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Seronegative brucella meningitis diagnosed by CSF PCR: report on seven cases

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Abstract

Introduction Neurobrucellosis (NB) can be associated with meningitis and present as a headache with or without meningeal signs. Pseudotumor presentation of NB has been reported to be accompanied by lymphocytic predominant cerebrospinal fluid(CSF) pleocytosis. NB is diagnosed by means of isolation of Brucella from blood or CSF and/or the presence of anti-Brucella antibodies in the CSF. Molecular techniques have been used in chronic or challenging cases of NB.

Clinical findings We report on seven cases of NB presenting with different types of headache and signs of meningeal involvement. In five cases, signs of intracranial hypertension were evident in the form of papilledema, sixth nerve palsy and blurred vision.

Diagnosis MRIs of the brain revealed signs of intracranial hypertension in three patients, basal meningeal enhancement in one patient and white matter lesions in one patient. Brucella serology in the blood and CSF was negative in all patients. It was interesting that four patients had normocellular CSF analysis with normal glucose and protein results. The diagnosis was made by Brucella PCR in all patients.

Conclusion NB should be considered in the differential diagnoses of pseudotumor cerebri syndrome in endemic areas. It is important to employ molecular techniques using sterile CSF samples in the investigation of Brucella.

Keywords Neurobrucellosis, Seronegative, Pseudotumor Cerebri, Brucella PCR

Introduction

Brucellosis is a common zoonotic infection worldwide and is an important public health problem [1]. It is highly transmissible through direct or indirect contact with diseased animals and from the consumption of unpasteurized dairy products. Brucellosis is endemic in Mediterranean and Middle Eastern countries. A lower disease incidence has been reported in more developed countries compared to low- and middle-income countries [1].

The yearly incidence of brucellosis in Iran has ranged from 0.73 to 141.60 per 100,000 individuals [1]. The burden of human brucellosis is significantly high in Iran and has increased in recent years, but is likely the result of improvements in the reporting system. The greatest

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burden of the disease has been observed in individuals aged 15–44 years [2].

Brucellosis, which can affect any organ system, has an incubation period of two weeks to several months. Nervous system involvement is an infrequent complication of brucellosis, but occurs in 3–10% of patients. This may be the result of the intracellular persistence of the microorganism or a possible immune mechanism triggered by the infection [3, 4]. Neurobrucellosis (NB) has classically been associated with meningitis, encephalitis, myelitis, radiculitis, neuritis and combinations of these diseases. Most patients with NB present with meningeal involvement. It generally occurs in patients without underlying diseases and may present as the first manifestation or at any time in the evolution of the disease. The illness can follow a subclinical course or manifest as acute or chronic infection [4].

The diagnosis of brucellosis is based on consistent clinical findings and direct or indirect evidence of Brucella in the cerebrospinal fluid (CSF). As cultures are positive in less than one-quarter of cases and conventional serological tests are not fully sensitive and specific, molecular techniques have been used in challenging cases of NB [4–6]. There have been limited reports of NB patients having negative serology where the diagnosis of brucellosis has been made by culture or PCR testing [7–11].

Pseudotumor presentation of NB has been reported in 1–4% of cases and is notably accompanied by predominant lymphocytic pleocytosis of the CSF. We report seven cases of seronegative NB with a pseudotumor presentation in most. These cases suggest that brucella should be placed high on the list of differential diagnoses of pseudotumor cerebri syndrome in endemic areas, especially in cases with atypical presentations or when the CSF analysis is abnormal, even in the absence of serologic findings [12–16].

Clinical findings

This study was conducted between November 2022 and August 2023 at a headache referral center of Sina Hospital in Tehran, which is affiliated with Tehran University of Medical Sciences. Seven patients diagnosed with NB were hospitalized during this period. The disease diagnosis was based on clinical symptoms consistent with neurobrucellosis and the extraction of Brucella DNA from the CSF.

The patient ages ranged from 38 to 59 years and comprised six females and one male. The patients were living in different cities in Iran. All but one patient had a history of consuming unpasteurized dairy products, including ice cream, cheese, yogurt and milk.

The duration of symptoms before diagnosis varied from 2 weeks to 3 years. All patients had sought medical attention at the hospital outpatient clinic or in the emergency room for headaches. Table 1 provides the headache characteristics of the patients. Most patients described the headache as generalized with moderate to severe intensity, but two had a pattern similar to trigeminal autonomic cephalalgia (TAC). Three patients experienced blurred vision and visual scotoma and two had diplopia.

