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Histopathological Insights Into Sino-Nasal Mucormycosis in COVID-19 Patients and Related Pathogenesis

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Abstract

Background: Rhino-orbital-cerebral mucormycosis (ROCM) is an aggressive fatal fungal infection in COVID-19 patients with uncontrolled diabetes and corticosteroids therapy.

Aim of Study: Early and accurate diagnosis of mucormycosis focusing on the pathognomonic histopathological features and related pathogenesis.

Patients and Methods: This retrospective study included 28 nasal biopsies of COVID-19 patients, between the first of January and the end of August 2021, data was collected from one tertiary center. Demographic, serological, radiological and clinical data were obtained from reports. Histopathological evaluation of H&E stained sections, PAS and Masson trichrome stains were done for each paraffin block.

Results: A spectrum of histopathological features was noticed; the pathognomonic ones are: mycotic abscess with multiple giant cells and Emperipolesis, mycotic bone necrosis and/or osteonecrosis, paucicellular fat necrosis and angioinvasion. Mucorales and associated Splendore-Hoeppli phenomenon were obvious in all examined sections. About 35% of patients were children (4-13 years) with pre existing or concomitant diabetes, all were positive COVID-19 (PCR and/or radiological findings).

Conclusions: Early histopathological diagnosis and new therapeutic modalities are safeguards against the co- morbidity of mucormycosis in COVID-19 patients.

Authors declare no conflict of interest.

Key Words: *Histopathology – Mucormycosis – COVID-19.*

Introduction

A WIDE spectrum of complications was reported in COVID-19 patients during the pandemic period. One of the most frequent opportunistic infection is the fungi especially Mucormycosis, with several cases being reported worldwide. WHO estimated the prevalence of COVID-19 associated Mucormycosis to be about 80 times higher in developing countries than developed ones with a surge in India [1].

Although Mucormycosis cannot spread directly from one person to another, inhalation, ingestion and inoculation are the main primary routes for transmission of spores. Nasocomial infections with contaminated tools are mandatory to spread infections [2]. The primary sites and spread of Mucormycosis in COVID-19 patients are illustrated in the following diagram (Fig. 1). 90% of sino-nasal mucormycosis is caused by Rhizopus Oryzae [3].

Uncontrolled diabetes (diabetic ketoacidosis) and improper use of corticosteroids in COVID patients are critical to development of Mucormycosis infection whatever the degree of pulmonary affection [1].

Mucormycosis induces a spectrum of histopathological changes in the nasal tissue; awareness of these features is the mainstay of understanding the underlying pathogenesis and subsequent proper management of the patients, leading to significant decrease in overall morbidity and mortality rates.

The overall mortality rate for mucormycosis remains more than 50 percent and approaches 100% among patients with disseminated disease or those with persistent neutropenia [1].

Aim of the work: Early detection of mucormycosis focusing on the pathognomonic histopathological features and its pathogenesis among uncontrolled diabetic COVID-19 patients during pandemic period. Promising therapeutic modalities will be suggested depending on the underlying pathogenesis.

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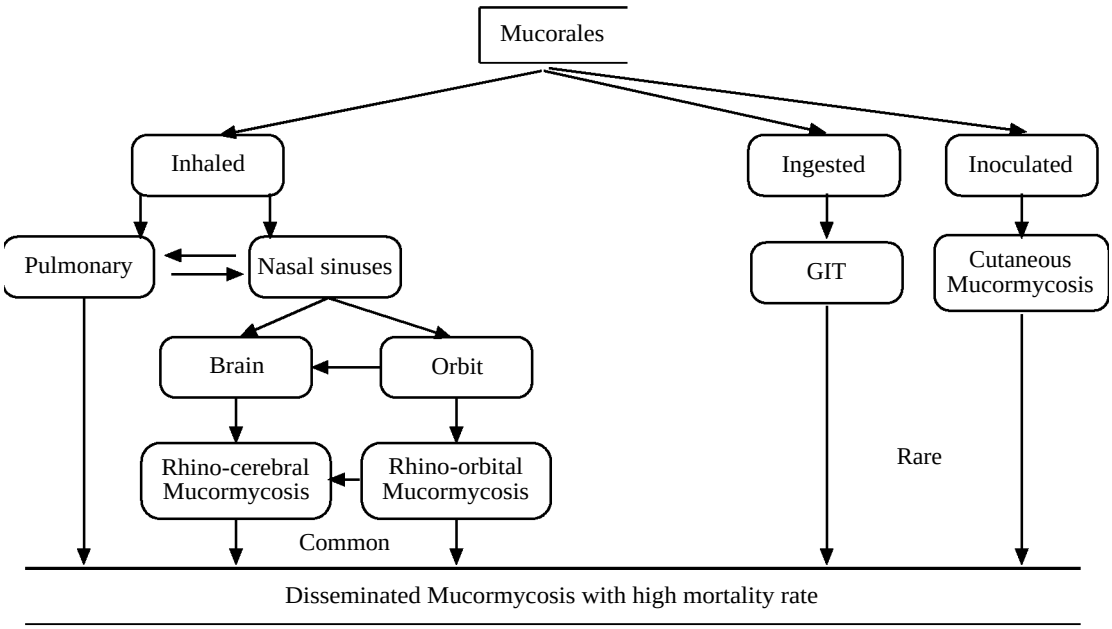

Fig. (1): Primary sites and spread of Mucormycosis in COVID-19 patients.

Patients and Methods

This retrospective study included 28 patients with mucormycosis, diagnosed during the pandemic of COVID-19, between the first of January and the end of August 2021. All data were collected from one University Hospital (tertiary center) in our locality.

These cases were admitted to the departments of pediatrics, ENT, ophthalmology and neurology for management of COVID-19 infection and its complications. Demographic, serological, radiological and clinical data were obtained from the reports.

COVID-19 infection was diagnosed by positive nasopharyngeal swabs (RT-PCR; real time polymerase chain reaction) and/or characteristic radiological CT findings of lungs. Data of CT or MRI of the nasal sinuses, orbit and brain were collected to assess the extension of mucormycosis.

Histopathological examination and revision of H&E stained sections from nasal biopsies were done. PAS and Masson trichrome stains were done for each block for accurate evaluation of fungal spores and hyphae.

Results

About one third (35%-10/28) of the patients were children in the range (4-13 years). All of the patients were diabetic either old or discovered during COVID-19 infection. All had variable degrees of pulmonary affection.

All examined sections (H&E/PAS/Masson Trichrome) revealed Mucorales fungi and a spectrum of histopathological changes.

