HUMAN LISTERIAL MENINGITIS: REPORTED FROM KARACHI, PAKISTAN

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SUMMARY : One hundred and twenty clinical samples comprising of cerebrospinal fluid (CSF) and blood of 60 patients showing symptoms of meningitis were screened for the presence of L. monocytogenes. One out of 60 CSF samples was found to harbor L. monocytogenes, incidence being 1.66%. This sample was procured from an 18 year old girl. Symptoms were indicative of meningitis or meningoencephalitis. There was no history of immunosuppressive therapy or any other underlying disease. The pathogen seems to have been transmitted through food. Organism was confirmed as L. monocytogenes by cultural, biochemical and serological tests recommended for identification of Listeria. Antibiotic susceptibility test showed that the organism was sensitive to penicillin, ampicillin, gentamicin and tetracycline. Ampicillin and gentamicin demonstrated a synergistic effect. The patient did not suffer from bacterimia as L. monocytogenes was not found in her blood. Blood samples of other patients were also negative in this respect. A comprehensive review of literature indicates that this is the first report of listerial meningitis from this region. The need of through screening is emphasized.

Key Words : Listeria monocytogenes, meningitis.

INTRODUCTION

Listeria is gm (+), microaerophilic, non-sporing, non-encapsulated, motile, coco bacillus. It has been isolated from a variety of environmental, food and clinical samples (1). For over fifty years the organism has been recognized as a cause of human disease, listeriosis. Most cases of human listeriosis appeared to be sporadic (5). Although the route of transmission of listeriosis is not exactly known but food borne out-breaks within past decades have established the Listeria as a food borne pathogen (6,22). Majority of listeriosis cases occur in those individuals who have suppressed T-cell immunity (15). Meningitis is a common manifestation of listeriosis in adults as well as in neonates, thus life threatening perinatal ailments have been reported by Buchdahl *et. al.* (2) and Gellin *et. al.* (7). In few food borne epidemics of listeriosis, pregnant women and neonates were the main target (4,22). There are also few reports of listerial focal infections resulting from bacterimia in immunocompromised patients. Focal infection include corneal ulcer, necrotizing ulcer, cataract extraction in eye, bacterial keratitis, septic arthritis, osteomyelitis, choleocystitis, peritonitis and pleuritis (7,11,12,25). Until recently it was thought that listeriosis is a rare illness in Asia but now it has crossed the geographical boundaries. Though percentage of listeric infection is still low but it's presence in raw and heated food samples is indicative of probable sporadic infection in this part of the world as well. We have previously reported contamination of milk, vegetables and fresh fruits by Listeria (23,24).

In the present study screening of clinical samples of

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potential patients of listeriosis was carried out for the presence of L. monocytogenes. We here report isolation of L. monocytogenes from one sample of CSF with an incidence rate of 1.66%. The organism was isolated from an eighteen year old girl. Meningitis was indicated by the characteristic symptoms viz stiffness in neck muscles, occurrence of fits and unconsciousness, CSF was found to be contaminated with small gm (+) rods which were subsequently identified as L. monocytogenes.

MATERIALS AND METHODS

Subjects and samples

Sixty individuals suspected to be at risk of listeriosis were included in this study. Patients were either admitted in or were visiting Out Patient Department of various local city hospitals. They were suffering from symptomatic meningitis or encephalitis. The ages of subjects ranged from three months to twenty years. Symptoms observed were rigidity of neck muscles, elevated body temperature, unconsciousness and in some cases vomiting. Samples of cerebrospinal fluid (CSF) were procured through lumbar puncture and transferred aseptically in sterile tubes. Two ml of peripheral venous blood was drawn and immediately mixed with EDTA in final concentration of 0.15% to prevent clotting. Breakdown of samples is given in Table 1.

Table 1: Statistics of samples screened for the presence of Listeria spp.

No. and type of samples	No. of sample revealing gm+ve rods	No of sample revealing L. monocytogenes	% of positive samples	Overall %
60-CSF	10	1	1.66	0.83
60-Blood	-	-	0	

Isolation and identification

All samples were directly streaked on Tryptose Phosphate Agar (TPA, OXOID) as well as subjected to cold enrichment. For cold enrichment 10 ml of TPB was inoculated with 1 ml sample of CSF and blood separately. All inoculated media were incubated at 4°C. Sub-culturing on TPA from these vessels were done first at 48 hrs then biweekly till 4th week. All the plates were examined using Henry's illumination technique (14). Colonies giving typical bluish tinge were considered to be of Listeria spp., and were purified and then subjected to standard microscopic and biochemical tests recommended for the identification of Listeria spp. (17). In vitro susceptibility of the isolate to a number of antibiotics tested using discs of ampicillin, tetracycline, erythromycin, ciprofloxacin, ampiclox, penicillin and gentamicin. The synergetic effect of any two antibiotics was evaluated through discs soaked in various combinations of antibiotic solutions. Inhibitory effect was measured in terms of diameter of the zones (mm).

