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# Somatosensory Evoked Potential for Detection of Subclinical Neuropathy in Egyptian Children with Acute Lymphoblastic Leukaemia

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**Abstract:** To evaluate neurological changes developing during paediatric Acute Lymphoblastic Leukaemia (ALL) therapy clinically and through electrophysiological Study of Somatosensory Evoked Potentials (SSEPs) changes in different phases of therapy. Thirty five-ALL patients with age range from 3-14 years were included compared to 30 healthy controls. History, neurological examination, complete blood counts, cytological examination of bone marrow aspirate and cerebrospinal fluid with Measurement of Serum Methotrexate (MTX) were done. The SSEPs were performed and patients subjected to another SSEP with measurement of serum MTX level before and 10 days after intra-thecal injection (IMTX). Clinical neurological findings in patients after induction were depressed deep tendon reflexes (43.3%), hypotonia (28.6%), lost pain sensation (28.6%), muscle weakness (17.1%) and movement disorders (17.1%). Percentage of delayed SSEPs after induction were at levels of brachial plexus (28.6%), spinal cord (68.6%), cortical conduction (31.4%), ERB-N13 Inter Peak Latency (IPL) (74.3%) and N13-N20 IPL (17.1%) in the studied patients. Significant prolonged latency of N13 ($p = 0.005$), N20 ($p = 0.04$) and IPL of ERB-N 13 ($p = 0.005$), N13-N20 ($p = 0.01$), Inter-Side Difference (ISD) of N13 ($p = 0.01$), ERB-N13 ($p = 0.02$) and N13-N20 ($p = 0.03$) after induction compared to values at diagnosis. Significant positive correlation were found between serum MTX after IMTX with N13-N20 IPL ($p = 0.01$), N20 ISD ($p = 0.03$) with significant prolongation in N20 latency, N13-N20 IPL and ISD of N20 compared to values before injection. ALL patients have prolonged latency of SSEPs at cervical cord and cortical levels which increased after IMTX due to axonal injury throughout the cord. SSEPs could be an early diagnostic tool for subclinical neuropathy.

**Key words:** Acute lymphoblastic leukemia, somatosensory evoked potential, methotrexate toxicity

## INTRODUCTION

Acute Lymphoblastic Leukaemia (ALL) is the commonest hematological malignancy in childhood. The improved survival of leukaemia patients leads to the importance of care about long term effects of chemotherapy (Díaz-Jaimes *et al*., 2009).

Therapy for childhood ALL is associated with adverse effects in both the peripheral and central nervous system. Peripheral neurotoxicity is a dose-limiting and disabling side effect of several important chemotherapeutic agents (Dicuonzo *et al*., 2009; Harila-Saari *et al*., 1997). In particular, vincristine, cisplatin and oxaliplatin are frequently used antineoplastic agents, which are known causes of a peripheral neuropathy. Acute neurotoxicity is observed in 5.8-18.4% of children undergoing therapy for ALL and is associated with the intensification of chemotherapy (Lo Nigro *et al*., 2000). Sensory and motor peripheral neuropathy caused by vincristine occur in nearly all patients treated for ALL with clinical manifestations as areflexia, weakness and atrophy of extremities, sensory impairment and muscle pain (Inaba *et al*., 2008).

Intrathecal methotrexate (IMTX) is commonly used in the treatment of leukaemia infiltrating the central nervous system. This form of therapy has been performed in the treatment of childhood ALL as a form of central nervous system prophylactic therapy in addition to intravenous chemotherapy and cranial irradiation (Bay *et al*., 2005; Kostiæ *et al*., 2009). Transient and permanent neurologic squeals have been reported in ALL patients with or without central nervous system leukaemia (Bota and Dafer, 2009; Drachtman *et al*., 2002).

Pathological and anatomical lesions in the spinal cord or brain tissue caused by IMTX and structural changes in magnetic resonance images in transient or permanent paraplegia caused by intrathecal chemotherapy have been described in patients with

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leukaemia (Finkelstein *et al*., 2005; Orken *et al*., 2009; Sandoval *et al*., 2003).

Neurophysiological studies may be more sensitive in the detection of subtle myelin disruptions than are structural imaging techniques. Evoked potential methods have been utilized successfully in the detection of even subclinical demyelinating processes (Díaz-Jaimes *et al*., 2009).

The aim of the study was to evaluate the changes within the nervous system both clinically and by the somatosensory evoked potentials (SSEPs) to detect subclinical neurological disease in different phases of chemotherapy in childhood ALL.