Criteria of mucorales fungi by different stains:

H&E stain sections revealed Mucorales as intravascular tangled mass or within the inflamed necrotic tissues. The hyphal forms of Mucorales were broad (ribbon like) with irregular variable thickness (6-50um), lacking microscopically visible septa, with lateral bullous protrusions, branching at variable degrees up to 90 angles (Fig. 2A,B).

They appear as negative white shadows with refractile esinophilic membranous borders on PAS stained sections (Fig. 2C). Masson Trichrome highlight the mucorales as esinophilic broad hyphae with thick esinophilic membrane (occasionally with double contour) contrasted with the surrounding blue collagen fibers (Fig. 2D).

Splendore-Hoeppli reactions could be seen as deeply esinophilic amorphous material with variable configurations (club-shaped or star like), around the mucorales or in the midst of inflammatory or necrotic zones (Figs. 2E,F).

Histopathological changes induced by mucorales fungi:

A wide spectrum of histopathological changes was detected, beginning with neutrophilic abscess and ending with Mycotic thrombosis followed by ischemic changes of tissues.

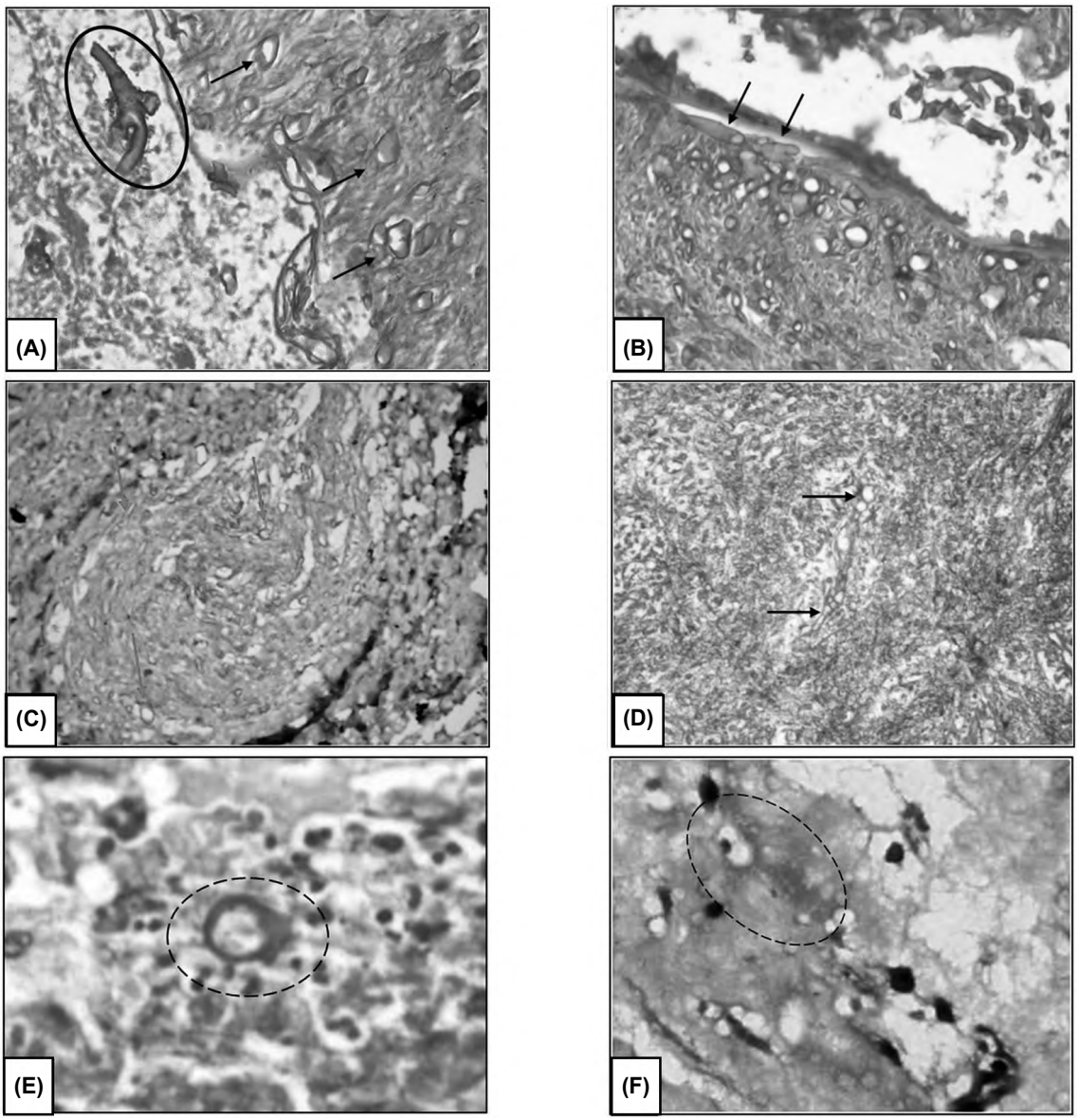

Fig. (2): Mucorales in nasal biopsy. (A&B) H&E stained sections showed transmural invasion of the vascular wall by thick, ribbon like, twisted (circle) hyphae with lateral bullous protrusions (black arrows). (C) PAS revealed tangled mass of mucorales; negative shadows with refractile esinophilic borders (red arrows). (D) Masson trichrome showed colonies of esinophilic broad hyphae contrasted with blue collagen fibers (black arrows). (E&F) Splendore-Hoeppli phenomenon (HE sections); esinophilic amorphous material around mucorales and in necrotic tissue (dotted circles). Original (x400 for all images).

1- *Inflammatory reactions:* Begin early in the form of diffuse mixed inflammatory infiltrate: Mainly neutrophils, pus cells and frequent eosinophils. Histocytes are predominant in various stages with formation of giant cells (F.B. & Langhan's like giant cells) (Fig. 3).

Later on, the inflammatory infiltrate tend to be localized to form esinophilic suppurative granuloma like reaction or abscess formation (Fig. 4).

Mycotic abscess could be seen with three zones: outer zone of fibrosis, mid zone of a mixture of acute inflammatory cells, giant, eosinophils and mucorales, central inner zone with colonies of mucorales and necrotic debris (Fig. 4).

Areas of granulation tissue with characteristic proliferating stag horn like vessels were frequently detected. Congestion and hemorrhage were diffusely noticed.

Emperipolesis: An interesting finding was noticed where neutrophils and/or eosinophils could be seen in the cytoplasm of macrophages (giant cells) (Fig. 3D).

2- *Vascular changes (Fig. 5):* They are recognized later; transmural invasion of the vasculature

by tangled mass of Mucorales is the main pathognomonic histopathological feature in all cases.