Behavioral changes were evident in one patient and three patients experienced systemic symptoms that included fever, chills, nausea, sweating, back pain and weight loss. Patients with a prior diagnosis of migraine or tension-type headache had received headache treatments before visiting the hospital. One patient had misused injectable corticosteroids to control her headaches, leading to Cushingoid symptoms.

The initial diagnoses for three patients were pseudotumor cerebri, two cases of TAC, one case of meningoencephalitis and one case of new daily persistent headache (NDPH). Neurological examinations were normal for two patients, while the rest had papilledema. One patient exhibited bilateral sixth nerve paralysis and another had visual field limitations.

Diagnostic testing

The initial diagnostic procedure for all patients included brain MRI and MRV with gadolinium injection. Brain imaging was normal in two patients and one patient showed increased mucosal thickness in the paranasal sinuses. Symptoms of raised intracranial pressure (RICP) were evident in three patients in the form of optic nerve tortuosity and increased optic nerve sheath diameter (ONSD) or flattening of the posterior globe. In one patient, meningeal enhancement, especially in the basal meningeal regions, was visible (Fig. 1). In another patient, white matter changes were observed in subcortical and juxtacortical areas (Fig. 2). The MRV was unremarkable, except for unilateral transverse sinus hypoplasia in three patients. Four patients had visual field limitations in perimetry.

Blood tests showed increases in C-reactive protein (CRP) and the erythrocyte sedimentation rate (ESR) in three cases and leukocytosis in two cases. Brucella serology, including Wright and Coombs-Wright tests of the blood, were negative for all patients, while using sequential dilutions to correct for a potential prozone effect (Table 2).

A lumbar puncture was performed on all patients and the results are presented in Table 3. In four patients, initial CSF examination showed normal cells, sugar and protein. All patient CSF samples were checked for Brucella using the PCR method due to its presence in the endemic area. The diagnosis in all seven patients was confirmed by the positive PCR results. It is worth noting that both the Coombs-Wright and Wright tests were negative in the

Table 1 Clinical findings of cases

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Age	45	38	49	46	44	41	59
Primary diagnosis	NDPH	Pseudo-tumor cerebri	TAC	Pseudo-tumor cerebri	Pseudo-tumor cerebri	Meningo-encephalitis	Pseudo-tumor cerebri, secondary TAC
Presenting symptoms	Refractory headache, blurred vision, diplopia	Daily headache, visual scotoma	Daily headache medication overuse	Daily headache, transient visual obscuration	Headache	Headache, disorient., behavioral change	Rt. temporal pain, blurred vision, diplopia
Symptom duration	8 months	1 month	3 years	20 days	2 weeks	2 weeks	1 month
Headache features	General. pulsatile/pressure, daily	Temporo-occipital pulsatile/pressure, mod. to severe, positional awaken.	General. pressure moderate to severe	General. pulsatile, severe, awakening	Bi-temporal daily severe positional awakening	Bi-temporal pulsatile, awakening	Rt. temporal pulsatile, severe
Assoc. symptoms	Nausea, photo-phobia	Nausea, photo/phono-phobia	Nausea, phono-phobia	Nausea, photo/phono-phobia	Nausea	Nausea, vomiting	Nausea
Autonomic symptoms	-	-	Bilateral red eye and tearing	-	-	-	Eye injection and tearing, ptosis, rhinorrhea
Systemic symptoms	Fever, chills	Nausea nocturnal sweating, back pain, palp.	-	-	-	-	Weight loss, sweating, musculoskeletal pain
Neurologic exam	Papille-dema, bilateral 6th nerve palsy	Papille-dema	Normal	Papille-dema	Papille-dema	Normal	Papille-dema, decreased rt. visual field, rt. ptosis
Use of unpast. dairy product	Ice cream, yoghurt	Ice cream	Yoghurt milk	Ice cream	-	Yoghurt, milk	Cheese
Geographical region of Iran	West	Center	North	Center	Center	South	Center

NDPH: New daily persistent headache

TAC: Trigeminal autonomic cephalalgia

serum and CSF of all patients. High CSF pressure (28–40 cm H_2O) was observed in five patients.