## MATERIALS AND METHODS

This study was conducted in the Pediatric Hematology/Oncology Unit of the Children's Hospital, Ain Shams University during the period from January 1st 2007 to December 31st 2009. Two groups of children were included.

**Group I (Patients group):** Thirty-five consecutive patients (21 males and 14 females) with ALL were included during their initial diagnosis of leukaemia. They were diagnosed and treated in the Pediatric Hematology/ Oncology Unit, Children's hospital, Ain Shams University, Cairo, Egypt. All included patients have achieved remission during the induction therapy. Their mean age was 7.51±3.02 years and ranging from 3 to 14 years. Diagnosis of ALL was based on peripheral blood film and bone marrow cytological examination, as well as immunophenotyping and cytogenetic study. All included patients were classified as standard and medium risk groups according to the BFM 90 ALL protocol of therapy. None had CNS leukaemia and none had received previous chemotherapy or cranial radiotherapy at time of recruitment. All patients had normal developmental history, no previous history of neurological disease or recent drug therapy with neurological adverse events.

Selected patients were taken treatment according to Ain Shams Protocol BFM 90 and all of them were standard risk category aiming to unify the doses of chemotherapy.

**BFM 90 Standard risk protocol:**

- **Induction:** Phase A; oral (PO) prednisone, intravenous (IV) Vincristine, IV Daunorubicin, intramuscular (IM) L-Asparginase and intathecal triple therapy (ITTT). Phase B; IV cyclophosphamide, subcutaneous (SC) Cytarabine and PO Mercaptopurine
- **Consolidation:** PO Mercaptopurine, Methotrexate and ITTT
- **Reinduction:** PO dexamethasone, IV Vincristine, IV Doxorubicin, IM L-Asparginase, IV cyclophosphamide, SC Cytarabine, PO thioguamine and ITTT
- **Continuation:** IV Vincristine, PO prednisone, PO Mercaptopurine, PO or IM Methotrexate and ITTT

**Group II (control group):** It included 30 healthy age, sex and height matched children. They were 15 males and 15 females, their ages ranged from 5 to 14 years, with a mean of 8.50±3.30 years. All were clinically healthy with normal developmental and neurological history.

**Methods:** All children included in the study were subjected to the followings:

Thorough clinical history especially concerning neurological disease as well as chemotherapy given for the leukaemia patients.

Clinical examination was done for anthropometric measures and organomegaly. Full neurological examination was done at time of initial diagnosis with follow up examination after induction therapy according to Touwen (1979). One leukemic patient had speech disorder which persisted during therapy.

Laboratory investigations included, Complete Blood Count (CBC) using Coulter counter Gene S (Coulter Corporation, Hialeah, Florida, USA) together with examination of leishman- stained Peripheral Blood (PB) smears for differential leucocyte count. Bone Marrow (BM) aspiration with examination of leishman-stained smears laying stress on the percentage and morphology of blasts and the BM cellularity together with immunophenotyping of BM and PB samples by flow cytometry using Coulter XL-flow cytometer (Coulter, USA) for diagnosing and risk categorization of ALL patients at diagnosis and at day 14 and day 28 of induction chemotherapy.

Cytological examination of cerebrospinal fluid (CSF) was done using Shandon Cytospin 3 Centrifuge (block Scientific, Inc. Bohemia, NY, USA) cytospan to diagnose central nervous system disease.

Assessment of serum level of methotrexate was done for patients using fluorescence polarization immunoassay (FPLA) technology.

**Assessment of serum level of methotrexate:** The methotrexate II assay utilizes fluorescence polarization immunoassay (FPLA) technology (Tietz *et al*., 1995). The TDx and TD x FL x software calculates a best-Fit curve

equation that is used to generate a calibration curve. This curve is stored in memory and concentration of drug in unknown samples are calculated from this curve using polarization values generated for each sample in the assay (Crom and Evans, 1992).

**Study of somatosensory evoked potentials:** The recordings of SSEPs were performed on all the studied 35 ALL patients at time of diagnosis and repeated immediately after the induction chemotherapy. SSEPs were repeated for 20 ALL before and 10 days after IMTX using Evomatic 8000 apparatus made by Dantec of Danemark.

**Median nerve somatosensory evoked potential methodology:** The apparatus used was the (EVOMATIC 8000 Apparatus) made by Dantec of Danemark.

**Stimulation:** Stimulation was applied to the median nerve at the wrist, using 2 saline soaked electrodes (made by Dantec of Danemark).