Mycotic thrombi, subsequent tissue necrosis and infarction are the main sequels in this stage.

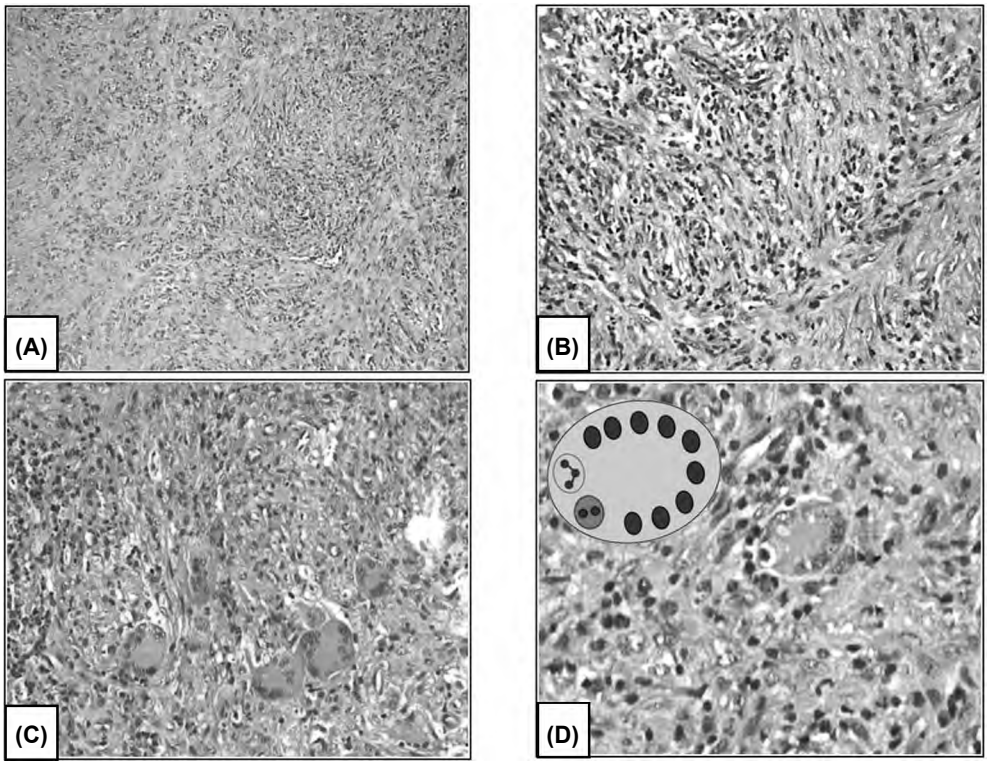

Fig. (3): A diffuse mixture of acute and chronic inflammatory cells. (A-C) Numerous neutrophils and eosinophils with multiple giant cell. (D) Emperipolesis: Macrophage engulfing neutrophils and eosinophil. Original (HE; x100,200,400,400).

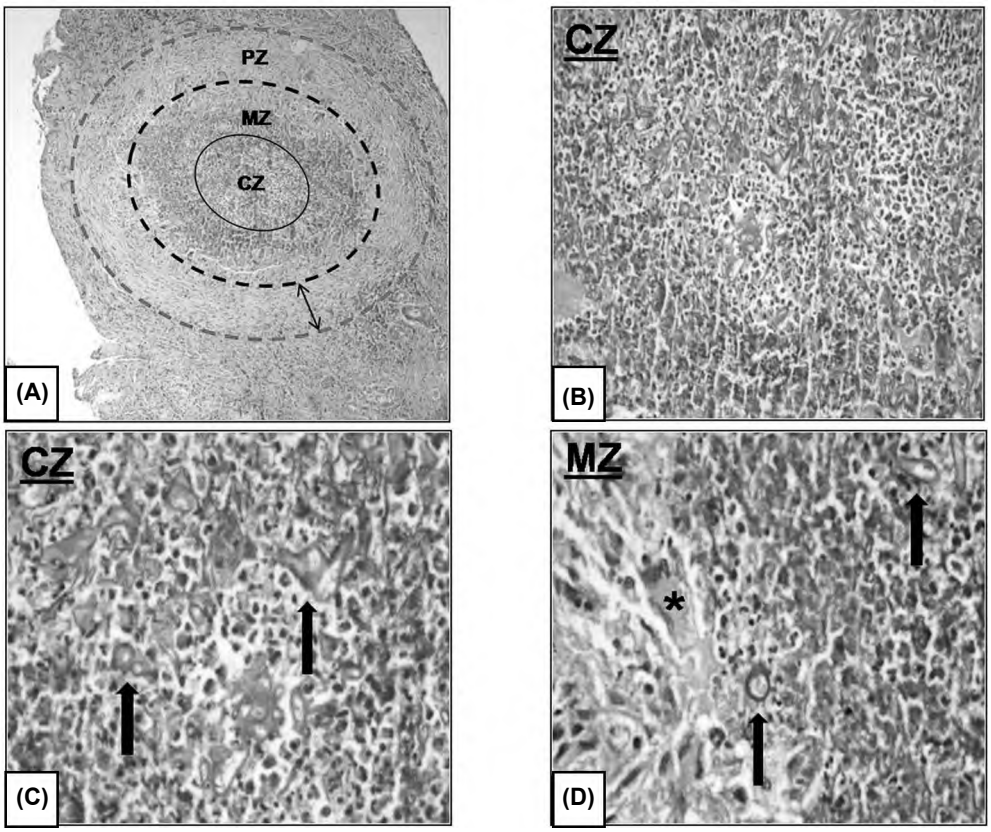

Fig. (4): Mycotic abscess. (A) Three zones were identified: Central (CZ), Mid (MZ) and peripheral (PZ) fibrotic zones (double ended arrow). (B-C) CZ: Central necrotic zone with colonies of mucorales (black thick arrows). (D) MZ: Mid zone of mixed inflammatory infiltrate and giant cells (astrix). Original (HE; x100,400,400,400).