Therapeutic interventions

All patients received intravenous ceftriaxone and cotrimoxazole as well as oral rifampin for one month. Oral treatment continued for five more months with doxycycline and rifampin. One of the patients experienced persistent headaches after one month of IV treatment and after being discharged with oral medications. Because of the persistent headache, the patient stopped treatment and, as a result, developed systemic symptoms of body pain, sweating and worsening of the headache. Reinitiation of the antibiotic treatment led to symptom improvement. Oral acetazolamide (500–750 TDS) was prescribed for four patients with high brain pressure and indomethacin was prescribed for one patient with a hemicrania continua pattern headache.

Follow-up

The clinical symptoms, including headache and systemic symptoms, improved after treatment. One patient developed skin lesions and another developed an oral ulcer after receiving intravenous cotrimoxazole. Both conditions resolved with corticosteroid administration. Mild gastrointestinal complications were observed in other patients. No other adverse effects were observed in the remaining cases, aside from the mild gastrointestinal complications.

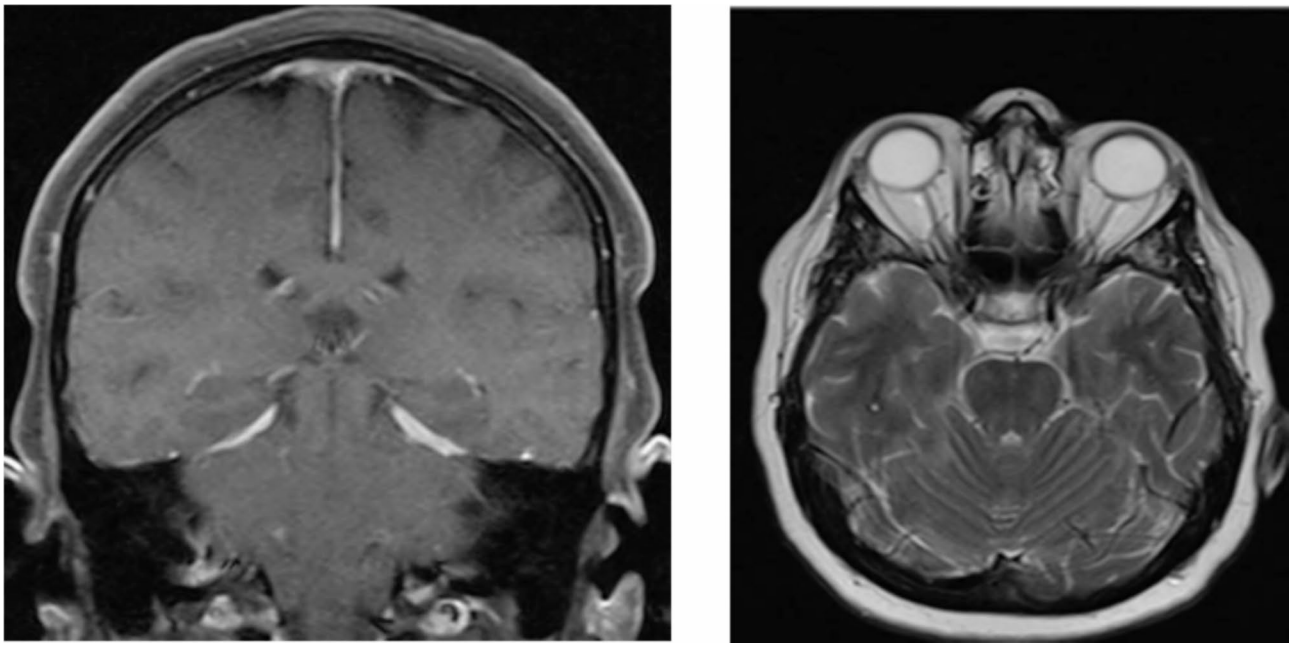

Fig. 1 Brain MRI with gadolinium of case 1: (left) meningeal enhancement; (right) increased ONSD

