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Caso clinico

Case report

Early diagnosis of cerebral aspergillosis with various methods: a case report

Diagnosi tempestiva di aspergillosi cerebrale effettuata con metodi differenti: caso clinico

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INTRODUCTION

Cerebral aspergillosis (CA) is a rare infection observed among immunosuppressed children with haematological malignancies, progressing with a mortality rate of over 95% [1]. The most common agent is *Aspergillus fumigatus;* however, cases with *Aspergillus niger* and *Aspergillus terreus* have also been reported [2]. The spread of *Aspergillus* species in the central nervous system (CNS) generally takes place via sinuses and is primarily observed in the frontal and the temporal lobes. Following this dispersion, the clinical manifestation is frequently presented as brain abscess. Meningitis, myelitis and carotid artery aneurysm are less common manifestations [3]. Since the cerebrospinal fluid (CSF) is non-diagnostic CA, the significance of serological and molecular methods in the early diagnosis and treatment is rising everyday along with the conventional methods [4]. The following case has been presented so as to indicate the significance of serological and molecular methods used in the early diagnosis of this pediatric patient with CA.

CASE REPORT

A 4-year-old girl with fever, sore throat and headache was referred to the General Pediatric Outpatient Clinic of Erciyes University Medical Faculty with signs of oedema in the eyelids, anemia, thrombocytopenia and neutropenia following the use of antimicrobials. The patient was referred to the Outpatient Clinic of Paediatric Haematology Oncology Department and was found to have pallor, ecchymosis and hepatosplenomegaly on physical examination. Upon detection of atypical cells in the peripheral smear, bone marrow aspiration (BMA) was performed.

Determination of L1 type lymphoblasts at a rate of 80% in BMA confirmed the diagnosis of acute lymphoblastic leukemia (ALL). The ALL treatment protocol was initiated. On the following days of chemotherapy, fever and neutropenia developed, in addition to focal seizures in the left arm, and Magnetic Resonance Imaging (MRI) was performed to clarify these findings. On the MRI, a lesion of 12 mm size in the right frontal lobe and nodular lesions on the left cerebellum and on the cingulate gyrus in the superior part of the left corpus callosum were detected.

The findings were regarded as brain abscess. Pathological and microbiological samples were obtained from the lesions. Tissue samples that were forwarded to the department of mycology were homogenized with physiological serum for direct inspection and fungal elements were found on microscopic examination (Figure 1). Culture from tissue sample was performed on Sabouraud Dextrose Agar (Oxoid, United Kingdom). The medium was incubated at 37°C and 25°C. The serological methods of serum galac-

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Figure 1 - Potassium hydroxide (KOH) 10% direct smears of brain biopsy showing 45-degree branching dichotomous hyphae and large septate hyphae (original magnification x400).

tomannan (GM) test (Platelia Aspergillus EIA; Bio-Rad) and (1→3)-β-D-glucan (BG) test (Fungitell, Associates of Cape Cod) were performed. The results of the GM test were displayed on the optic reader (ELX808, Bio Tek, USA) at a wavelength of 50 nm.

The Index value was calculated for serum samples using the control sera and samples with ≥0.5 ng/ml were regarded as positive.

The BG test results were interpreted with a reading for 40 minutes at one minute intervals at a wavelength of 450 nm, performed using the incubated enzyme-based immunological method (ELISA) reader (ELX808, Bio Tek, USA). The provided detection range of the kit was indicated as 31.25-500 pg/ml. As per recommendation of the manufacturer, <60 pg/ml was regarded as negative, >80 pg/ml as positive, and 60-79 pg/ml as a suspicious result. In our case, the consecutive serum GM values were determined as 3.39 ng/ml and 0.72 ng/ml, while the consecutive serum BG values were 93 pg/ml and 356 pg/ml.

Figure 2 - Colonies on Sabouraud dextrose agar.

The real time polymerase chain reaction (Rt-PCR) test was performed, both on tissue and serum samples.

The tissue sample was homogenized by 100 µl Buffer ATL (Qiagen) tissue lysis buffer and prepared for DNA isolation.