The cathode (negative stimulating electrode, designated with a black connector) was placed 2 cm proximal to the wrist crease.

The anode (positive stimulating electrode, designated by a red connector) was placed on the wrist crease. The stimulus intensity was adjusted to just over the motor threshold, so it did not produce pain.

**Recording electrodes placement:** Recording electrodes were placed over ERB's point bilaterally, skin over laying the fifth cervical spine, the contralateral central scalp region and the forehead.

ERB's point is located within the angle formed by the posterior border of the clavicular head of the sternocleidomastoid muscle and the clavicle, 2-3 cm above the clavicle.

The spinal electrode was located over the fifth cervical spinous process, two spines above cervical spinous process and two spines above the seventh spinous process.

The central scalp electrodes were located 2 cm posterior to the C3 and C4 positions of the 10-20 international system for EEG electrode placement.

The forehead reference site was chosen was electrode site FZ of the 10-20 system.

Before applying the recording and stimulating electrodes, their sites were properly cleaned using gentle rubbing with phenol.

**The ground electrode:** A grounding electrode was applied between the constant current stimulation and the recording electrode (Mare *et al*., 1994).

**Technique:** A 200 $\mu$ sec$^2$ wave electrical pulse was delivered with sufficient intensity to cause a 1 cm thumb movement. The stimulus intensity was altered as needed to maintain fairly constant thumb movement through out the test. The low filters were set to 20-30 Hz and the high filters were set to approximately 3000 Hz. Analysis time base was about 30 m sec and was extended to 50-60 m sec when significant delay of N20 was anticipated.

Two hundred responses were averaged for each SSEP measurement. The latencies of N9, N13 and N20, the inter peak latencies of N9-N13 and N13-N20 and interside differences were measured (Bleyer and Griffin, 1980).

**Interpretation:** The peak latency of the N20 (sensory cortex) component of the median nerve was measured from the first channel, the latency of the N13 peak (spinal potential from the C7 level) was measured from the third channel and the latency of the EP peak (the compound action potential at ERBs point) from the fourth channel.

The means of the latencies and amplitudes from both averages (averaging was performed twice on both sides) were calculated. The interpeak latencies (IPLs) EP-N13, N13-20 in the median nerve SSEPs were calculated from those means.

**Statistical analysis:** Standard computer program SPSS for Windows, release 10.0 (SPSS Inc, USA) was used for data entry and analysis. All numeric variables were expressed as mean  Standard Deviation (SD). Comparison of different variables in various groups was done student t test or Mann Whitney test according to the type of variable. Paired t or Wilcox on signed ranks tests were used to compare variables before and after therapy. Pearson's and Spearman;s correlation tests were used for correlating different variables. For all tests a probability (p) less than 0.05 was considered significant.

## RESULTS

In the present study, ALL patients at time of diagnosis had no clinically manifest neurological disease and their initial values of the median nerve SSEPs did not show significant difference from healthy controls (Table 1).

After 4 weeks of induction remission therapy using vincristine, adriamycin, prednisone with CNS prophylaxis using weekly intrathecal methotrexate, cytosine arabinoside and hydrocortisone; none of the studied 35 ALL patients had reported symptomatic neurological disease. However the follow up neurological examination done separately by two consultants of pediatric neurology revealed; hypotonia in 10 patients

Table 1: Comparison between 35 ALL patients at diagnosis versus 30 healthy controls regarding the results of SSEP (Parametric values) (unpaired t test)

| Latencies (m sec) | Controls (n = 30) | Patients at diagnosis (n = 35) | t-value | p-value |
|---|---|---|---|---|
| ERB RT | 9.24±1.32 | 9.41±0.51 | 0.70 | 0.48 |
| ERB LT | 9.32±1.09 | 9.54±0.35 | 1.13 | 0.26 |
| N13 RT | 12.62±1.36 | 12.36±0.34 | 1.09 | 0.28 |
| N13 M | 12.87±1.22 | 12.49±0.29 | 1.79 | 0.08 |
| N20 LT | 18.54±2.18 | 18.96±0.38 | 1.12 | 0.27 |
| **Inter peak latency** | | | | |
| ERB N13 LT | 3.12±1.89 | 3.09±0.46 | 0.09 | 0.93 |
| ERB N13 M | 3.18±1.03 | 3.02±0.48 | 0.82 | 0.41 |
| N13N20 RT | 6.84±1.48 | 6.25±1.22 | 1.76 | 0.08 |
| N13N20 LT | 6.58±1.15 | 6.33±0.45 | 1.19 | 0.24 |
| N13N20 M | 6.44±1.63 | 6.29±0.46 | 0.52 | 0.60 |

IPL: Inter peak latency; ISD:Inter side difference; m sec: Milli seconds; Erb(N9): Arises from distal brachial plexus; N13: Cervical peak generated from dorsal or branches from dorsal column; N20: Cortical peak, RT: Right side, LT: Lift side and M: Mean values of RT and LT

(28.6%), muscle weakness in 6 patients (17.1%) depressed deep tendon reflexes in 12 patients(43.3%) and cerebellar signs in 6 patients (28.6%).

