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Severe hypoxia and multiple infarctions resembling Creutzfeldt-Jakob disease

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Abstract

Although neuropathological examination is still required for the definite diagnosis of Creutzfeldt-Jakob disease (CJD), specialised clinical assessment predicts probable CJD. Here we present a 73-year-old female patient presenting with rapid cognitive decline, visual, acoustic and cerebellar disturbances, ataxia and EEG changes compatible with early CJD stages. MRI revealed hyperintensities within the thalami, hypothalami, corpora mammillaria, the tectum and the cortex. Initial neuropathological examination showed severe cortical and subcortical spongiosis. However, both immunohistochemistry and Western blotting showed no pathological prion protein. Finally, small infarctions affecting the tectum, tegmentum, corpora mammillaria and global hypoxic-ischaemic changes could be identified as the probable reason for the changes interpreted as CJD-related pathology. Hypoxic-ischaemic CNS alterations mainly affecting the supply area of the basilar artery should be ruled out in case of probable CJD. In addition, severe spongiosis can be misleading in the histological examination, suggesting the diagnosis of a prion-induced spongiform encephalopathy.

Key words: Creutzfeldt-Jakob disease, hypoxia, infarction, mesencephalon, prion.

Introduction

Neuropathological examination is still the gold standard for the definite diagnosis of Creutzfeldt-Jakob disease (CJD) [9]. However, emerging data lead to the conclusion that probable CJD can be clinically diagnosed. For that purpose, the WHO established clinical parameters leading at least to the diagnosis of probable CJD. Together with the clinical symptoms, further tests such as the 14-3-3 protein test seem to have a positive predictive value of about 99% in the case of probable CJD [4]. The tau protein showed a sensitivity of 87% and a specificity of 97%, setting a threshold at 1300 pg/ml, and is therefore considered as a quite specific biomarker for CJD [14]. Furthermore, EEG can be used to estimate the stage or subtype of CJD [15]. Recently,

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MRI investigations have been proposed to be included in the diagnostic criteria of CJD [13]. Here we present a unique case with clinical symptoms of CJD, EEG changes, elevated tau and 14-3-3 proteins as well as even histological neuropathological changes compatible with a spongiform encephalopathy. However, final histological evaluations revealed mainly mesencephalic infarctions and severe global hypoxia while immunohistochemistry and Western blotting showed negative results for a prion-induced disease.

Case history

The 73-year old female patient showed a history of hypothyroidism, type II diabetes, polyneuropathy of unknown origin and gastrectomy. Two weeks before admission to the hospital, she complained of abnormal fatigue and loss of appetite, which was attributed to a pre-existent depressed mood. However, within a few days, she developed rapid cognitive decline and was disorientated in time and space. On admission, the patient showed severe amnestic aphasia with confabulation, both vertical and horizontal gaze palsy and hearing loss. She was unable to stand or walk alone. Movements were atactic and dysmetric. On the first CCT, no cerebral abnormalities could be detected. In MRI, which was performed one week after the CCT, mild hyperintensities within the white matter were seen. An additional MRI was conducted 6 days later, now exhibiting signal hyperintensities within the thalami, the hypothalami as well as in the dorsomedial tegmentum and the tectum, especially within the periaqueductal grey matter, being compatible with CJD (Fig. 1a, b). EEG examination revealed continuous bilateral fronto-temporal theta-delta activity, which is reported in early stages of Creutzfeldt-Jakob disease [6,16]. The differential diagnosis of Hashimoto encephalopathy was ruled

