

Infectious Sources of Histoplasmosis and Molecular Techniques for its Identification

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Abstract

Histoplasmosis, a fungal infection caused by *Histoplasma capsulatum* (*H. capsulatum*), acquired from contaminated soil with droppings of chicken or birds and found to be distributed in many parts of the world. The prevalence of histoplasmosis has not well studied in Nepal. The common symptoms of acute and epidemic histoplasmosis include high fever, cough, and asthenia and weight loss. Most of the infections associated with histoplasmosis are asymptomatic. People with compromised immune systems such as HIV/AIDS (PLWHA), cancer, and organ transplant recipients are at risk of developing this disease. In this review, we have summarised the current status of histoplasmosis in Nepal and molecular techniques available for its identification. To date, the significant outbreak is not reported in Nepal, but the risk of infection for the vulnerable population cannot be undermined. Appropriate preventive measures and treatment on time can reduce the burden of this fungal disease. Further, this review is also focused on molecular identification of *H. capsulatum*. Hence, careful considerations by concerned stakeholders for national surveillance programs and the treatment of patients on time after proper diagnosis is highly recommended.

Keywords: Histoplasmosis, asymptomatic, vulnerable, treatment

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Introduction

Histoplasmosis is acquired by inhalation of spores of *H. capsulatum*, which is usually found in the soil contaminated by bird droppings, which reveals the higher risk of disease in farmers, gardeners, poultry keepers, construction workers, pest control workers and in some instances travelers visiting caves and tunnels[1]. *Histoplasma* is a dimorphic fungus, which produces mycelial form in the soil environment and converts to the yeast form at host body temperature (37 °C), and it usually does not develop the symptomatic infection. So people need not concern about the infection [2]. However, for immune-compromised people, the fungus can result in severe infection. Very young children and elderly people who have a weak immune system; are more likely to get histoplasmosis disease [3].

The development of infection and the dissemination of *H. capsulatum* are initially dependent on the condition of the host body[4].

The majority of infected persons could have either no symptoms or a very less mild sickness, which is hard to recognize as the cause of histoplasmosis [5]. The clinical symptoms generally occur only in a small number (around 1%) of the population when exposed with *H. capsulatum* spores [6]. Persons who are usually immuno-compromised and are unable to develop effective cell-mediated response are likely to develop symptomatic disease during the period of acute dissemination in body [5], which includes infant child, patients with HIV/AIDS, organ transplant recipients, and those with hematologic deficiencies, and also those patients, who are on corticosteroids drugs treatment [7]. An individual is under the risk of developing symptoms even after years leaving the endemic vicinity of histoplasmosis if the man or woman was in an immuno-suppressive situation at that time [8]. Histoplasmosis is of four major types: pulmonary histoplasmosis, progressive disseminated histoplasmosis,



cutaneous histoplasmosis, and African histoplasmosis [9, 10].

Pathogenesis of Histoplasmosis

The fungal infection starts after the inhalation of spores produced by the mycelial form of *H. capsulatum* and the spores deposited inside the alveoli of the lungs [11]. After some time, the spores start to germinate inside the alveoli at normal body temperature to become dimorphic form. Then pulmonary macrophages engulf that yeast form of dimorphic fungus [12]. After the engulfment, the yeasts become a parasite and multiply within the alveolar cells, then travel to hilar and mediastinal lymph nodes [13]. When yeast form fungus gets access to the blood circulation system, then disseminates more rapidly and swiftly across various organs of the body. About after two weeks of exposure, the macrophages become fully fungicidal, then the cellular immunity starts a defense against the fungal particles [14]. It leads to necrosis at the site of infection like in the lungs, liver, spleen, lymph nodes, and on bone marrow, adrenal glands, and mucocutaneous membranes, which results in progressive disseminated histoplasmosis [4] as shown in Figure 1. The progressive type of histoplasmosis is much more lethal and severe as compared to other kinds of histoplasmosis [15].