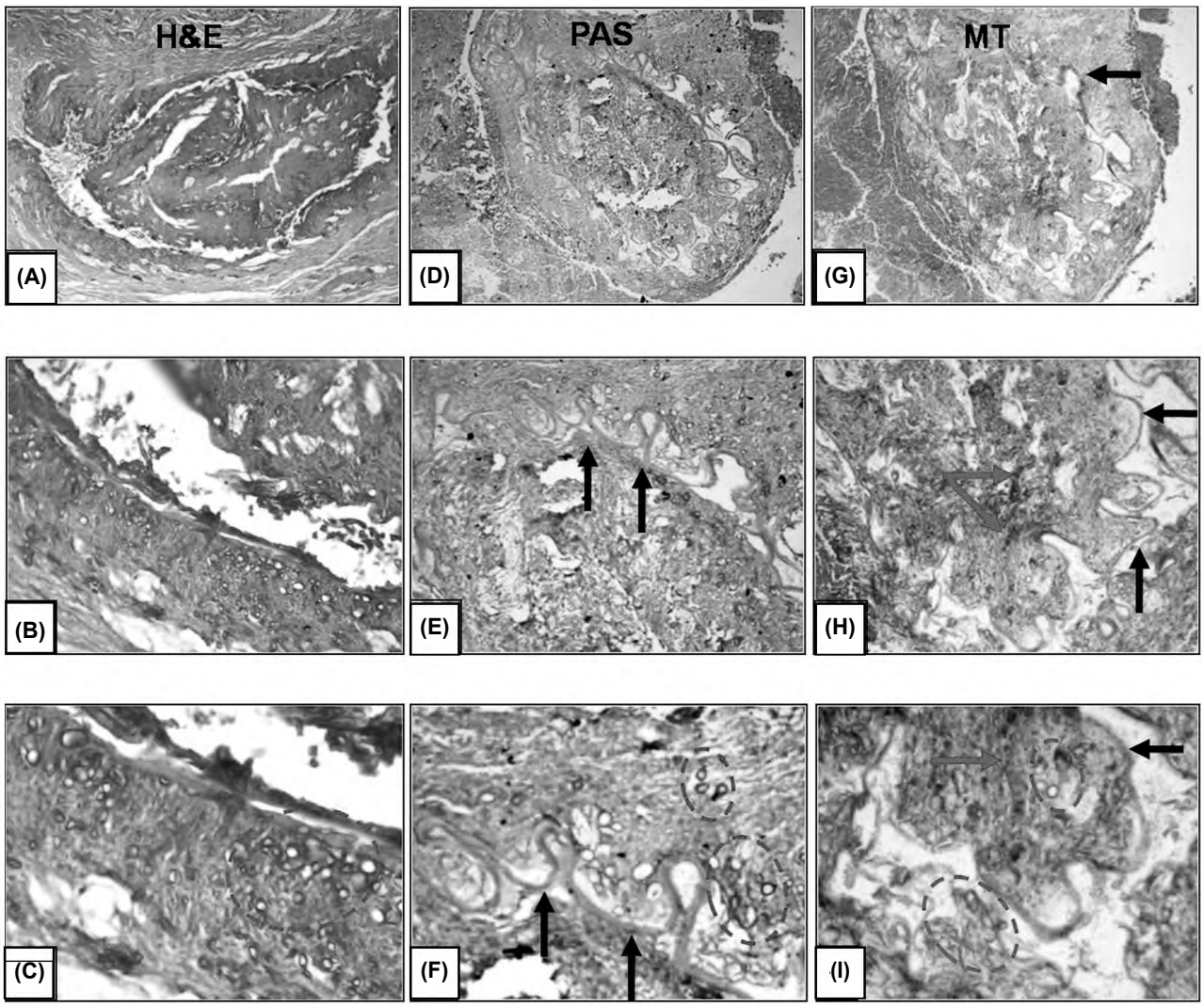

Fig. (5): Intra vascular mycotic thrombi and detailed steps for transmural angioinvasion; Mucorales (thick hyphae; red arrows) and (rounded spores; dotted red circle) invade the vascular wall leading to detached basement membrane (black arrows). Original (x100,400,400/ 200,400.400/ 200,400,400).

3- *Bone reaction (Fig. 6):* Recognized a later complication of previous stages. Two patterns were identified: Pyogenic fungal osteomyelitis and osteonecrosis. Areas with osteomyelitis showed brisk inflammatory reaction, reactive new bone formation, stromal fibro-myxoid reaction and periosteal fibrosis. Sequestrated necrotic bony spicules with adjacent colonies of invasive mucorales were observed.

Areas with osteonecrosis (avascular necrosis) showed empty lacuna (loss of osteocytes), absence of osteoclasts and presence of reparative process (new bone formation with preiostael fibrosis).