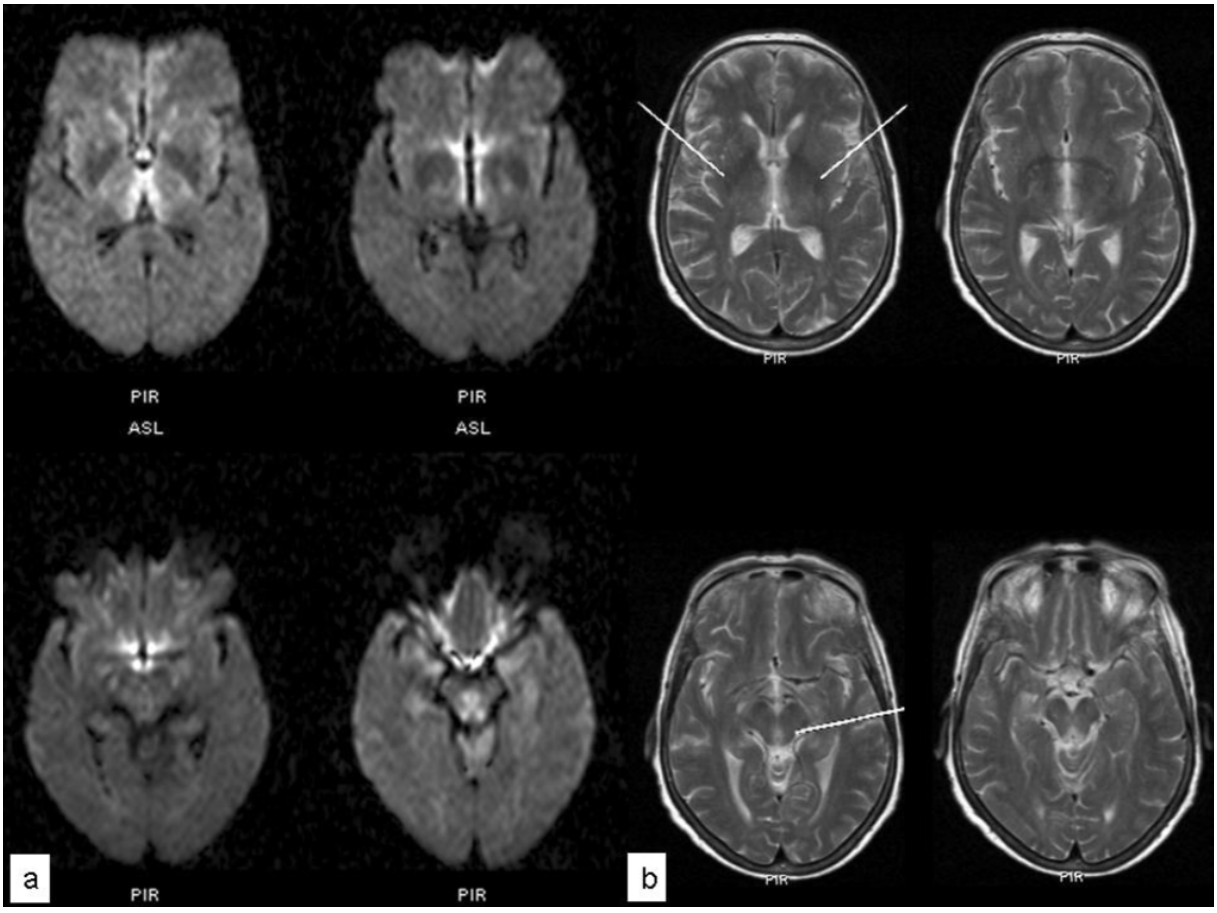

Fig. 1. (a) Diffusion-weighted cranial MRI showing bilateral restricted diffusion in the mamillary bodies and in the periaqueductal grey matter. (b) T2-weighted imaging with hyperintensities in the same regions

out by negative findings of specific auto-antibodies. The patient died one month after admission to the hospital.

Cerebrospinal fluid (CSF) studies

14-3-3 protein was positive in the CSF. In addition, multiple brain-derived proteins such as tau protein (1789 pg/ml; reference cut-off (rco): 195 pg/ml), NSE (47.6 ng/ml; rco: 12.5 ng/ml), and S 100b (10.5 ng/ml; rco: 2 ng/ml) were highly elevated. Cell count and protein were normal. No oligoclonal bands. Lactate was rather elevated with 5.2 mmol/l (normal 0.6 – 2.2 mmol/l).

Neuropathological findings

Since the clinical diagnosis was CJD, no visceral but only brain autopsy was performed. On macroscopic examination, the brain presented with mild global atrophy. No further pathological alterations were detected. Four areas (frontal, central, occipital and cerebellar) of one hemisphere were immediately stored unfixed at –80°C for Western blotting. The other parts of the brain were fixed in 4% buffered formalin for histological, immunohistochemical and paraffin--embedded tissue blot (PET blot) investigations. Histological examination revealed marked telencephalic cortical (Fig. 2a), subcortical (Fig. 2b) as well as cerebellar (Fig. 2c) spongiosis. Only very mild hypoxic changes were encountered within the hippocampus. More pronounced hypoxic neuronal changes were detected in the frontal lobe, caudate nucleus, the hypothalamus and the cerebellum (Fig. 2d). In the tectum and the tegmentum, especially dorso-medially from the aqueduct, pseudocystic changes (Fig. 2e), few macrophages (Fig. 2f) and marked reactive gliosis (Fig. 2g) were present. No other specific pathological alterations could be detected. PrP immunohistochemistry with antibody L42 was performed and was negative for pathological accumulations of the prion protein (Fig. 2h). No pathological prion protein deposits could be found within the hippocampus and the cerebellum using the PET blot technique (data not shown).