Epidemiology

Histoplasmosis is distributed worldwide, but incidence cases are often reported around the periphery of river valleys [16]. The majority of infections in humans are reported from the central United States, whereas the small number of cases are also reported from Brazil, Argentina, India, and South Africa [17]. Currently, due to an increase in the occurrence of histoplasmosis worldwide, scientists have been developing several diagnostic strategies. Histoplasmosis endemicity can be evaluated by population-based use of a histoplasmosis skin test [18]. The skin test was found to be useful when a major endemic area of histoplasmosis identified in the north-eastern and Midwestern United States [19].

Increased number of infectious diseases like histoplasmosis is being reported in areas where

it was previously not thought to be prevalent, and the changing distribution of infectious diseases can become an important step to guide diagnostic workup and direct public health awareness [17]. Notably, in tuberculosis-endemic regions, disseminated histoplasmosis can easily be misdiagnosed as tuberculosis because of its similar clinical symptoms and must be considered as histoplasmosis infection in patients who do not respond to empiric anti-tubercular therapy [20].

An estimated discern indicates that nearly 3,000 people develop kidney failure yearly in Nepal [21]. Similarly, there are additionally a high number of the population in Nepal with a diabetic condition; the 2016 Diabetes Profile has shown that 9.1 % of the Nepali people are dwellings with diabetes [22]. Likewise, the cases of HIV/AIDS is also in excessive number inside the country [23]. According to National Centre for AIDS and STD Control (Nepal), there are around 31,020 HIV/AIDS sufferers in the country, and it is estimated to upward thrust at a rate of 2 patients per day. Those persons having conditions like a diabetic, organ transplant, and the person with HIV/AIDS have a weak immune system, which means that they are at high risk of developing other secondary infections like histoplasmosis during their lifetime.

According to the published reports, the geographical distribution of histoplasmosis has not been clearly understood [4]. The occurrence of histoplasmosis in Asia has not fully appreciated until recently. Malaysia is the first country in Asia, where *H. capsulatum* was isolated from soil samples [24]. India, a close neighboring of Nepal, has also reported a high number of histoplasmosis cases in West Bengal and Assam till 2018 [24]. From, 1995 to 2018, about 388 cases of *H. capsulatum* has been reported, and the number is in increasing order in India [24]. In recent years, an increasing number of histoplasmosis cases have been recognized among HIV-positive and/or diabetic patients in India [1]. In case of Nepal, histoplasmosis has only been described four times, Amatya et al., (2014) reported that the

cases of histoplasmosis in an Indian national who traveled to Nepal for medical care and once in a Nepali citizen, making this the second case described in the literature by Subedi et al., (2016) [25]. Recently, another case has been published by Sharma and Adhikari (2019). According to them, a 52 years old man with diabetes shows adrenal involvement in histoplasmosis [26].

Additionally, one case has reported in a Nepalese migrant to the USA with evidence of infection being acquired in Nepal by Gandi et al., in 2015 [27]. Nepal and India have an open border, and a large number of Nepalese migrants is directly dependent on Indian economic and cultural aspects such as employment, education, social relationship, and trade among other elements. Such socio-economic relationship directly has an impact on the dissemination of the diseases, including histoplasmosis.

Identification of *H. Capsulatum* and diagnosis of histoplasmosis

Traditionally, identification of causative agent of histoplasmosis is carried out through tissue culture or body fluids at 25°C on Sabouraud's dextrose agar to allow the growth of mycelial phase of *H. capsulatum*. For the proper growth of mold, it takes about six weeks. After the incubation period, two different types of conidia are formed. The tuberculate or macroconidia are of about 8 – 15 µm in diameter and have a thick wall. Similarly, the microconidia are tiny about 2 -4 µm in diameter and are more infectious when compared to the more abundant conidia. However, the procedure of culturing is time-consuming [28].

Likewise, histopathological examination is another technique for detection of the *Histoplasma*. It is generally done for severely ill patients [29]. The method includes tissue biopsy. During the histopathological observation, other organisms also could mimic the appearance like that of *H. capsulatum*. So, this could be eliminated by using another stain like methenamine silver or periodic acid- Schiff stains, which visualize *H. capsulatum* [30]. Detection of circulating *Histoplasma* associated antigen in urine and serum is another diagnostic option to detect the presence of the fungi using

an immunological technique like sandwich enzyme immunoassay (EIA), and it was first described in 1986 [31]. This technique generally uses *H. capsulatum* antigen, which is a polysaccharide, a polyclonal rabbit anti-*Histoplasma* immunoglobulin G linked to biotin, and horseradish peroxidase [4, 18]. The assay also shows the highest sensitivity against AIDS patients who had disseminated histoplasmosis [9].