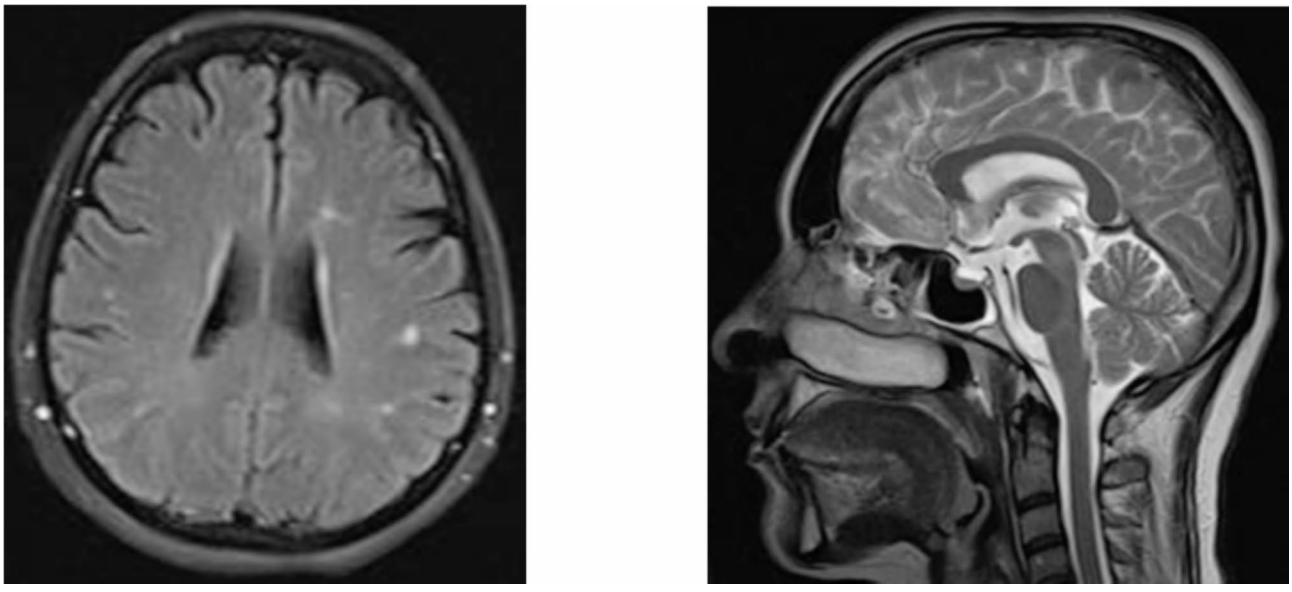

Fig. 2 Brain MRI of case 3: (left) axial image of multiple abnormal signal intensity in subcortical and juxtacortical white matter; (right) sagittal T2 image with partial empty sella

Discussion

We report on seven cases of seronegative NB who presented with progressive subacute or chronic headaches. They were diagnosed by molecular PCR-based methods on CSF and the findings were confirmed by clinical improvement after starting appropriate treatment.

The age range of our patients was 38–59 years with a predominance of females. In a review of 187 cases with NB in Turkey, the average age was 40.3 years (range 10–77 years) with males being slightly more affected than females [17]. Another review of 221 NB cases showed a mean age of 36 years with a male-to-female ratio of 1.68 [18]. The duration of patient symptoms before diagnosis in our series varied from two weeks to three years. In other studies, signs and symptoms that were related to CNS involvement were found to occur early in the course of the disease or up to one year after the onset of systemic symptoms [3, 18, 19]. In a review of 221 NB cases, 11.2% of the cases were sub-acute and 7.1% were chronic [18].

Table 2 Paraclinical findings

		Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
Brain MRI +/-		Basal meningeal enhance-ment, increased ONSD, tonsillar descent		Multiple abnormal signal intensity in subcortical and juxtacortical white matter, no abnormal enhance-ment, mucosal thickening of paranasal sinuses, optic nerve tortuosity, partial empty sella		Optic nerve tortuosity	Bilateral muco-sal thickening and polypoid changes in maxillary sinuses	Post-globe flatten., in-creased ONSD
Blood tests	**CRP**	59.9	7.4	14.5	11	7.4	3.3	8.1
	ESR	62	21	56	55	25	26	26
	Coombs Wright	neg.	neg.	neg.	1/80	neg.	neg.	neg.
	Wright	neg.	neg.	neg.	1/40	neg.	neg.	neg.
	2ME	neg.	-	-	1/40	neg.	neg.	neg.
	WBC	11,500	5600	7700	6900	13,300	6300	5000