Rapid slide agglutination test

For serological identification the isolates as well as standard cultures of L. monocytogenes and L. ivanovii were typed with antilisteria 'O' type l, type 4, and 1:4 polyvalent commercially prepared antisera (DIFCO). Organisms were grown in TPA, growth was suspended in FA buffer (DIFCO), heated at 80°C for 1 hr and then centrifuged at 3000-4000 rpm. Organisms were resuspended in small amounts of buffer. One drop of each heat treated organism was exposed to Listeria 'O' type l, type 4 and 1:4 polyvalent antisera. One drop of each heat treated culture was mixed with 0.3% normal saline and served as negative control. Slides were moved back and forth for proper mixing and then observed microscopically.

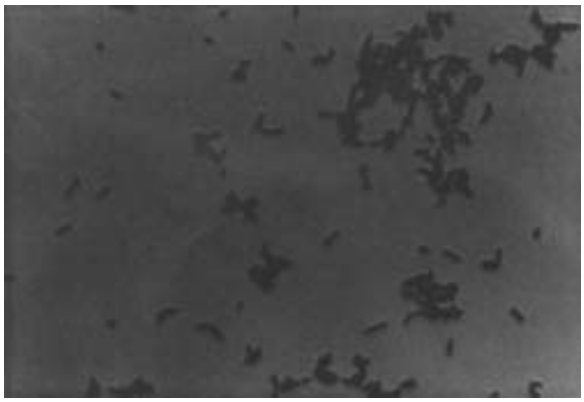
Immunofluorescent antibody technique

For further confirmation of our isolate immunofluorescent microscopy was performed using commercially prepared anti-Listeria monocytogenes 'O' sera type l, type 4 and 1:4 polyvalent antisera conjugated with fluorescein isothiocynate (FITC). The suspected cultures as well as standard cultures were smeared on glass slides. Fixed in alcohol (70%) for 10 minutes, then dried in air. One drop of each antiserum was placed on smears of each culture, incubated in moist chamber, washed with PBS and placed in the same. Slides were air dried mounted with mounting buffer, one drop of glycerin was placed on cover slip and then observed using U.V. fluorescent filters. Photography was performed using high speed (100 Din) Kodak film.

RESULTS

One hundred and twenty clinical samples comprising of CSF and blood were collected from 60 patients with symptomatic meningitis or encephalitis. These samples were screened for the presence of Listeria spp., 17/120 samples revealed gm (+) rods. They were purified and further tested for their characteristic motility at 20°C and 37°C. Only one isolate was found to be motile at 20°C and not so at 37°C. In gram stain preparations it appeared as gm (+), coco bacillus with palisade arrangement (Figure 1). The morphology was comparable with that of L. monocytogenes NCTC 7973 (Figure 2). As this was isolated from

Figure 1: Isolate CSF17 stained with Gram's method showing morphology similar to Listeria (x1000).



CSF it was designated as CSF17. The isolate was catalase (+) oxidase (-), urease (-), it failed to reduce nitrate to nitrite, produced acid but no gas from dextrose, dextrin, maltose, salicin and trehalose. It hydrolyzed esculin, hemolysed 5% sheep red blood cells (SRBCs) and gave positive CAMP test with Staphylococcus aureus (17). The culture was sensitive to penicillin, ampicillin, gentamicin and tetracycline. Ampicillin and gentamicin behaved well synergistically as compared to other combinations used, giving largest inhibitory zone. None of the blood cultures revealed any bacterial growth.

Serological identification of isolated CSF17 was performed using commercially prepared antilisteria sera. The organism was found to be L. monocytogenes as it gave prominent agglutination reaction with antilisteria type 1/2 a sera. CSF17 also showed strong agglutination when FITC conjugated antisera were employed (Figure 3). The results were comparable with standard cultures of L. monocytogenes serovariety 1/2 a NCTC 7973 (Figure 4). The results were also compared with L. monocytogenes serovariety 4a NCTC 5214m and L. ivanovii (Table 2).

Table 2: Serological reactions of some standard Listeria cultures and the isolate CSF17.

Antisera	L. ml/2a	L. m and 4a	CSF-17	L. iv.
Antilisteria 'O' antisera :				
Type 1	+	±	+	-
Type 4	±	+	±	-
Polvalent	+	+	+	±
Antilisterial FITC Conjugated :				
Type 1	++	+	++	-
Type 4	+	++	+	-
Polyvalent	++	++	++	±

DISCUSSION

Listeria infections can take a variety of courses ranging from virtually no observable symptoms to involvement of vital organs like brain and systems like central nervous system, often with high mortality (9-10). Meningitis and meningoencephalitis are most common manifestations of the disease in adults (3,19). Pleural fluid infection caused by L. monocytogenes has been reported by Mazzulli and Salit (16). There is high percentage of lethal outcome in the absence of prompt diagnosis and early antibiotic therapy. Serological tests alone are of low diagnostic value. Biochemical and cytological investigations on CSF are also not characteristic of Listeria brainstem involvement. CSF may show mild changes (13) or may appear normal (21). Computed tomography (CT) and magnetic resonance imaging (MRI) might supply evidence of brainstem involvement and contribute to diagnosis. However, their utility for diagnosis of listeriosis could be restricted as they are not available to common man in a developing country. It would also not be easy to differentiate between the pathological changes produced by a variety of etiological agents. Moreover, until now majority of CT investigations have been reported to be normal. Certain diagnosis can

Figure 2: L. monocytogenes NCTC 7973 stained with Gram's method showing normal morphology x 1000. Typical palisade V and Y shaped arrangement.