The Heliosis DNA isolation system (Metis Biotechnology, Turkey) was used to obtain nucleic acid from serum and tissue samples. The obtained DNAs were processed with RtPCR (Light Cycler-Roche, USA) in order to increase the number of targets specified for *A. fumigatus*. The Light Cycler Fast Start DNA Master Hybridization Kit (Roche, USA) and the specific primary probe mixture (TıbMolbiol, Germany) were used to prepare the reaction mixture. For each reaction, 15 µl of mixture was transferred to capillaries (Light Cycler 1.5/2.0). A sample of 5 µl was added to this mixture to obtain a final concentration of 20 µl.

The denaturation cycle was repeated as per the following scheme: 45 times, 10 seconds at 95°C, 15 seconds at 58°, and 18 seconds at 72°C. The following step was the melting curve from 95°C to 40°C.

The serum RtPCR value was determined to be negative, while the tissue RtPCR was found to be positive.

In the culture, white colonies were observed in the initial stage, which gradually adopted a green color (Figure 2). On microscopic examination of the slide culture, hyphae with septa, domed vesicles, phyllites covering half of the upper portion and conidia arranged in columns were observed and defined as *A. fumigatus*. The species-based description was made as per microscopic image.

Antifungal sensitivity tests were carried out using the E-test (AB Biodisk, Solna, Sweden) method which is faster and simpler than the CLSI method for routine use in laboratories as per recommendations of the manufacturer, and the minimal inhibitor concentration (MIC) values were determined as 0.25 µg/ml, 0.16 µg/ml and 0.125 µg/ml for amphotericin B, caspofungin and voriconazole, respectively. Treatment was commenced with amphotericin B and

voriconazole and maintained for 14 days. With improvement in the general health status and negative control serum BG and GM values, the patient was discharged with recommendations.

DISCUSSION

CA is a major clinical manifestation observed in immunosuppressed paediatric patients, carrying a high rate of mortality [5]. The agent pathogen generally spreads via the bloodstream following a systemic infection or alternatively, it may spread to adjacent structures following a rhinocerebral infection.

Neutropenia and long term steroid use in patients are the main factors which influence the spread of the disease [6].

Symptoms such as fever, headache, mental state disorders and focal neurological deficits are common in patients with cerebral aspergillosis, while meningeal symptoms are quite rare [4]. Similarly, fever and focal seizure were found in the current case and neutropenia developed in association with the ALL treatment protocol.

CSF is non-specific in terms of diagnosis of CA. In general, no growth is seen in CSF cultures prepared for *Aspergillus* [4]. Diagnosis of CA requires histopathological evidence of tissue invasion, eventually supported by microbiological cultures.

However, in immunocompromised patients, biopsy samples may not always be obtained due to neutropenia and coagulation issues [7]. Since early diagnosis and treatment are crucial, alternative diagnostic methods alongside conventional methods should be developed. For this purpose, serological and molecular methods may be used as supplemental techniques.

The GM test is an enzyme-based immunological method used for determination of the GM exo-antigen of *Aspergillus* species in the cell wall.

The serum GM cut-off value is 0.5 as per FDA recommendations [8]. Viscoli et al. reported, a trial on bone marrow transplant patients suspected to have CA and indicated that serum GM values were beneficial in terms of the early diagnosis of CA [9].

In another trial, no growth was seen in the culture of brain tissue samples of a patient with cerebral aspergillosis caused by *Aspergillus flavus,* while the serum GM, BG and PCR values were reported as positive [10].

Another serological test is the BG test, which determines the mould and yeast components, apart from *Zygomycetes* and *Cryptococcus* species, found in the cell wall [11]. The only disadvantage of the test is that it is not specific for a single fungus species.

False-positive results may be seen in the BG test associated with gastrointestinal colonization, mucositis and use of various antimicrobials. In a previous trial, false-positive results were reported with the use of certain cephalosporins, carbapenem and ampicilline-sulbactam; however, they indicated that these results should be supported with further trials [12].

Similarly, carbapenem and cephalosporin administered in this patient were not associated with a false-positive status.

A number of molecular methods are used in the diagnosis. Unresolved issues reported in association with molecular methods include as use of samples (tissue, serum, bronchoalveolar lavage) extraction protocol, amplification systems (nested, conventional) protocol (real time, quantitative and conventional) or selection of primer (specific for *Aspergillus* or for all moulds, panfungal) [13].

