

## Subacute sclerosing panencephalitis (SSPE) In Iraq

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### Abstract

**Objective:** Subacute sclerosing panencephalitis (SSPE) is a progressive inflammatory disease of the central nervous system with poor prognosis and high mortality, caused by the persistent infection with measles virus (MV). Despite much research into SSPE, its pathology remains obscure. Only 5% of individuals with SSPE undergo spontaneous remission, with the remaining 95% dying within 5 years of diagnosis. The prevalence of the disease varies depending on the measles vaccine immunization status. The clinical manifestations occur, on an average 6 years after measles virus infection and may initially present with a varied clinical picture ranging from psychiatric to ophthalmological to neurological symptoms and signs. The diagnosis is based upon characteristic clinical manifestations of myoclonus, the presence of characteristic periodic EEG discharges, and demonstration of raised antibody titer. We are reporting 8 cases of Iraqi patients with SSPE and we describe the test methodology used for detection of MV in those patients

**Methods:** Cerebral spinal fluid (CSF) and 5 ml of blood sample obtained from eight patients from those attended the neurology unite- Baghdad Teaching Hospital in the Medical City during the period from October 2010 to November 2013, were enrolled in this study, their ages were ranged between 7-10 years, with an average age of 8.2 years.

Enzyme linked immunosorbent assay (ELISA) were used for the detection of anti-measles virus IgG by the use of Bio ELISA Kits (Biokit S.A.Spain) , the positive results were confirmed by (RT-PCR) technique((DYNAL kits,USA) .

Protein electrophoresis were done for all CSF samples and oligoclonal bands (OCB) were detected by isoelectric focusing (IEF).

**Results:** ELISA technique showed that, 8(100 %) of SSPE patients were positive for serum and CSF anti- MV IgG . Those patients were subjected to PCR techniques as a confirmatory test. The results showed that all patients with positive result for IgG was positive for MV genomic DNA. Five patients (62.5%) showed evidence of oligoclonal bands (OCB).

**Conclusion:** This study showed that,The increased risk of developing SSPE after measles virus infection in young children underscores the importance of childhood immunization programs that decrease measles virus transmission and, therefore, reduce the risk of exposure to measles among infants. The success of global programs to eliminate measles will not only prevent the severe complications and death associated with acute cases of measles but will also prevent the devastating disease SSPE . in addition, the overall relative sensitivity of PCR for detection SSPE were found to be 100%.

**Keywords:** Measles virus; Polymerase chain reaction (PCR); Subacute sclerosing panencephalitis ( SSPE), oligoclonal bands (OCB).

### I. Introduction

Subacute sclerosing panencephalitis (SSPE) is a progressive fatal disease of the central nervous system that is caused by a persistent measles virus infection. Early clinical characteristics of SSPE may be variable, but they often include behavioral changes, cognitive deterioration, sporadic episodes of falling, and optic disturbances such as optic atrophy, chorioretinitis (1,2). As the disease advances, neurologic symptoms, such as myoclonic jerks or spasms, become more pronounced, and the patient develops severe physical and mental impairment. SSPE has an average period of latency of 7–10 years (range, 1 month to 27 years) after measles virus infection, and death generally occurs ~1–3 years after the onset of symptoms. There are known risk factors for SSPE, such as the development of measles virus infection at an early age (i.e., before 2 years of age), and most studies have noted that a greater proportion of cases of SSPE occur among males. The reason why measles virus persists in some individuals and results in deadly consequences is unknown, but it is likely to be host related (3,4).

MV belongs to the family of single stranded paramyxoviruses. It is the causative agent for several diseases including subacute sclerosing panencephalitis (SSPE) and measles inclusion body encephalitis (5,6). Measles ranked as one of the leading causes of childhood mortality in the late 20<sup>th</sup> century (7). In developing countries, 1 million deaths each year are related to MV infections.