To recognize the patients with ALL developing abnormal SSEP after the induction therapy, the cut-off value of each SSEP potential was calculated as three standard deviations above the mean values for the healthy age, sex and height matched control children. The conduction times for the peripheral parts of the median nerve SSEPs in the studied leukaemia patients were delayed indicating vincrisitine neuropathy.

The percentage of the ALL patients developed delayed latencies in their median nerve SSEP after induction therapy was shown in Fig. 1. Among the 35 ALL patients examined after induction therapy; 10 patients had prolonged ERB latency N9 (28.6%), 24 had prolonged N13 latency (68.6%), 11 had prolonged N20 latency (31.4%), 26 had prolonged IPL of ERB-N13 (74.3%), 6 had prolonged IPL of N13-N20 (17.1%), 19 had prolonged ISD ERB-N13 (54.3%), 20 had prolonged ISD of N13-N20 (57.1%), 15 had prolonged N13 I SD (60%) and 11 patients had prolonged N20 interside difference (31.4%).

Comparison between the results of the mean parametric values of the median nerve SSEPs in the 35 ALL patients done at diagnosis and immediately after the induction therapy (on day 28 of chemotherapy) revealed statistically significant prolongation of N 13 latency after therapy (13.80±1.95 m sec) compared to values at diagnosis (12.36±0.34 m sec, $p<0.001$). Moreover a prolonged ERB-N13 inter-peak latency was found after therapy (4.88±2.0 m sec) compared to values at diagnosis (3.09±0.46 m sec, $p<0.001$). The inter-peak latency of N13N20 was significantly prolonged in patients after induction therapy (6.29±0.46 m sec) compared to initial values (4.46±2.63 m sec, $p<0.05$) (Table 2).

Table 2: Comparison between 35 ALL patients after induction versus at diagnosis regarding the results of SSEP (parametric values)

| Latencies (m sec) | Patients at diagnosis (n = 35) | Patients at day 28 induction (n = 35) | t-value | p-value |
|---|---|---|---|---|
| ERB RT | 9.41±0.51 | 9.091±1.65 | 1.09 | 0.28 |
| ERB LT | 9.54±0.35 | 9.111±1.48 | 1.67 | 0.10 |
| N13 RT | 12.36±0.34 | 13.80±1.95 | 4.30 | 5.51E-05 |
| N13 M | 12.49±0.29 | 12.89±1.53 | 1.52 | 0.13 |
| N20 LT | 18.96±0.38 | 18.54±3.07 | 0.80 | 0.43 |
| **Inter peak latency** | | | | |
| ERB N13 LT | 3.09±0.46 | 4.88±2.006 | 5.16 | 2.32E-06 |
| ERB N13 M | 3.20±0.48 | 3.80±1.81 | 1.90 | 0.06 |
| N13N20 RT | 6.25±1.22 | 5.36±2.87 | 1.69 | 0.09 |
| N13N20 LT | 6.33±0.45 | 5.55±2.76 | 1.65 | 0.11 |
| N13N20 M | 4.46±2.63 | 6.29±0.46 | 4.05 | 0.0001 |

Paired t-tests 5.51E-05 means 0.0000551

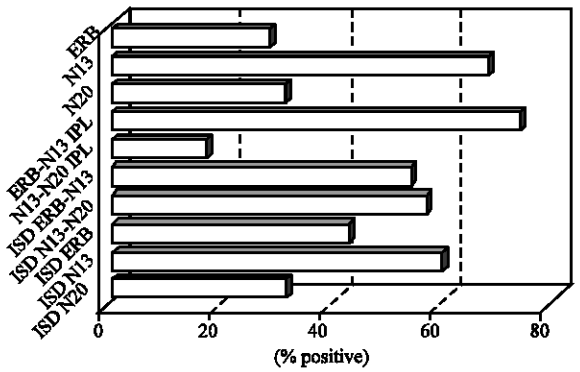


Fig. 1: Percentage of ALL patients with affected SSEP at day 28 (The cut-off value of each SSEP potential was calculated through adding the 3 standard deviation above the mean values of the healthy age, sex and height matched children)