The RtPCR serum may be processed in sterile body fluids and tissues [13]. However, it is not yet standardized for fungal diagnosis. In the current case, the RtPCR was also performed, both in tissue and in serum samples, and the tissue RtPCR was found to be positive while the serum RtPCR was negative.

Liposomal amphotericin B was indicated as the most efficacious antifungal agent in the medical treatment of CA; however, cases successfully treated with voriconazole as a single agent or combination of voriconazole with caspofungin have also been reported [15, 16].

Besides, the combination of amphotericin B with antifungals like flucytosine and voriconazole or has also been reported [17]. Similarly, the current case was successfully treated with a combination of amphotericin B and voriconazole.

In conclusion, serological GM and BG tests and the molecular RtPCR method were shown to be significant in terms of the early diagnosis of CA in our case, together with culture-based methods; these findings should be supported with further large scale trials to be conducted in the coming years.

Keywords: cerebral aspergillosis, galactomannan, (1→3)-beta-D-glucan, PCR.

SUMMARY

The clinical and laboratory diagnosis of cerebral aspergillosis (CA) is problematic and mortality is quite high, even in cases receiving appropriate treatment. Therefore, an early and accurate diagnosis may prove to be life saving in patients with the diagnosis of CA. In this report, a case of CA which developed in a pediatric patient with acute lymphoblastic leukemia (ALL) is presented. Upon development of neutropenia and focal seizures in the left arm during implementation of the ALL treatment protocol, brain MRI was performed in the patient and nodular lesions compliant with brain abscess were detected in the frontal lobe, left cerebellum and the cingulate gyrus on the superior aspect of the left corpus callosum. Direct assessment of brain tissue revealed fungal elements, while consecutive serum galactomannan (GM) values were determined as 3.39 ng/ml and 0.72 ng/ml, and the consecutive serum (1⊠3)-beta-D-glucan (BG) values were 93 pg/ml and 356 pg/ml. Negative serum real-time polymerase chain reaction (RtPCR) and positive tissue RtPCR were determined, with growth of *Aspergillus fumigatus* in the culture.
Treatment was initiated with amphotericin B and voriconazole; upon disappearance of symptoms and negative control serum BG and GM values, the patient was discharged with recommendations. In conclusion, this case is presented with the objective of indicating the significance of serological and molecular methods used in the early diagnosis of patients with CA.

RIASSUNTO

La diagnosi clinica e di laboratorio di aspergillosi cerebrale (CA) pone notevoli difficoltà e il tasso di mortalità è alquanto elevato, anche quando il trattamento è appropriato. Pertanto, una diagnosi tempestiva e accurata può rivelarsi fondamentale per la sopravvivenza nei pazienti con diagnosi di CA. Nel presente articolo riportiamo un caso di CA verificatosi in un paziente pediatrico affetto da leucemia linfoblastica acuta (LLA). In seguito allo sviluppo di neutropenia e focal seizures *al braccio sinistro nel corso del protocollo di trattamento della LLA, il paziente veniva sottoposto a MRI cerebrale che evidenziava lesioni nodulari compatibili con un ascesso cerebrale a livello del lobo frontale, del cervelletto sinistro e del giro cingolato del corpo calloso sinistro. L'esame diretto del tessuto cerebrale mostrava elementi fungini; i valori sierici consecutivi di galattomannano (GM) erano pari a 3,39 ng/ml e 0,72 ng/ml, e quelli di (1 3)-beta-D-glucano (BG) a 93 pg/ml e 356 pg/ml. La PCR* real-time *condotta sul siero risultava negativa mentre quella tessutale dava esito positivo; l'esame colturale era positivo per* Aspergillus fumigatus*. Veniva quindi iniziato un trattamento con amfotericina B e voriconazolo. In seguito alla scomparsa dei sintomi e all'esito negativo del controllo dei valori sierici di BG e GM, il paziente veniva dimesso. In conclusione, questo caso clinico è stato presentato nell'intento di indicare l'importanza dei metodi sierologici e molecolari utilizzati nella diagnosi tempestiva dei pazienti con CA.*

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