Table 3 CSF findings

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7
OP	36	40	32	35	22	15	28
protein	101.9	65.9	25.8	25	22.5	125	44.1
WBC	280	64	2	2	0	72	2
Lymph (%)	40	85	-	-	-	90	-
GLC	63	59	52	64	54	50	50
Wright	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Coombs Wright	neg.	neg.	neg.	neg.	neg.	neg.	neg.
Brucella PCR	pos.	pos.	pos.	s.	pos.	pos.	pos.
Cytology	Lympho-cytic rich smears with some atypical cells	Some degen. monocytes and lympho-cytes in a clear back-ground	Some degencells admixed with a few mature lympho-cytes in a clear back-ground	-	Some lympho-cytes in a clear back-ground	Many mature lympho-cytes in a clear back-ground	Some degen, cells and few lymphocytes in a clear background

The most common route for transmission of infection was the consumption of yogurt and ice cream that contained unpasteurized milk. Other studies have reported unpasteurized milk as the most common source of infection [20]. Systemic manifestations of NB are highly variable and not always present [3, 4, 17]. Three of our patients complained about systemic symptoms of fever, chills, nausea, sweating, back pain and weight loss.

The initial complaint by our patients was headache, that has been reported to be the most common presenting symptom in NB [17, 18, 20, 21]. None of the available studies have mentioned a specific pattern for headache in NB; however, we noted a TAC-like pattern of headache in two patients and nonspecific headaches with migrainous features in the rest of our patients. These headaches could have been caused by meningeal inflammation with or without increased intracranial pressure, as five of our patients showed signs of RICP that included papilledema and sixth cranial nerve palsy.

Pseudotumor presentation of NB is very uncommon and only has been reported in 1–4% of cases [4–8]. Intracranial pressure may increase in NB as a consequence of cerebritis or impedance of the flow of the CSF caused by basilar meningitis. Al-Deeb et al. [14] reported four cases of pseudotumor cerebri out of 400 cases of brucellosis with a CSF pressure of 370 mm as well as 32–35/mm^3 cells (all lymphocytes) [12].

Diaz Espejo et al. presented an unusual case of NB in which intracranial hypertension syndrome developed over a period of one month [13]. The CSF pressure was 400 mm and it contained 195 white cells/mm^3 (92% lymphocytes) and 119 mg/dl protein. Panagariya et al. [14] reported a case of NB with a presentation similar to pseudotumor cerebri but with abnormal CSF results. Hence, it may be reasonable to consider NB in differential diagnoses of pseudotumor cerebri syndrome in areas with endemic NB, especially in cases with atypical presentations or when the CSF analysis is abnormal.

Examination of the CSF in NB typically reveals an elevated protein concentration, decreased glucose and moderate leukocytosis composed mainly of lymphocytes. It was interesting that four of our patients had

normocellular CSF with normal glucose and protein; however, three of these cases had increased opening CSF pressures and one had systemic symptoms consistent with brucellosis.

CSF pleocytosis has been reported in 91% of cases of brucella meningitis [15]. However, in a study by Naderi et al. [16] of 54 hospitalized adults with NB in Iran, about half of the patients had mild CSF pleocytosis of fewer than 50 leukocytes per microliter of CSF, including 17% with five or fewer leukocytes per microliter.

We have reported on seven patients with NB, all of whom had negative serologic test results for serum and CSF. A diagnosis of NB requires direct or indirect evidence of Brucella in the CSF. It depends on the demonstration of meningeal inflammation and detection of specific antibodies in the CSF, as cultures are positive in less than one quarter of cases, take time for assessment and are not always reliable [4, 5, 11]. The conventional serological tests also lack full sensitivity and specificity. The serum agglutination test (Wright test) has shown a high rate of false-negative results in chronic and complicated cases [20, 22, 23]. In limited reports of NB patients with negative serology in the literature, the diagnosis of brucellosis was made by culture or PCR test. Few of these cases had NB and the rest had systemic involvement [7–11]. In a study of 43 hospitalized patients with NB in Iran by Pourhassan [5], lumbar puncture confirmed lymphocytic pleocytosis in all patients; however, the CSF serology was negative in 40% of cases. A central nervous system (CNS)-specific immunological reaction due to persistent antigenic local stimulation could be the case in seronegative individuals.

The enzyme-linked immunosorbent assay (ELISA) can detect immunoglobulin classes including IgM, IgG, and IgA. It is the test of choice in the diagnosis of patients with brucellosis, especially those with chronic or CNS infection [22, 24]. However, this test was not performed in any of our patients, which is considered a laboratory defect of the study.