Table 3 shows comparison between ALL patients after induction therapy (at day 28) and at diagnosis regarding their non-parametric values of the median nerve SSEPs. The inter-side difference ERB-N13 was significantly prolonged in treated patients (1.40±1.19 m sec) compared to patients at diagnosis (0.34±0.31 m sec, $p<0.01$).There was statistically significant prolonged inter-side difference N13 in treated patients (1.42±1.11 m sec) compared to initial values (0.35±0.13 m sec, $p<0.01$). Inter side difference N13-N20 was significantly prolonged in treated patients (1.34±0.98 m sec) compared to initial values (0.58±0.23 m sec, $p<0.05$).

Comparison between parameters of median nerve SSEPs before and after 10 days of intrathecal chemotherapy which were done for 20 ALL patients were presented in Table 4. A conduction delay was detected as there was statistically significant prolongation in N20 latency R (19.28±3.6, 18.06±2.90 m sec, $p = 0.041$), N20 latency L (19.93±3.36 and 18.54±3.07 m sec, $p = 0.009$)

Table 3: Comparison between patients at day 28 and at diagnosis regarding their non-parametric values of SSEP

| Variable | Mann-Whitney | Z | Exact Sig. [2(1-tailed sig)] |
|---|---|---|---|
| ERB M | 159.500 | -0.423 | 0.677 |
| N13 LT | 70.500 | -2.859 | 0.003** |
| N20 RT | 97.000 | -2.131 | 0.033* |
| N20 M | 135.000 | -1.093 | 0.286 |
| IPLERB N13 RT | 80.500 | 2.581 | 0.008* |
| ISD ERBN13 | 69.500 | -2.894 | 0.003** |
| ISD N13N20 | 94.500 | -2.203 | 0.026* |
| ISDERB | 171.500 | -0.096 | 0.925 |
| ISD N13 | 35.500 | -3.822 | 0.001** |
| ISD N20 | 168.000 | -0.193 | 0.861 |

Mann-Whitney test is used. *p < 0.05, **p < 0.01

Table 4: Comparison between parameters of SSEP before and within 10 days after ITMTX for 20 patients

| Comparison | N20 RA N20RB | N20 LA N20LB | N13N20RA N13N20RB | ISDN20A ISDN20B |
|---|---|---|---|---|
| Patient $\bar{X}B$ | 18.06±2.90 | 18.54±3.07 | 4.36±2.76 | 0.91±1.01 |
| Patient $\bar{X}A$ | 19.28±3.6 | 19.93±3.56 | 5.04±3.10 | 1.41±1.34 |
| Z-value | -2.040 | -2.599 | -2.371 | -2.70 |
| p-value | 0.041* | 0.009** | 0.018* | 0.007** |

A: After ITMTX; B: Before ITMTX. *p < 0.05, **p < 0.01

Table 5: Correlation between MTX level and parameters of SSEP in ALL patients (n = 35) after intrathecal injection

| Parameters | MTX |
|---|---|
| **ERB M** | |
| r value | 0.647 |
| p value | 0.165 |
| **N13 M** | |
| r value | 0.118 |
| p value | 0.824 |
| **N20 M** | |
| r value | -0.088 |
| p value | 0.868 |
| **IPL ERB N13 M** | |
| r value | 0.118 |
| p value | 0.824 |
| **IPL N13 N20 M** | |
| r value | 0.912 |
| p value | 0.011* |
| **IPD ERB N13** | |
| r value | 0.470 |
| p value | 0.347 |
| **IPD N13 N20** | |
| r value | 0.618 |
| p value | 0.191 |
| **ISD ERB** | |
| r value | 0.702 |
| p value | 0.120 |
| **ISD N13** | |
| r value | 0.265 |
| p value | 0.612 |
| **ISD N20** | |
| r value | 0.746 |
| p value | 0.038* |

N13-N20 inter-peak latency (5.04±3.10 and 4.36±2.76, p = 0.018) and N20 inter-side difference (1.41±1.34 and 0.91±1.01, p = 0.007) after intrathecal chemotherapy compared to values before intrathecal injection.