The diagnosis of SSPE requires the fulfilment of at least three of the five criteria (8), which include i) a typical clinical picture of progressive subacute mental deterioration with typical signs like myoclonus; ii) characteristic EEG changes; iii) elevated CSF globulin levels greater than 20% of total CSF protein; iv) raised titers of measles antibodies in blood and CSF and v) typical histopathological finding in brain biopsy or autopsy

We are reporting eight cases of SSPE in the Iraq and we describe the test methodology used for detection of MV in those patients

## **II. Patients, Materials And Methods**

This study was conducted at the Teaching Laboratories- Medical City- Baghdad National and involved eight SSPE patients evaluated during the period from October 2010 to November 2013.

Eight patients aged 7-10 years at first symptom onset were considered to have adult onset SSPE and their data regarding measles vaccination, any history of measles, age at onset of symptoms and diagnosis, presenting symptoms, initial diagnosis, clinical staging, course of the disease, electroencephalographic changes, and measles anti-body status were recorded. Ethical approval letter to all patients was done.

Diagnosis was based on the characteristic clinical features of progressive cognitive and/or behavioral changes, myoclonus, electroencephalographic evidence of periodic complexes, and raised IgG (40-80 IU/ml) anti-measles antibody in cerebrospinal fluid (CSF) and (60-160 IU/ml) in serum, detected by ELISA in all patients.

Classical protein electrophoresis was done as described by Teitez. The formula used was  $\text{IgG (CSF)} \times \text{Albumin (serum)} / \text{IgG (serum)} \times \text{Albumin (CSF)}$ . The principle of electrophoresis is electrophoretic separation on agarose medium with a subsequent blotting with nitrocellulose membrane and visualization of separated protein bands with gold stain. As a routine 5 micro liters unconcentrated CSF was used. Reagents used were Agarose gel (usually serum protein gel is used), Nitrocellulose membrane, Filter papers, Gold Chloride (Chloroauric acid), Stannous Chloride, HCl Tween20 and Anhydrous Citric acid. Instruments in use were Beckman electrophoretic chambers, Electric shakers and heavy weights to press the gel.

### **Procedure**

One positive and one negative control with each strip containing 8 patient samples were prepared. Electrophoresis procedure was performed for 30 minutes at constant voltage using the setting of 100mV. For blotting, nitrocellulose membrane was used. After electrophoresis completion, the gel was removed from the chamber and placed in a clean dry surface. The gel was overlaid by sheet of Nitrocellulose membrane and then pressed with few absorbent papers by keeping heavy weights on them for 30 minutes. Incubation Gold Stain was prepared by adding to 75ml of deionized water the following reagents drop wise with vigorous mixing: 1 ml of 1% aqueous gold chloride, 0.4 ml of 1% stannous (prepared by dissolving 0.1gm stannous chloride in 0.5ml of 1M HCl and mixing to 10ml water to give a cloudy solution), 10 ml of 2% aqueous Tween20 and finally 25 ml of 9.6g/l of anhydrous citric acid.

After removing the filter papers, the lower sheet was immersed in the Gold Stain at room temperature. Staining was completed within 1 hour; after which the membrane was washed thoroughly with water and interpreted and then stored after labelling. The results were visually interpreted by finding a characteristic OCB in the gamma globulin region.

### **Detection and analysis of measles virus genome from CSF**

RNA was extracted from CS samples according to the procedure of the kits. RNA was extracted by use of the guanidinium-acid phenol method. The supernatant was then acidified and extracted using phenol-chloroform [27]. The RNA pellet was resuspended in 30 µL of RNase-free water, and the RNA concentration was determined by ultraviolet spectroscopy

