**Objectives**:

The main goal of the present study was to investigate the diagnostic value of molecular methods specific for the detection of *Aspergillus* species and *Mucorales* order in fresh tissue samples from Acute invasive fungal rhinosinusitis (AIFRS) patients and compare it with conventional techniques. Secondarily, to correlate between different risk factors and the infecting fungus.

**Materials & Methods:**

This cross-sectional study was carried out on 50 clinically, endoscopically and radiologically diagnosed AIFRS patients who were managed in a tertiary referral hospital from October 2021 to January 2023. Fresh tissue samples were collected during surgical debridement and subjected to histopathological examination, culture and microscopic identification, and semi-nested PCR with specific primers for *Mucorales* and *Aspergillus*.

**Results**:

Among the 50 studied patients, 56% were males while 44% were females with mean age of (53.3 ± 15.3) years**.** Histopathology confirmed the AIFRS diagnosis in 47 cases (94%), while it was only able to identify *Mucorales* hyphae in 21 samples and *Aspergillus* hyphae in 7 samples. Cultures yield only 39 positive samples (78%), *Mucorales* in 27 samples, *Aspergillus* in 9 samples, and mixed colonies of both in 3 samples. Microscopically, *Rhizopus* was the most common organism in followed by *Mucor* and *Aspergillus Fumigatus*. Species-specific seminested PCR was able to identify the causative fungi in all samples (100%). *Mucorales* seminested PCR was positive in 11 culture-negative samples. Also, the 19 samples, that histopathology diagnosed them as AIFRS-positive but failed to specify the fungi type, were diagnosed by semi-nested PCR as Mucorales in 15 samples, Aspergillus in 3 samples, and mixed infection in 1 sample. Moreover, seminested PCR detect Mucorales in all 3 histopathologically-negative samples. Also, the 3 mixed infection detected by both culture and seminested PCR were wrongly diagnosed by histopathology as *Mucorales* only in one sample, *Aspergillus* only in another one, and histopathology couldn’t identify the hyphae type in the third. Finally, by reviewing the clinical data, we found that the most common risk factors were diabetes, steroids intake, and Covid-19 in 70% of the cases and they were more susceptible to *Mucorales* infection.

**Conclusions**:

Histopathological examination of tissue samples is the cornerstone of confirming the invasive nature of fungal infection; however, it couldn’t distinguish between Aspergillosis and Mucormycosis in many cases. Although culture and microscopy techniques are important for detecting the causative fungi and antifungal susceptibility testing, they yield high percentage of false negative results even with avoiding tissue homogenization. Seminested PCR with species specific primers for *Mucorales* and *Aspergillus* is an effective tool in identifying the causative fungi of AIFRS from fresh tissue samples and it is recommended to confirm the negative histopathology and culture results. Still, extreme caution must be taken to avoid false positive results by environmental contamination during the molecular detection. Finally, the most frequent causative fungi of AIFRS during the study period was the *Mucorales* order and it was more common in patients with neutrophil dysfunction and favoured milieu for fungal survival and invasion resulted from comorbidities as uncontrolled diabetes, prolonged use of corticosteroids and Covid-19 infection.