Antibody tests are also performed to detect several forms of *Histoplasma* cases. There are two standard assays for antibody detection they are, (i) complement fixation (CF) test, which is based on the use of two separate antigens- yeast and mycelial (or histoplasmosis)- and (ii) immunodiffusion (ID) assay [32]. The ID assay tests generally detect the presence of M and H precipitin bands. An M band often existing in persistent types of histoplasmosis and lasts for many months to years after the infection. An H band is also indicative of the severe form of histoplasmosis [17]. The ID assay is approximately only 80% sensitive but is more specific than CF assay because in CF assay, cross-reaction may occur with other fungal infections like sarcoidosis [33].

Molecular identification of Histoplasmosis

In recent days, the molecular approach is widely used as a reliable and rapid technique for the detection of diseases with higher sensitivity and specificity. At present, the use of chemoluminescence labeled DNA probe for the detection of specific sequences of ribosomal RNA (rRNA) has been able to detect and confirm all the *H. capsulatum* isolates from the culture [32]. AccuProbe is one of them, which is based on the principle of nucleic acid hybridization. A single-stranded chemiluminescent labeled DNA probe is complementary to the sequence of ribosomal RNA of fungus. After the rRNA released from fungi, the steady DNA-RNA hybrid formed and that hybrids are detected and measured by using Hologicluminometer. A positive result is given by reading equal to or higher than the predetermined cut-off values, and an adverse

effect is the cost less than that of cut-off values [39].

Polymerase chain reaction (PCR) Assay

PCR is based on the amplification of the fungal gene for identifying mycoses. In 2003, Guedes et al. designed a nested PCR based technique for the rapid detection of fungi. The oligonucleotides used in this method is based on the M- antigens of *H. capsulatum var. capsulatum*. The oligonucleotides *Msp1F* - *Msp1R* (pair 1) and *Msp2F* - *Msp2R* (pair 2) results amplicon of size 111 bp and 279 bp, respectively [34]; (*Msp1F*: 5' - ACA AGA GAC GAC GGT AGC TTC ACG - 3' and *Msp1R*: 5' - GCG TTG GGG ATC AAG CGA TGA GCC - 3'), (*Msp2F*: 5' - CGG GCC GCG TTT AAC AGC GCC - 3' and *Msp2R*: 5' - ACC AGC GGC CAT AAG GAC GTC - 3'). The PCR reaction was performed with 100ng DNA amplified in a 25 µl reaction using 20 pmol of oligonucleotides (35 cycles of denaturation, annealing, and chain extension) [34].

By using this protocol, 100% specificity and accuracy were mentioned in the 31 examined strains from the animal, soil, and human [34]. The results also confirmed the absence of other fungal strains such as *H. capsulatum var. farciminosum*, *Paracoccidioides brasiliensis*, *Candida spp.*, *Sporothrix schenckii*, *Cryptococcus neoformans*, etc in the amplified products [34].

Real-time PCR (RT-PCR)

The real-time RT-PCR or qPCR shows promising results in the diagnosis of histoplasmosis infection, which currently identified the *H. capsulatum* among several other fungi [35]. Simon et al., (2010) carried out research for the detection of *H. capsulatum* based on real-time PCR using the TaqMan probe [36]. For this study, specific primers and probe (TaqMan labeled with fluorescent dye 6-carboxyfluorescein at 5' end and a nonfluorescent quencher at the 3' end) were used for the analysis of the internal transcribed spacer (ITS) region of the rRNA. The oligonucleotides HcITS-167F (5'-AACGATTGGCGTCTGAGCAT-3') and HcITS-229R (5'-GAGATCCGTTGTTGAAAGTTTTGA-3') were used. The following probe HcITS-188P

5'-6-FAM-AGAGCGATAATAATCC-MGB-3') was used. The specificity of RT-PCR was calculated to be 95.4% in comparison to the culture method. During the process, there is no PCR inhibitor detected. Among the 275 samples which were previously reported to be negative in culture method, 11 samples were reported as positive by RT-PCR with a specificity of 96.0%. Similarly, among the 341 samples tested, zero nonspecific signals were recorded [36].