### **RT-PCR and sequence analysis**

To prepare templates for sequencing, RT-PCR was performed using the Superscript One Step RT-PCR kit (Gibco BRL). Primers were designed to amplify the 450 nucleotides coding for the COOH-terminal 150 amino acids of the nucleoprotein. PCR products were purified using the PCR Preps DNA Purification System (Promega) and were analyzed by agarose gel electrophoresis followed by ethidium bromide staining. Templates were sequenced using a cycle sequencing reaction with fluorescent dye terminators (Applied Biosystems Division, Perkin-Elmer), and the reaction products were analyzed using an ABI 3100 automatic sequencer

(Applied Biosystems Division, Perkin-Elmer). Sequence data from multiple reactions were analyzed using the Genetics Computer Group Package (version 10.1; Accelrys) [//29]  
 The PCR primers used in the RT-PCR are known to amplify all genotypes of measles virus.

SPSS v10 software was used for statistical analysis.

Student's t test and Pearson x2 test were applied to compare various parameters. Data were considered significant for p values less than 0.05 (p(0.05).

### III. Results

#### Imaging

Two patients had normal CT while others, the CT changes were as follows: white matter hypodensities involving frontal/parietal/occipital/temporal regions (two), cerebral atrophy (one), thalamic hypodensities (one), and diffuse oedema (two). MRI was carried out in all patients. One patients had normal MRI. In the remaining seven patients, the MRI abnormalities included focal or diffuse white matter signal changes in three patients and cerebral atrophy of varying degrees in the other two, gray matter in one patient, u fibers generally spared.

#### EEG finding:

Digital acquired EEG showed paroxysmal activity in the form of generalized giant slow wave complexes which were time locked to the myoclonic jerks, and occurred at 4-6 seconds interval and slow back ground activity giving the typical periodic pattern which is seen in SSPE, ( figure -1).

#### Demographic profile

The demographic profile of those 8 patients (6 males, 2 females) were presented in table-1

No. of patients	8
Male/ female	6/2
Age range (mean)years	7-10 years (8.2)
Duration from onset to diagnosis	3 months
Interval between measles symptoms	6-8 years
Myoclonus	8 patients
Behavioral changes	8 patients
Visual disturbance	3 patients
Seizures (myoclonic, generalized tonic-clonic, atonic )	5 patients
Lateralizing deficits	4 patients

#### Clinical profile

Those presented with history of behavioral changes followed by myoclonic body jerks involving the upper limbs and facial muscles for 8 months-1 year. These myoclonic jerks were involuntary, more frequent during wakefulness and less frequent during sleep. Initially, these myoclonic jerks were infrequent but became worse after one year.

Currently, it is repeated more than hundred times per day; there is no clear loss of consciousness or eye deviation. Other seizure types described by parents include generalized tonic-clonic seizures and atonic seizures.

The drop attacks and generalized tonic-clonic seizures were reasonably controlled but the myoclonic jerks continued to occur frequently. During the course of the illness, the patient showed clear decline in her educational performance with poor attention span and emotional instability. No clear visual and hearing impairment was noted and ambulation was maintained. Past medical history was unremarkable, except that five of them had measles at around one year of age; the patient was fully vaccinated including MMR vaccine.

Family history was negative for epilepsy or other chronic neurological disorders. The patients' mini mental examination was on the lower side with non fluent slurred speech; there was no ophthalmoplegia and the rest of cranial nerves were normal. Fundoscopic examination show that one patient has optic atrophy, one with chorionetinitis , other normal.

Motor examination showed pyramidal signs in the lower limbs with brisk deep tendon reflexes and upgoing plantares; the gait was maintained; ataxia, no organomegaly or neurocutaneous stigmata. Systemic examination was unremarkable.

#### Laboratory studies

All patients underwent CSF analysis. The results presented in table-2:

Table-2: The laboratory finding of SSPE patients

Lab Findings	
Protein (mg/100ml)	20-96 (mean=30.6)
Glucose (mg/100ml)	48-78 (mean=50.2)
Total WBCs/C.mm	0-30
% of Lymphocytes	55-90
Conc. Of anti-Mesales (IgG-serum)	60-160 IU/ml *
Conc. Of anti-Mesales (IgG-CSF)	40-80 IU/ml *

\*Significant Conc. For anti-measles (IgG)=10.0 IU/ml

CSF oligoclonal band was positive in 5 patients (62.5%) indicating intrathecal immunoglobulin synthesis with high level of IgG index.