Immunoblotting

Tissue handling and Western blots were carried out as described [11]. Samples were homogenized and PK digested (100 µg/ml, 37°C, 1 hour). Soluble proteins were separated on SDS-PAGE (NuPAGE gel, Bis-Tris 12%, Invitrogen, Karlsruhe, Germany). The immunodetection of PK-resistant PrPSc was followed by using monoclonal antibody 3F4 (1:5000, Dako-Cytomation, Hamburg, Germany), which recognizes the human PrP residues 109 to 111. The bound antibodies were visualized with secondary antibody conjugated to alkaline phosphatase and nitroblue tetrazolium salt (NBT) with 5-bromo-4-chloro-3-indolyl phosphate (BCIP). No pathological prion protein could be detected (Fig. 3).

Discussion

Here we report the case of a 73-year-old female patient of whom the clinical symptoms, neuroradiological, EEG and especially CSF findings as well as routine histology were compatible with Creutzfeldt-Jakob disease, although the clinical course was faster than usually observed in prion diseases. However, immunohistochemistry, PET blot and Western blotting performed on CNS tissue specimens taken at autopsy showed negative results for pathological prion protein, and therefore CJD could be excluded in this case. Histologically, main findings included infarctions in the stage of resorption within the tectum measuring approximately 2 mm in diameter and also extending to the periaqueductal grey matter in a circular manner. Further, severe multifocal spongiosis was encountered as well as higher grade hypoxic-ischaemic changes in the midbrain, thalamus, hypothalamus, mamillary bodies, the cerebellum and also the frontal lobe. The age of the infarct correlates well with the onset of clinical symptoms. The ischaemic changes, located bilaterally in the midline of the dorsal mesencephalon, indicate a watershed infarction between the supply areas of the posterior cerebral and the superior cerebellar artery. These changes most probably originate from disturbed blood circulation in the basilar artery. The clinical presentation of patients with disturbances in the supply area of the basilar artery is very variable and depends on vascular anatomy [1]. Symptoms which are constantly found in these patients include visual, oculomotor and behavioural abnormalities often accompanied by somnolence and vivid hallucinations [3]. Motor problems are usually absent, although in uncommon variants of basilar artery disturbances severe bilateral ataxia with dysmetria in all four extremities has been reported [12]. In contrast, poor data about changes in the CSF protein content of 14-3-3, tau, S100b or NSE in human brainstem infarction are available. Positive protein 14-3-3 detection in the CSF was supposed to be a sensitive marker in case of clinical suspicion of CJD, although positive protein 14-3-3 was detected in patients with stroke or infectious CNS diseases [8]. Both 14-3-3 and tau protein have also been shown to be