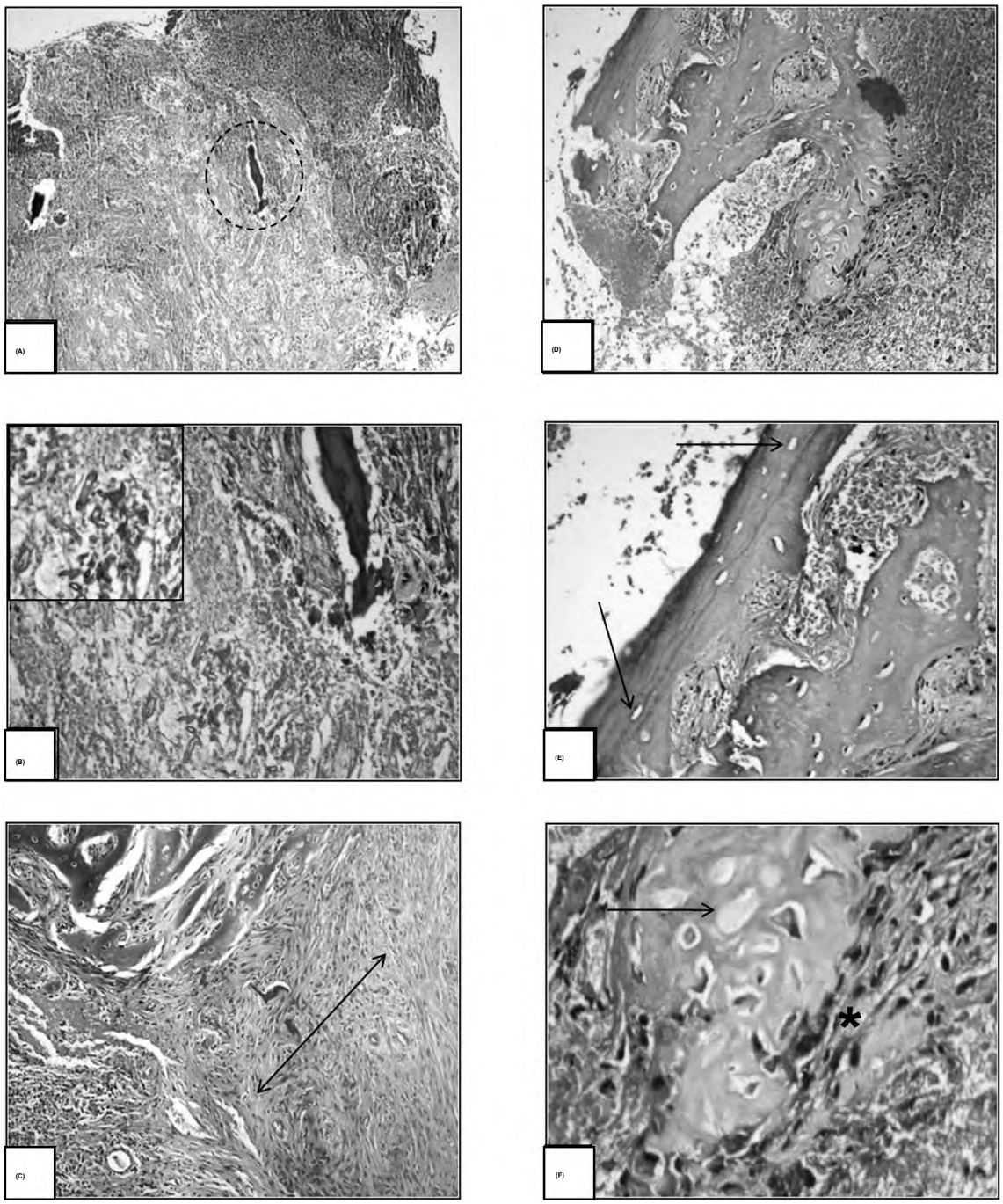

Fig. (6): Nasal biopsy showed a mix of Mycotic osteomyelitis (left side) and Osteonecrosis (RT side). (A-C) Mycotic osteomyelitis: Bony specule (dotted circle), Mucorales (insight) and fibrosis (double ended arrow). (D-F) Osteonecrosis (ischemic bone necrosis): Empty lacuna (arrows) and reactive bone formation (astrix). Original; (HE x100,400,200/x200,400,400).

4- *Paucicellular fat necrosis (Fig. 7):* Either ischemic or enzymatic. Wide areas of necrotic fat appear as ghosts of fat lobules with scarce cells were seen. Numerous Mucorales could be seen within the necrotic fat or within the surrounding inflammatory reaction.

5- *Reactive hyperplastic changes of mucosal glands (Fig. 8):* Increased numbers, excessive intra luminal secretions, thickened and hyalinized basement membrane (Exudative changes) were noticed.

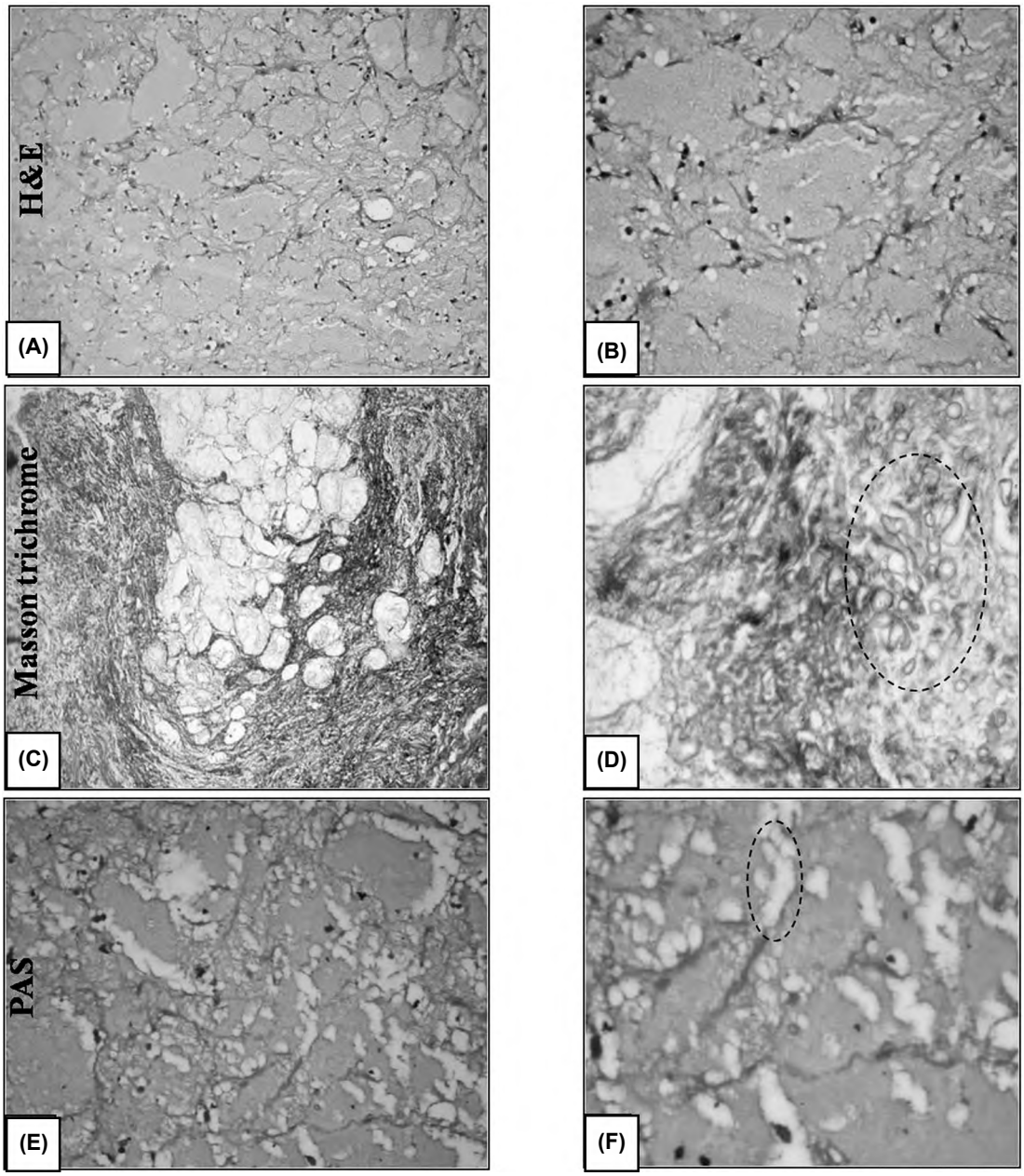

Fig. (7): Paucicellular fat necrosis. (A-B) Diffuse areas of wiped out necrotic fat and a few inflammatory cells. (C-D) Esinophilic broad hyphae of mucorales (dotted circle) surrounded by blue collagen (fibrosis). (E-F) Negative shadows (dotted circle) of mucorales in necrotic fat. Original (x200,400,200,400,400,400).