Table 5 demonstrates the results of correlation studies between MTX blood level after intrathecal injection and parameters of SSEPs in ALL patients (10 days after ITMTX). There was statistically significant positive correlation between the serum MTX level and each of N13-N20 inter peak latency ($r = 0.912$, $p<0.05$) and N20 inter side difference ($r = 0.746$, $p<0.05$)

In the present study, there was no significant correlation between the different parameters of SSEP with the age or sex of the patients (data not shown).

Comparing ALL patients with and without abnormal motor function (abnormal neurological examination) and those with depressed deep tendon reflexes regarding their parameters of SSEP revealed no significant difference between them. Also the mean values of the median nerve SSEPs in ALL patients who had muscle weakness and those with signs of cerebellar affection did not significantly differ from patients with normal neurological examination. Interestingly the parameters of SSEP in the studied ALL patients did not correlate with serum MTX level before intrathecal injection, cumulative dose of ITMTX or cumulative dose of vincristine (data not shown).

## DISCUSSION

Both peripheral and Central Nervous System (CNS) pathways have been shown to be affected during treatment for childhood ALL, but evaluation and location of the lesions, particularly those affecting the sensory pathways is difficult especially in young children (Harila-Saari *et al*., 1998a; Karabacak *et al*., 1997). Objective information regarding the nerve lesions is needed for the evaluation and comparison of the various treatment modalities and dosage, which could be of great importance in developing neurologically safer modes of therapy (Osterlundh *et al*., 1997; Ramchandren *et al*., 2009). The measurement of SSEPs provides one method for assessing nerve function and for evaluating treatment modalities in this respect (Toopchizadeh *et al*., 2009). It is a non invasive, objective, quantitative and practically painless method of obtaining information on lesions of entire nervous system. Abnormalities may be associated with axonal or neuronal loss or with demylination. Axonal loss reduces the amplitudes of SSEP whereas demyelination produces latency prolongation (Moleski, 2000).

The present study was designed to find an objective method to evaluate the effect of chemotherapy treatment on nerve function in children with ALL which could be of great importance in modulating chemotherapy protocol to be neurologically safer.

In the present study, depression of deep tendon reflexes (DTRs) was found in 43.3% of patients, hypotonia

was found in 28.6%, lost pain sensation of stock-glove distribution was found in 28.6%, muscle weakness in 17.1% and movement disorder of cerebellar type in 17.1% of patients during neurological examination after induction chemotherapy. These clinical data are similar to the earlier described findings by Harila-Saari *et al.* (2001) as they reported that; clinical signs of vincristine neuropathy are early loss of Achilles tendon reflexes followed by loss of other DTRs and sensory disturbances. Also the reported muscle weakness in the present study is exactly referred to that; motor impairment, specifically muscle weakness of the extensor muscles of fingers and wrists and dorsiflexors of the toes and ankles is the most severe manifestation of vincristine neuropathy.

Vincristine causes an axonal sensory-motor neuropathy often early during treatment, heralded by paresthesias and followed by severe motor weakness if treatment is continued, these findings explains the sensory manifestation in the studied ALL patients (Gursel *et al.*, 2009). Pain is not a prominent feature. Autonomic neuropathy is often prominent (ileus, orthostatic hypotension). The pathogenesis of the neuropathy is partially explained by that, vincristine-induced interference with microtubules, affecting axonal transport. Neurotoxicity of vincristine is a dose-limiting toxicity, in particular related to dose per cycle (Van den Bent, 2005).

In concordance with our results Vainionpaa (1993) reported that the gross motor disturbances observed in ALL patients were thought to be caused mainly by a vincristine sensory-motor neuropathy in the early phases of chemotherapy (Vainionpaa, 1993). Moreover our findings were supported by the delay in the peripheral conduction time in the median and tibial SSEPs in the studied leukemic patients and were related to the vincristine therapy (Vainionpaa *et al.*, 1995). Similarly Vainionpaa *et al.* (1997) reported walking difficulties due to myelopathy and motor clumsiness and weakness due to vincristine neuropathy affecting the peripheral nerves are manifested simultaneously (Vainionpaa *et al.*, 1997).

Similar to our data, Vainionpaa (1993), reported depression of DTRs and fine motor disorders in 9 out of 31 children with ALL (29%) whereas gross motor difficulties were experienced in 29% of the patients and dysdiadochokinesia in 50% of the studied patients.

In the present study the 35 ALL patients were evaluated during treatment for leukemia. So, acute toxicity might explain the higher percentage of neurologic dysfunction while, Vainionpaa *et al.* (1995), study was conducted on ALL patients during follow up. So, the toxic effects of some drugs might be reversed after discontinuation of therapy.