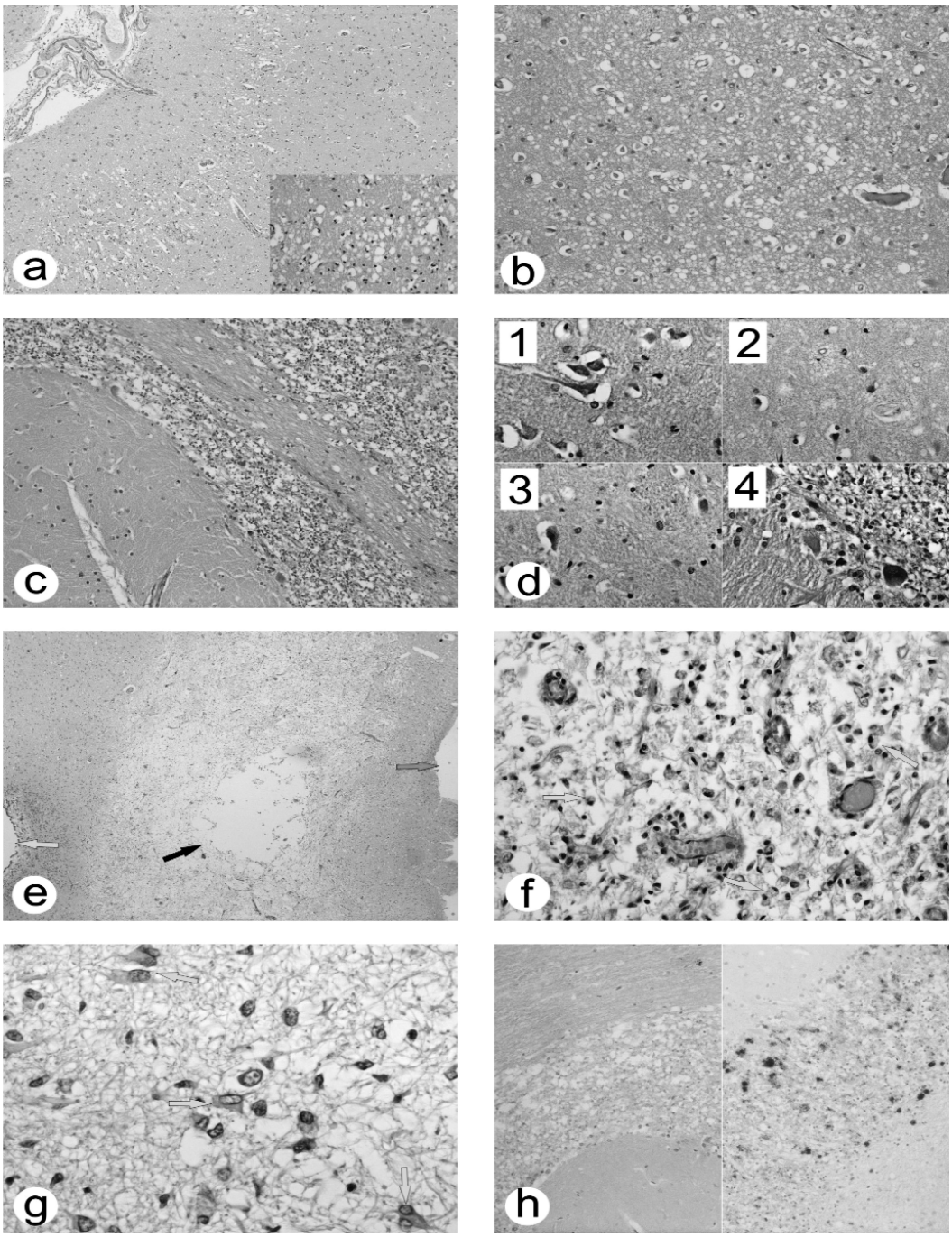

Fig. 2. Histology and immunohistochemistry. (a) Regionally, marked spongiform degeneration was observed in the frontal cortex (magnification 20×, inlet 200×) and subcortical ganglia (b, here in caudate nucleus; 100×) whereas the cerebellar spongiosis was milder (c; 100×). Hypoxic neuronal changes were found in several regions (d) including the frontal lobe (1), caudate nucleus (2), the hypothalamus (3) and the cerebellum (4). (e) Tectum with large pseudocystic changes (black arrow) dorso-medially of the aqueduct (yellow arrow) and the anterior lamina quadrigemina (green arrow). (f) PAS positive macrophages in the periphery of the cystic change (yellow arrows). (g) Marked reactive gliosis was also present with several multinuclear cells (yellow arrows). (h) Immunohistochemical analysis revealed no expression of PrP^{Sc} in any of the investigated regions (left, here shown for cerebellum). The positive control demonstrated strong staining for PrP^{Sc} (right)

elevated in multiple sclerosis patients, but the tau levels were clearly lower than in our presented case [2]. Tau is further released into the CSF after ischaemic CNS lesions and correlates with size and duration of the infarct [7]. In our case, tau protein level was again far higher than in the study of Hesse et al., where large infarcts were included, although the infarct size of the case reported here was very small. This might reflect the fact that neuronal damage in our case must have been severe, with the morphological correlate of multifocal spongiosis, a feature frequently seen in hypoxic-ischaemic brain lesions [10]. Elevated lactate in the CSF is also compatible with ischaemia. In summary, this case of severe CNS hypoxia with especially small brainstem infarctions is unique in terms of clinical manifestation, EEG findings, neuroradiology, CSF studies and routine histology, which were all compatible with CJD. Our findings therefore can add an important, but difficult differential diagnosis to the clinical spectrum of CJD, since thromboembolic events – the most frequent cause of basilar artery syndromes – require a fast clinical intervention and have a much better prognosis than CJD [5].

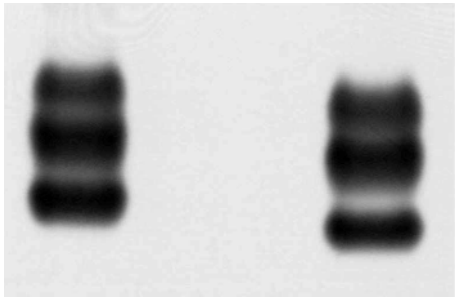

Fig. 3. Western blotting with proteinase K digestion. Lanes I and IV show two control cases of CJD patients exhibiting proteinase K resistant prion protein (Lane I: M/M1 variant; Lane IV: V/V2 variant). In contrast, in brain tissue of the presented patient neither in the frontal cortex (FC) nor in the cerebellum (CE) could proteinase K resistant prion protein be detected

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