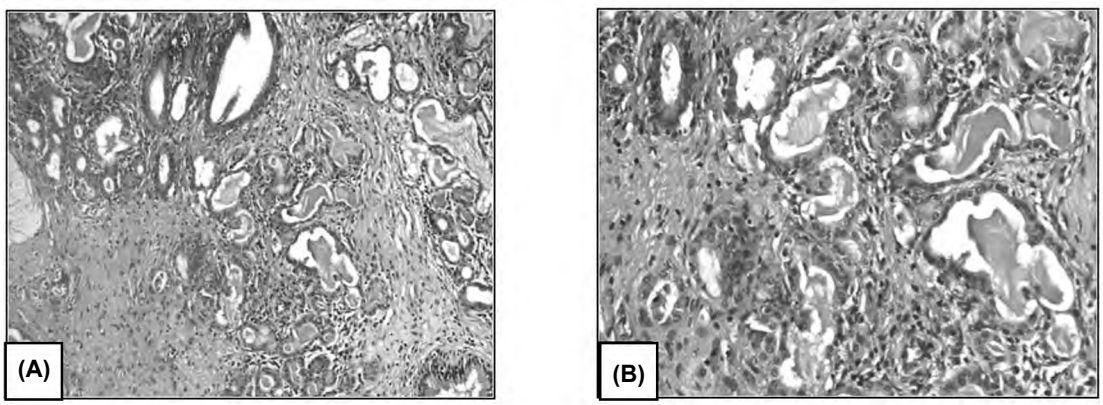

Fig. (8): Reactive hyperplastic changes of nasal mucosal glands with exudative changes. Original (HE; x200,400).

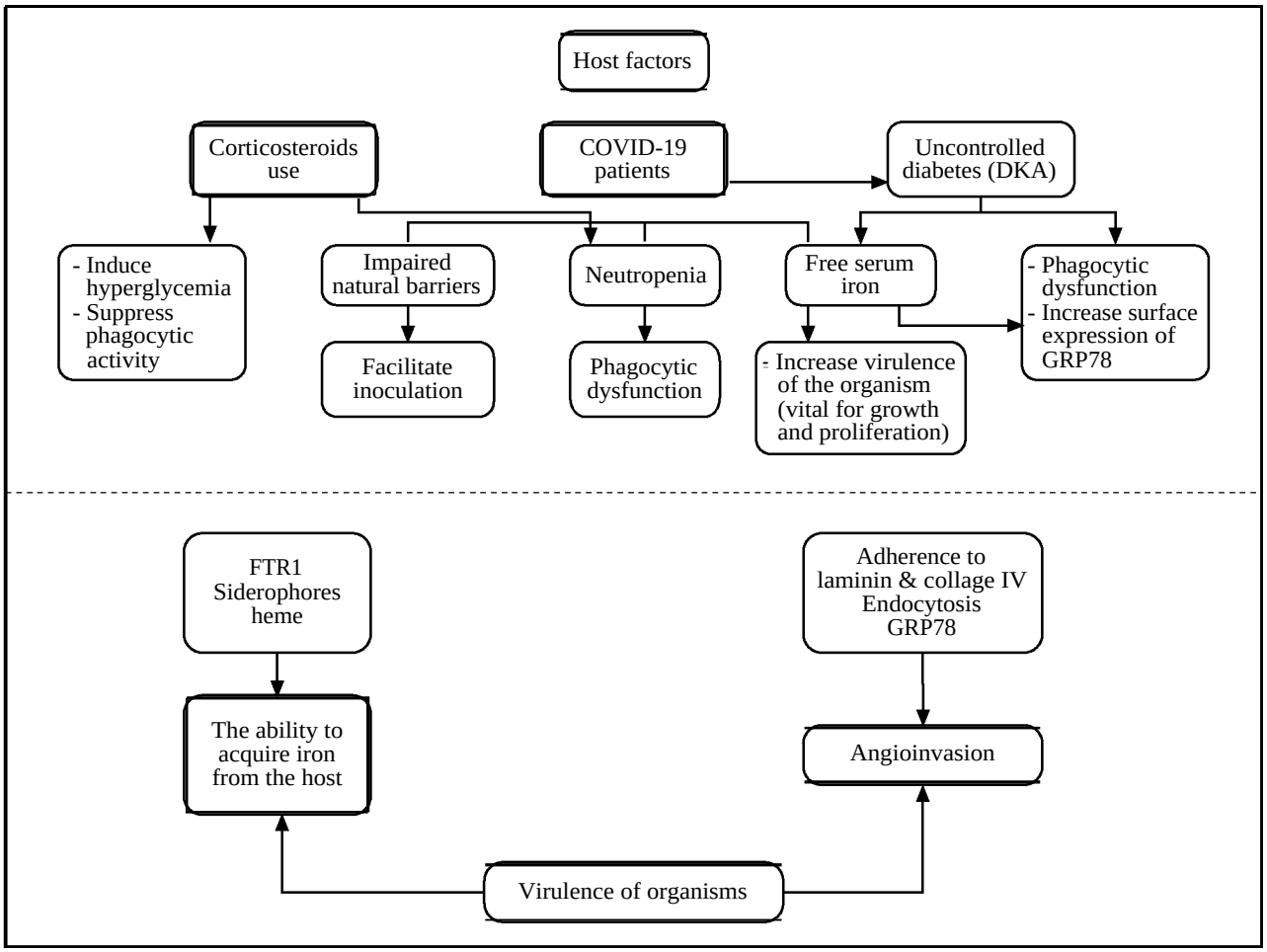

Fig. (9): Pathogenesis of mucormycosis in COVID-19 patients; important risk factors.

Discussion

WHO reported Mucormycosis as an aggressive fatal fungal infection, caused by Mucorales (bread mould fungi). They can be inhaled or ingested and then infect nasal sinuses, lungs and GIT. Less often Mucorales cause cutaneous infection as primary site **[2]**.

We detected 28 cases of Mucormycosis from one tertiary center, still considerable numbers of cases were lost due to either improper diagnosis or underreporting. In the past, the main risk factors of mucormycosis were organ or bone transplantation, renal dialysis and malignant hematological disorders. Now uncontrolled diabetes mellitus and abuse of corticosteroids in COVID-19 patients are the main insults for development mucormycosis infection. Singh et al., 2021 **[1]** published a systematic review of 101 cases of mucormycosis in COVID-19 patients worldwide, they found about 90% of cases involved nose and sinuses, they confirmed that the corticosteroids use and associated diabetes are important linked risk factors.