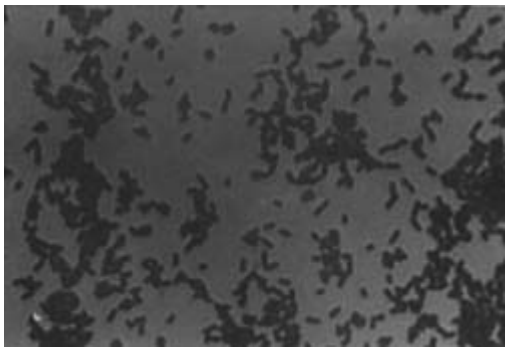
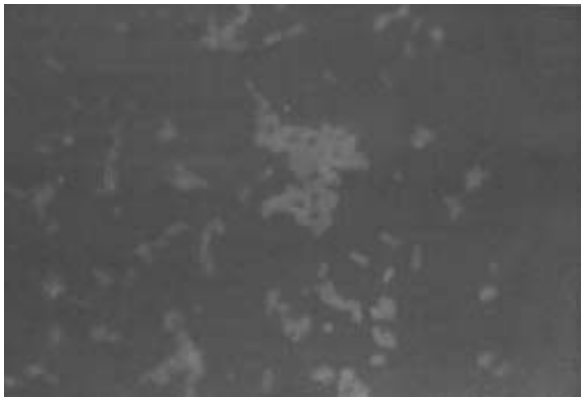


Figure 3: Immunofluorescent staining of isolate CSF17 in pure culture, employing anti L. monocytogenes 1/2a serum conjugated with FITC (x1000).



only be achieved through isolation of Listeria from body fluids like CSF and blood (15). Patients with signs and symptoms of meningitis should be examined for Listeria, their CSF and blood should be screened for L. monocytogenes, an approach adopted in the present study. Routine approach in the hospital laboratories is to screen the CSF of meningitis patients for gram (+) cocci which usually grow well within 24 hrs, a practice which does not reveal Listereae. Therapeutic doses of only the tested antibiotics should be administered.

Until recently there were no reports upon the incidence of listeriosis from Pakistan. Studies of Vahidy *et. al.* (23) revealed a 3% incidence in food and fodder samples screened over a period of one year. 4% samples of fresh fruits and vegetable to be consumed raw as salad, was also reported by the same group (24). The present study concerns one hundred and twenty clinical samples comprising of CSF and blood. These were screened for the presence of L. monocytogenes. While all other samples turned out to be negative in this respect. One out of sixty CSF sample revealed the pathogen. Since the CSF samples were from suspected cases of meningitis/encephalitis the incidence rate of listerial meningitis being reported here in is 1.66% with an over all incidence in clinical samples studied as 0.83%.

The patient, from whose CSF L. monocytogenes was isolated, was an eighteen year old girl admitted in a local city hospital. She was brought to the hospital unconscious. Her body temperature was elevated to 101°F and blood pressure was 90/75 mm/Hg. She demonstrated neurological symptoms like stiffness of neck muscles and spasms of limb muscles. In addition to the routine biochemical,

Figure 4: Immunofluorescent staining of L. monocytogenes in pure culture, employing homologous antiserum conjugated with FITC (x1000).

cytological and bacteriological testing, CSF was also screened for the presence of L. monocytogenes. A 48 hrs incubation on TPA at 37°C, revealed pure growth of gm (+) coco bacilli which was identified to be L. monocytogenes. The patient was discharged from the hospital against the advice of the doctor and thus her fate could not be ascertained. Her personal data revealed that there was no history of drug addiction, alcoholism, diabetes, malignancies, Acquired Immunodeficiency Syndrome (AIDS), blood transfusions or organ transplantation, conditions that usually predispose and individual to listerial infection (18). Thus it is presumed that consumption of Listeria contaminated food was the most probable cause of meningitis in our patient. Oh *et. al.* (20) have described well established listeriosis in one of their patients, in whom they could not pin point the source of infection. They suspected it to be correlated with consumption of raw boiled milk which the patient had consumed while he visited India. Since pasteurization is not a common practice in developing countries, we believe that a similar source may have been responsible for transmission of disease to our patient. Though there are only few reports of Listeria infection in our environment, in view of the high fatality rate attention should be focused on correct and early diagnosis of the disease.

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