Earlier studies (Harila-Saari *et al.*, 2001; Harila-Saari *et al.*, 1998b) reported that; the clinical signs of vincristine neuropathy are to a great extent reversible and there are only few studies for the long term effects of this drug. Reinders-Messelink *et al.* (2000) observed persistent fine motor and handwriting difficulties in examinations performed 2 years after cessation of therapy in approximately 67% of their patients treated for childhood leukaemia and suggested vincristine as a causative agent. In concordance with our findings, signs of peripheral neuropathy such as depression of reflexes or fine motor disorders occurred in approximately 33% of the studied ALL patients in a study done by Harila-Saari *et al.* (1998a) and they reported that the clinical findings were more common in the standard risk patients, who received the larger doses of vincristine.

An important finding in the present study is that we found no statistically significant difference in SSEPs parameters, between patients with clinical neurological findings and those without. This was in agreement with Fagan *et al.* (1987), who reported that SSEPs changes do not always correlate with mild disturbances observed in traditional clinical sensory examination. Thus SSEPs could detect abnormalities in nervous system before the related clinical neurological signs appear by examination.

In the present study SSEP was selected for evaluation of the chemotherapy effect on spinal cord function in the studied leukaemia patients as it is a sensitive tool for assessing the spinal and brain stem posterior columns and medial lemniscal tracts and near by structures (Bay *et al.*, 2005). The present study reported that; there was a significant delay in the latency of N9 (28.6%), which represent the ERB's point representing the brachial plexus, delay in the latency of N13 (68.6%), which represent cervical segment of spinal cord, delay in latency of N20 (31.4%),which represent the cortical level. Also, it was detected that there is delay of the inter-peak latency of (ERB-N13) in 74.3%, delay in the inter-peak latency of (N13-N20) in 17.1%. Also, there was delay of inter-side difference between the corresponding waves of right and left side of N9 in 42.9%, of N13 in 60%, N20 in 31.4%, ERB-N13 in 54.3% and N13-N20 in 57.1% in the studied leukemic patients after induction therapy compared to values at diagnosis.

The CNS therapy is directed to the brain tissue and therefore plays its own role in deteriorating the function of the sensory pathways and cortex

(Kwong *et al*., 2009; Mahoney *et al*., 1998). In a prospective study done for 38 ALL patients during treatment, the intracranial central conduction measured via median nerve SSEPs was not significantly delayed, but the amplitudes of the median nerve cortical SSEPs were significantly decreased in patients after the CNS therapy, which included both intravenous and intrathecal methotrexate. They attributed this amplitude reduction to axonal and neuronal loss in the somatosensory pathways or sensory cortex. The CNS effect of chemotherapy is also supported by the finding that cortical function is disturbed in positron emission tomography during intrathecal and intravenous chemotherapy in ALL patients. The pathogenesis of these myelin lesions is supposed to be a toxic oedema or a demyelinating process (Clark *et al*., 1982; McLean *et al*., 1994; Vainionpaa *et al*., 1997).

The toxic effect of vincristine involved the entire length of the nerves measured by the SSEPs and the most impressive conduction slowing indicative of evident demyelination occurred in the proximal part of the nerves. Vincristine neuropathy has been identified mainly in the distal parts of peripheral nerves by electromyographic examination, which disclosed consistently reduced potentials of the distal sensory and motor nerves of the hands, indicating axonal neuropathy and only a slight reduction on conduction velocity (Koh *et al*., 1999), which explains our finding of lost pain sensation of stock-glove distribution in 28.6% of the studied leukemic patients.

In the present study; there was a significant prolongation of the latency at the N13 peak (spinal potential) in patients after 28 days of chemotherapy compared to the latency at time of diagnosis. Also there is a significant delay in the inter-peak latencies of ERB-N13 LT and N13N20M in patients after 28 days of chemotherapy compared to these values at time of diagnosis.

In accordance to the present study, Vainionpaa *et al*. (1997), studied SSEP in 31 children with ALL during and after therapy, they reported significant delay in the mean conduction time of the median nerve SSEP between the wrist and central nervous system (spinal cord and brain) and the delay was located mainly within the spinal cord. The earlier significant prolongation of the latencies in the peripheral nerves observed in their patients had disappeared after cessation of therapy indicating remyelination.

In the present study, SSEP was done for 20 patients before and 10 days after ITMTX, together with measurement of serum MTX level. No significant correlation was found between SSEP parameters and serum methotrexate level before ITMTX. Whereas, statistically significant correlation was found between serum MTX level after ITMTX and each of the delay of IPL N13-N20 and ISD between right and left side of N20. This denotes delay in conduction between spinal cord and cortex. This delay can explain acute encephalomyelitis after ITMTX which reverses rapidly (Pascual *et al*., 2008).