ROCM (rhino-orbital-cerebral mucormycosis) is still the most common clinical pattern in COVID-19 patients. They included variable clinical degrees: Limited Sino-nasal invasion, rhino-orbital invasion and disseminated rhino-orbital-cerebral affection as it was reported during the pandemic of COVID-19 **[1]**.

The current work will answer and discuss two important questions. Firstly, is the pathogenesis the same or different? Secondly, what are the diagnostic histopathological features?

Several papers **[4-6]** mentioned the complete clinical, radiological and serological data of COVID-19 patients with Mucormycosis but till now no one discussed in details the specific histopathological features.

If Mucormycosis-induced histopathological changes are overlooked by the pathologist, it can end in disaster. Therefore we aimed to correlate the pathogenesis with the pathognomonic histopathological features for early detection of cases and early management.

Pathogenesis of mucormycosis in COVID-19 patients (Fig. 9):

Why are patients with COVID 19 an ideal environment for germination of mucorales spores? Several contributing factors were reported to determine the severity and complication of Mucormycosis infections: Host, virulence of the organism and environmental factors **[3]**.

Several studies **[1,3]** reported uncontrolled diabetes with ketoacidosis as an independent risk factor during the pandemic of COVID-19. Another important factor is abuse of corticosteroids whatever the duration of therapy.

The fungus invades, then proliferates and later disseminates within the host. Several initiating and predisposing factors contributed to these three processes. The major players in our journey are microphages (neutrophils), macrophages (histocytes) and endothelial cells.

1- Invasion of the host barriers: Necrotic or ulcerated mucosa and skin facilitate the penetration of the organism to the host body **[3]**.

2- Evasion the immune system and proliferation: Two predisposing factors establish and augment this step (Phagocytic dysfunction and Iron overload).

A-Defect in the phagocytic system **[7-9]**: Both mononuclear (macrophages) and polymorphnuclear phagocytes (microphages/neutrophils) have important roles in the normal defense against mucorales. Macrophages provide the initial defense by phagocytosis and oxidative killing of Mucorales, while neutrophils have the key role in killing fungi during the established infection.

COVID-19 patients had severe neutropenia reflected as a major defect in phagocytosis which declares the important role of neutrophils in inhibiting fungal spore proliferation.

Additionally, Diabetic ketoacidosis (hyperglycemia and low PH) lead to dysfunctional Phagocytosis as they impaired neutrophilic chemotaxis. Furthermore, there is marked impairment in intracellular killing either by oxidative or non oxidative mechanisms. Phagocytic dysfunction alone cannot explain the high incidence of mucormycosis in COVID-19 patients with DKA.

Some authors **[7-9]** emphasized the role of neutrophils in mucormycosis as the most important immune cell; they stated that patients with severe neutropenia are at increased risk of mucormycosis. Not involving patients with AIDS in that risk group suggests that neutrophils not T lymphocytes are the most critical for inhibiting Mucorales proliferation.

Abuse of corticosteroids leads to augmentation of phagocytic dysfunction and hyperglycemia whatever the duration of therapy as stated by Singh et al., 2021 **[1]**.

B- Iron overload: The main vital processes of mucormycosis depend mainly on iron. Patients with COVID-19 had serum iron overload which was exaggerated by acidic PH (diabetic ketoacidosis) which augmented the growth of mucormycosis [10].

Several mechanisms increased the virulence of mucormycosis via increase in iron uptake from host serum; FTR1-gene encoding high affinity iron permease, siderophores and heme **[11-13]**.

So the suggested novel therapeutic modalities are: Anti-FTR1 passive immunotherapy, iron chelator. They can decrease the virulence of Mucorales via reducing iron uptake from the patient's serum [11].

Previous literature **[14]** before COVID-19 pandemic stated that hematological malignancy were the main source of iron overload due to frequent blood transfusion.

3- Vascular dissemination: Easy access of vasculature is achieved via Glucose-regulated protein (GRP78) which is a receptor that mediates penetration and damage of endothelial cells by Mucorales **[15]**.

The associated hyperglycemia and iron overload in COVID-19 patients increased the expression of GRP78 in different tissues (nasal sinuses, lung and brain) resulting in more and more penetration of vascular endothelial cells **[16]**.

Another novel therapeutic line is the use of anti-GRP78 immune serum to minimize the angioinvasion property of mucorales **[15]**, but still need more molecular studies.

Endocytosis and early attachment of mucorales to the extracellular matrix (laminin and type IV collagen) were mentioned in the literature as different mechanisms of mucormycosis angioinvasion **[17,18]**.

Mycotic angioinvasion lead to Mycotic thrombus followed by ischemic necrosis of the affected tissue and hematogenous spread of mucormycosis

to other organs. Ibrahim and his colleagues [3] stated that localized tissue necrosis interferes with delivery of leukocytes or antifungal treatment to the infected foci. That leads to severe damage with more co- morbidity.

As we mentioned the main virulence of mucormycosis depends mainly on its ability to pick up iron from patient's serum in different mechanisms and its powerful angio-invasive property. Several other putative factors render mucormycosis more virulent e.g.: Lytic enzymes (proteinases and lipolytic) [19], mycotoxins and thermo tolerance [3].

Mucormycosis mimics malignancy in its behavior; it can attack and penetrate any tissue irrespective of the anatomical or histological barriers [20,21]. We reported that Sino-nasal mucormycosis can attack and invade the epithelium, mucous glands, muscle, fibrous tissue, cartilage, bone and eventually the vessels (angioinvasion).

In the current work, mucorales could be seen in all examined sections with characteristic irregular broad hyphae and surrounding Splendore-Hoeppli phenomenon. Tissue processing creates artifactual changable angle of branching of mucorales (45-90 degree) which is modified by surrounding interstitial pressure. Furthermore, tissue folding during sections processing creates false septations (artificial lines) of the hyphae. Cornely and his colleagues [2] confirmed the previous findings and reported that the irregular wide thickness of hyphae is more reliable criteria than septations and angle of branching for diagnosis of mucorales.

Splendore-Hoeppli phenomenon could be seen in the current work as deeply esinophilic hyalinized-like material around mucorales. Prof Hussein, 2008 [22] emphasized the role of antigen antibody complex deposition (Type III hypersensitivity like reaction) in inducing this phenomenon. We found a fibrinoid-like material within the detached basement membrane of the invaded vessels and necrotic nasal tissue.

A few literatures [1,2] described a little about the histopathological features that help in diagnosis of mucormycosis in COVID-19 patients during the pandemic.

A wide spectrum of histopathological features was detected in COVID-19 patients with Sino-nasal mucormycosis. We categorized them into five reactions; inflammatory, vascular, bone, fat and reactive hyperplastic changes.