Comparison of PCR results was made with other investigative techniques like MRI, CT scan, EEG, OCB, ELIZA which could be supportive to the diagnosis of SSPE. Most of these were concordant with the final clinical diagnosis (Table-3).

Table-3: comparison of PCR with other investigative techniques

	No. of patients	%
MRI	7	87.5
CT scan	6	75
EEG	8	100
PCR	8	100
ELIZA (IgG)	8	100
OCB	5	62.5

#### IV. Discussion

SSPE is a progressive neurological disorder caused by persistent measles infection and failure to clear up primary infection due to defective measles virus replication cycle.

The mutated measles genome produces abnormal matrix protein (M-protein) which is important for viral budding and establishing extracellular infection. The preserved hemagglutinin and fusion protein allow spreading of measles virus from cell to cell (9).

SSPE patients develop a hyperimmune response to measles virus, which fails to control persistent infection (10).

The patients go into several stages. In stage I, behavioral and intellectual changes are noted; rarely SSPE is diagnosed at this stage (11,12).

In stage II, continued intellectual deterioration and onset of typical pattern jerk, both are hallmarks of SSPE. Visual impairment and dressing apraxia may be apparent indicating parieto-occipital involvement (13).

In stage III, prominent hyperkinetic movements, chorioathetosis are noted indicating basal ganglia involvement. Ambulation is usually clearly affected at this stage. Autonomic dysfunction is a prominent feature (14).

In stage IV, the patients become completely bedridden, the myoclonic jerks and seizures may disappear with the appearance of pathological laughter, persistence of autonomic dysfunction and startle reflex.

Ten percent of patients, develop slower progressive course, some may survive up to 10 years; but in another 10%, the disease has a more fulminant course (15).

EEG findings are quite characteristic of SSPE. The slow wave complexes occur in a periodic pattern with interburst interval of 4-12 seconds, the jerks are time locked to the

complexes. With the progression of the disease, the interburst interval gets shorter with further background activity slowing. Neuroimaging study correlates with the stage of the disease (16,17).

The white matter changes progress from occipital lobe to the frontal lobe, with subsequent loss of white matter volume. Basal ganglia show abnormalities. In neuroimaging, we can see areas of wedge like appearance of cortical and subcortical white matter involvement mimicking stroke (18).

Many studies have been performed in Western countries, to determine the role of PCR in the diagnosis of SSPE and all these studies found that MV was detectable in 100% of the patients (19).

Positive measles antibodies in serum and CSF are common, positive oligoclonal band in the CSF indicates intrathecal immunoglobulin synthesis.

The viral burden between patients differed up to four fold by quantitative PCR and corresponded with detection of measles virus protein. The level of both viral RNA and antigen in the brain may correlate with disease progression (20).

The introduction of measles vaccination program reduced the SSPE incidence. There is no proof that SSPE caused by measles vaccine as it was thought by some. The possible mechanism of SSPE in vaccinated

children is either due to the preceding primary measles infection or aborted vaccine. Currently, the patient has follow up with neurology service and infectious team was involved.

## V. Conclusion

We are reporting 8 cases of SSPE diagnosed and confirmed through serology and neuroimaging. Subacute sclerosing panencephalitis is a rare complication of measles infection. We need to keep it in mind during evaluation of any child presenting with progressive myoclonic epilepsy and behavioral and intellectual changes seek a history of previous measles infection as it may cause a latent infection. Although the treatment options are not promising but the introduction of measles vaccination program reduced the prevalence of the disease. PCR technique have high sensitivity for detection of MV in SSPE. This study is probably the first reported study in Iraq.

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