Despite this promising research result, for the detection of *H. capsulatum* in clinical samples, there are no currently FDA-approved molecular assays available. Moreover, to date, the molecular approach for the detection of histoplasmosis is in a developing stage. Similarly, an improved and reliable technique has to be designed as a dependable approach for the detection of the disease. Regarding the future accessibility of molecular techniques for the detection of histoplasmosis is not unclouded in resource-limited countries like Nepal [37].

Treatment of Histoplasmosis

Based on the current status, most patients with mild acute histoplasmosis limited to lungs will be resolved after a month without specific treatment. However, the disseminated or the severe form of histoplasmosis should be treated with antifungal agents [17].

The treatment of chronic and severe form of pulmonary histoplasmosis is generally done by prescribing amphotericin B (3.0–5.0 mg/kg daily for 1–2 weeks, intravenously), followed by itraconazole (200 mg 3 times per day for 3 days and after that 200 mg for two times a day, for a total of 3 months) is required and recommended [38]. Similarly, for progressive disseminated histoplasmosis amphotericin B (3.0 mg/kg daily) is recommended for about 7–15 days, which is followed by oral drug itraconazole having 200 mg concentration for daily three times for three days and after that 200 mg daily for two times for a total of at least a year). Likewise, for the patients with the immunosuppressed condition, itraconazole daily (200 mg) is recommended as prophylaxis [19,38].

Research status of histoplasmosis in Nepal

In Nepal, there is poor hygienic practices and sanitation in major parts of the country and is offering the home for the continuous emergence and re-emergence of several life-threatening infectious diseases. So, Histoplasmosis could be a serious issue of public health in days to come. In Nepal, the majority of the population lives in rural areas with minimal health care facilities. The burden of the disease is much higher in rural settings compared to the urban areas. Although the pattern of diseases might change regularly, infectious diseases remain the leading cause of mortality and morbidity in Nepal. In many rural settings, cases of some diseases are often identified only through their clinical signs and symptoms. Lack of proper public health awareness and treatment of infectious diseases made people prone to several fungal infections, including Histoplasmosis, which may be a severe issue [40]. As the pervasiveness of HIV/AIDS and Tuberculosis are budding swiftly, the infection from the *Histoplasma* may lead to a significant effect [41]. Thus, careful diagnosis and treatment on time are paramount to control the future outbreaks of histoplasmosis. Moreover, the probability of undiagnosed histoplasmosis dissemination also urges for the need of proper diagnostic strategies of *Histoplasma* spp. infection in Nepal. As per institutional information, there is no routine examination and treatment of histoplasmosis infection in Tribhuvan University Teaching Hospital and National Public Health Laboratory (NPHL-Nepal), while both of them are considered as reference health service centres of the country.

Challenges in Nepal

As Nepal is under the way of development, the budgeting for the health and its related sector is very less. Lack of proper investment in the health sector for the diagnosis and treatment of diseases is a significant problem in Nepal. Death of hundreds of people by unknown disease is also prevalent. Lack of proper government strategies and operative ventures in the health sector has been a cardinal cause of those strange

deaths. Allocation of the budget on the health sector is only attentive to the treatment of some severe disease, which shows that the country has less interest in disease diagnosis. It is one of the significant consequences of the death of people from unknown conditions. Therefore, early diagnosis of histoplasmosis is mandatory to reduce the burden of illness.

Conclusion

The clear and accurate picture of the dissemination of histoplasmosis is complicated to understand in the resource-limited clinical sector of Nepal. Many factors could contribute to this, like lack of proper lab facilities, lack of expertise on fungal infections, etc. Additionally, not much reliable data are available from the government and academia due to incompetence in the diagnosis strategies. The published data are also in the form of case reports based on a hospital visit. It is already emphasized that the pockets of endemicity do exist in Nepal, which could lead to a significant impact on public health. Hence, careful considerations and the molecular approach of disease identification may be a suitable alternative. To address this problem, further research should be done immediately in Nepal

Conflict of Interest

The authors declare no conflict of interest.

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