The inflammatory reactions are mainly a mixture of acute and chronic inflammatory cells with a tendency to form a characteristic Mycotic abscess with eosinophils and multiple giant cells.

One of the newly detected histopathological features in mucormycosis cases in the current research is Histocytic Emperipolesis; neutrophilic or esinophilic cell penetration of inflammatory giant cells with intact cell membrane for both the invading and the host cells [23]. The exact mechanism is unknown and in need for more molecular research.

We found necrotic bone to be another pathognomonic feature of mucormycosis. We detected bimodal areas of bone necrosis; the first revealed dense mixture of inflammatory reactions, reactive bone formation and sequestrated bony specules which are consistent with pyogenic (fungal) osteomyelitis. The second demonstrated necrotic bone tissue with empty lacuna, absence of osteoclasts and presence of periosteal fibrosis suggesting avascular osteonecrosis which is induced and exaggerated by Mycotic thrombosis. Both processes are contributing to development of bone necrosis in nasal mucormycosis.

Both pyogenic osteomyelitis and osteonecrosis are considered late sequence of mucormycosis infection, the mucorales spread either by direct invasion of the adjacent bone or by hematogenous spread [24].

We relied on certain histopathological features to differentiate between both types of bone necrosis. Hansen and his colleagues [25] mentioned the previous histopathological criteria for differentiation between different types of bone necrosis.

We noticed wide areas of wiped out fat; paucicellular fat necrosis. Two factors contributed to the fat necrosis of nasal tissue when overloaded by COVID-19 infection, the first is direct viral infection and the associated neutrophilic enzymes which lead to enzymatic autolysis of fat. The second is an ischemic fat necrosis due to Mycotic thrombi. Areas of fibrosis and inflammatory reaction were usually detected around fat. Mucorales could be seen within the necrotic fat and in the surrounding inflammatory reaction.

Definite fat necrosis in COVID-19 patients could not be illustrated except in a research which was conducted by Lagana and his colleagues [26], they mentioned steatosis of hepatic cells as main finding in COVID-19 patients.

Rapid dissemination of mucormycosis is an extraordinary phenomenon as reported by Maartens et al., [27]. They stated that even a delay few hours in diagnosis could be fetal. This feature of mucormycosis is attributed to its virulent property of angioinvasion. We detected multiple transmural invasions of variable-sized blood vessels by mucorales, followed by formation of intravascular Mycotic thrombi which lead to ischemic necrosis and infarction.

Angioinvasion lead to rapid systemic dissemination of the fungus and local exaggerated destruction of the affected tissue due to ischemia and necrosis. Inflammatory influx rich in immune cells and antifungal therapy could not reach the ischemic tissue leading to exaggerated destruction and more vicious circle of fungal dissemination.

We recommended golden histopathological features for the diagnosis of mucormycosis in COVID-19 patients: Mycotic abscess with multiple giant cells and Emperipolesis, Mycotic bone necrosis and/or osteonecrosis, Paucicellular fat necrosis and angioinvasion.

Furthermore, a few guidelines should be mentioned for early identification of mucormycosis in nasal biopsy of COVID-19 patients: Firstly, awareness and present thinking of fatal mucormycosis in dealing with patients of COVID with exaggerated nasal symptoms. Secondly, combined use of Masson trichrome and PAS stains highlights the broad esinophilic hyphae with more brightness in Masson trichrome stained sections. Thirdly, avoiding confusion with other mimickers e.g. Aspergillus by constellation of the previous mentioned criteria.

Unfortunately, the late diagnosis and the usual lines of management (disfiguring surgical debridement and aggressive systemic antifungal) made the overall mortality and morbidity high. Early biopsy, proper diagnosis and new therapeutic modalities are safeguards against the co-morbidity of mucormycosis in COVID-19 patients.

Multiple suggested therapeutic modalities should be considered: Passive immunization with anti-FTR1p immune serum to protect from infection with R. oryzae, administration of iron chelator to prevent profuse growth of mucormycosis, breakdown of link between the endothelial cell and the organism via administration of anti-GRP78 immune serum to minimize the angioinvasion property of mucorales, in addition to the usual modalities of therapy which include the correction of neutropenia and hyperglycemia.

Conclusion:

Early histopathological diagnosis and new therapeutic modalities are essential to decrease the morbidity and mortality rates in COVID-19 patients with mucormycosis.

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Declarations:

- Competing interests: Author declares no conflict of interest.
- Funding: No specific funding was included or contributed.
- The study protocol was reviewed and approved by Ethical Committee of Sohag, Faculty of Medicine.
- Availability of data and material: All raw data and materials were available.
- Authors Contribution: Fatma El-Zahraa Salah El-Deen Yassin carried out manuscript writing & design, figures analysis & manipulation and histopathological diagnosis. Fatma Mohammed Hamdan collected the clinical, demographic and radiological data with archival revision of paraffin blocks and slides.

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نظرة هستوباثولوجية فى تشخيص الفطر الأسود الأنفى فى مرضى الكوفيد وطريقة تطوره

يعد الفطر الأسود لمرضى الكوفيد ١٩ الذى يصيب الأنف ويتمد إلى العين ثم الجهاز العصبى من أشد الفطريات فتكا ويكون مصحوباً بمرض السكرى الغير منضبط واستخدام مثبطات المناعة.

ويهدف البحث إلى التشخيص المبكر والدقيق لمرض الفطر الأسود معتمداً على الصفات الهستوباثولوجية له وعلاقتها بتطور المرض.

ولقد شملت الدراسة ٢٨ عينة من الأنف لمرض الكوفيد فى الفترة من يناير إلى أغسطس ٢٠٢١. ولقد تم جمع البيانات الإكلينيكية والفحوصات والاشعاعات من التقارير فى الأرشيف وتم فحصها هستوباثولوجيا بصبغة الهيماتوكسلين والباث والماسون ترياكروم.

وتم إيجاد مجموعة من الصفات الهستوباثولوجية المميزة لتشخيص الفطر الأسود وهى : الخراج الفطرى وموت أو تنخر العظام الناتج من الإصابة بالفطر أو نتيجة قطع الدم وتخثر الدهون وغزو الأوعية الدموية. ولقد وجد الفطر الأسود محاطاً بهالة حمراء خاصية (سبلندر هويل).

كما تم إثبات إيجابية عينات المرضى للكوفيد بالمسحة أو بالأشعة.

ولقد ثبت أن التشخيص المبكر وابتكار أنواع جديدة من العلاج للفطر الأسود هى الحلول المثلى لتقليل المضاعفات والموت الناتج عن إصابة مرضى الكوفيد بالفطر الأسود.