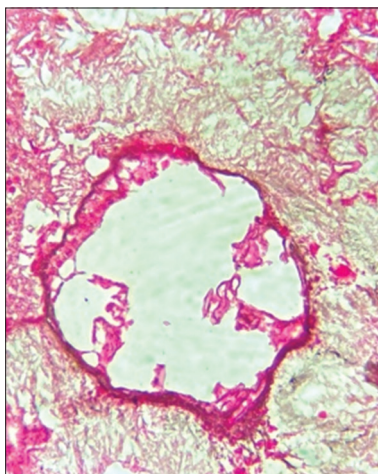


Figure 3: Vascular invasion and growth of fungal hyphae in blood vessel demonstrated by periodic acid Schiff staining

however, it stained the fungi strongly so that it was easy to detect by inexperienced residents.

We know positive surgical margins are associated with worst outcomes in the oncosurgeries.^[10] Even after tremendous advances in molecular-level diagnosis and pharmacology, mucormycosis involving bone is not treated solely with pharmacotherapy; surgical removal is needed in 100% of cases to date, which reveals the need for complete removal of the pathogen in its vegetative state from the bone tissue. The distribution rates of antifungal drugs in soft tissue show probabilities of non-surgical therapy of isolated

soft-tissue mucormycosis only. The literature shows a non-surgical cure in an isolated soft-tissue mucormycosis case in the cerebrum without bone involvement,^[11] lung,^[12] and gastric tissue.^[13] To the best of our knowledge, we could not find a case report of mucormycosis with bone involvement cured without surgical intervention.

Possible Future Direction of this Study

We know that theories and practices bloom and fade in time according to the necessity and phase of development. At some stages, very primitive ways may be helpful in treatment. If continued at a multi-center level, this study can give knowledge on recurrent mucormycosis, whether it is recurrent or residual. Further, improvements in the processing, as follows, may be applied.

- i. Standardization of sampling areas
- ii. Centrifugation of the dissolved solution for a better yield of diagnostic material on a wet mount
- iii. Developing a “number of samplings to the number of positive” ratio and analyzing its relations to the
- iv. Disease progress and treatment outcome.

CONCLUSION

Technology and research are speeding at a high rate to find a better combination of antifungal mucormycosis cases to find a better combination of antifungal drug therapy for mucormycosis cases; still, this study can reveal answers to the aforementioned unanswered questions in the effect

of treatment on bony involvement in mucormycosis cases, especially in the maxillofacial region. Our KOH wet mount intraoperative guidance for maxillofacial bony fungal infections could be an easy intraoperative surgical margin cross-check procedure, which anyone could do with minimal expertise and bring about a major change in the prognosis of the patient. Further, exploration of possible application of this hypothesis and standardization could be done on a multi-center basis to get more samples and statistically arguable data.

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