Molecular techniques have been used in cases where diagnosis of NB has been difficult, as well as in cases of seronegative organ-specific brucellosis. CSF PCR for Brucella has been proposed for diagnosis and follow-up of NB. It has been proposed that PCR assays are more sensitive than serological testing for detecting relapses of brucellosis [9, 25]. PCR assay in CSF samples is more rapid and sensitive than conventional microbiological tests [6, 26]. Molecular methods allow for the diagnosis of brucellosis in a few hours with high sensitivity and specificity. They remain positive for a long time in patients who are apparently asymptomatic and when clinical relevance is unclear. Conventional and real-time (RT) PCR assays directly detect Brucella -specific genes, including: BCS P31, BP26, 16 S rRNA, and the insertion sequence rRNA. However, the sensitivities of these assays are quite variable, ranging from 50 to 100%. The interpretation of molecular PCR-based results also requires careful attention because it may not necessarily indicate an active infection, but rather a low bacterial inoculum, the presence of DNA from dead bacteria or is from a patient that has recovered [27].

Paraclinical findings in the serum also showed increased ESR and CRP in two of our patients and leukocytosis in another two. ESR has been reported to increase in <25% of patients and the white blood cell count is often normal or low [5]. Abnormal imaging findings of NB include leptomeningeal involvement, basal meningeal enhancement, cranial nerve involvement, spinal nerve root enhancement, brain abscess or granuloma, signs of RICP, white-matter involvement with or without demyelinating lesions, vascular involvement, hydrocephalus and brain edema [28]. Postcontrast 3D Fluid-Attenuated Inversion Recovery (FLAIR) may provide more additional information for the depiction of leptomeningeal diseases [29]. Brain MRIs of our patients revealed signs of RICP in four of the seven patients that was accompanied by basal meningeal enhancement in one and white matter lesions in another one. Three patients had normal imaging of the brain. Erdem et al. [30] in Istanbul also reported normal CT or MRI scans in 54.3% of patients with NB.

There is no consensus for the choice or dosage of antibiotic used or the duration of treatment for NB. Combined therapy with doxycycline, rifampicin, trimethoprim-sulfamethoxazole or ceftriaxone for more than two months has been recommended [20, 31]. All of our patients were treated with intravenous ceftriaxone and cotrimoxazole along with oral rifampin for one month, followed by five months of oral doxycycline and rifampin. Clinical improvement was observed in all patients.

This study was limited by the small number of the reported patients. Furthermore, we did not perform ELISA testing in the serum or CSF of patients, which could be a highly sensitive and specific examination. We included neurobrucellosis cases who were referred to the headache clinic by prolonged undiagnosed headache complaint. More studies are suggested in different geographical parts to discover different forms of neurobrucellosis.

Conclusion

This case series reported on seven cases of seronegative neurobrucellosis with a pseudotumor presentation in most patients. It adds to the literature by demonstrating the importance of employing molecular techniques for the investigation of Brucella in sterile CSF samples. We suggest the consideration of NB in the differential diagnoses of pseudotumor cerebri syndrome in NB-endemic areas, especially in cases with atypical presentations or

when the CSF analysis is abnormal, even in the absence of serologic findings.

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Author contributions
M.T. contributed to patient diagnosis, study design, data collection and manuscript revision. E.J. contributed to the acquisition of data and writing of the manuscript. Z.S. contributed to writing the draft of the manuscript and data entry. N.R. contributed to the acquisition of data. S.A contributed to data entry. J.A. contributed to patient diagnosis.

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Data availability
Regarding the availability of data and materials, it is necessary to request access due to privacy and ethical restrictions. Rest assured, the authors are committed to protecting the confidentiality of the data and will provide access upon request, within the boundaries of these restrictions.

Declarations

Ethics approval and consent to participate
This study was a retrospective review, which, according to established guidelines, did not require review by an Ethics Committee (EC). The authors adhered to these guidelines and ensured that the study was conducted in an ethical manner. All authors have provided their consent for the publication of this article. Their collective agreement demonstrates their confidence in the research findings and their commitment to sharing this valuable information with the scientific community.

Consent for publication
Written informed consent was obtained from all patients for publication of their clinical details, and paraclinical data.

Competing interests
The authors declare no competing interests.

Conflict of interest
The authors declare no potential conflict of interest with respect to the research, authorship and/or publication of this article.

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