In the present study, significant prolongation of the latency of N20, IPL of N13-N20, ISD between right and left side of N20 after ITMTX compared to the readings before ITMTX which denotes affection within spinal cord and cortex.

Children with ALL are treated with IMTX which may affect the nerve roots in the spinal canal. Knowledge about the possible radiculopathy is based on histological findings in fatal cases and has been contradictory. Both axonal swelling and myelin loss were (Koh *et al*., 1999).

In the present study, there was no change in serum MTX level after ITMTX. The findings in SSEP could be explained by the direct damaging effect of ITMTX on CNS and not the serum level of the drug. Methotrexate used intrathecally and at high doses intravenously, is an essential part of the CNS treatment provided for patients with ALL, but it entails a variety of neurotoxic effects, principally meningeal irritation, seizures, encephalopathy and paraplegia (Bleyer and Griffin, 1980).

Bleyer (1981), found chemical arachnoiditis in 5-40% of patients and cases of transient or permanent paraplegia have been reported after intrathecal methotrexate. Microvaculization in the myelinated long tracts of spinal cord, swelling and loss of axons was found at autopsy. It has been suggested that neuronal loss leads to secondary loss of myelin (Pascual *et al*., 2008).

Development of neurotoxic effects was also correlated with a high CSF level of methotrexate. Progressive myelopathy and arachnoiditis developed after the first dose as well as the 10th dose of intrathecal therapy. These findings suggest that the toxic effect of methotrexate does not seem to be dose related but may be related to individual sensitivity (Shuper *et al*., 2000, 2002).

Vaimionpaa *et al*. (1997), reported prolongation in nerve conduction for ALL children. Decreased amplitudes were observed within the spinal cord after IMTX, findings that persisted until the end of therapy. There had been no improvement in the conduction time between the spinal cord and cortex during the 2 years of continuation therapy. According to their findings, both the axonal damage and the injury to the myelin sheaths within the spinal cord were of long duration.

In the same way of our study, Toopchizadeh *et al*. (2009) reported that; Vincristine is commonly used in treatment of Acute Lymphoblastic Leukemia (ALL) and

peripheral neuropathy is its dose-related toxicity. Motor impairment, specifically muscle weakness is the most severe manifestation of neuropathy. They perform a prospective cohort study to evaluate the electrophysiological consequences of vincristine-contained chemotherapy in 42 children (25 cases of ALL, 17 cases of non-ALL malignancies) before and five weeks after chemotherapy. In the ALL group, there was no significant change in motor and sensory nerve conduction velocity and amplitude of sensory nerve action potential after five weeks. However, the amplitude of Compound Muscle Action Potential (CMAP) was significantly decreased both in upper and lower extremities. Decreased CMAP amplitude was detected in 96% of the ALL cases after induction. Sixteen (66.7%) patients suffered from gait abnormality as well. They reported a significant decrease of CMAP amplitude with increasing dose of vincristine. This study showed that the electrophysiological changes due to weekly administration of vincristine are common in children with ALL during the induction phase, which usually presented in the form of decreased CMAP amplitude (motor-axonal neuropathy), however routine sensory studies were normal. Gait abnormality is accompanied in 66.7% of ALL cases.

In the present study, there was no significant correlation between SSEP parameters and disease duration or age. Also there was no difference in cumulative dose of IVMTX, ITMTX and vincristine, when patients with prolonged latency were compared to those with normal latency.

Khalifa *et al.* (1999), studied the toxic side effects of IVMTX and reported elevated serum methotrexate and toxic adverse side effects in 4 out of 14 patients who received 3 g $m^{-2}$ IVMTX, While, in patients who received 2 g $m^{-2}$ IVMTX none of them showed any elevation in the serum level of methotrexate.

Vainionpaa *et al.* (1997), found that, 4 ALL patients who received high cumulative doses of vincristine showed decrease in amplitudes and prolongation in latencies of SSEP relative to their controls. The decrease in peripheral amplitudes indicates axonal injury.

In the present study, clinical examination and SSEP findings denote delayed conduction at peripheral nerve, within spinal cord and at cortical level. These findings are reported during treatment with IVMTX, IMTX and vincristine. The prolongation in SSEP parameters was more after ITMTX. There was no relation between any of SSEP parameters and neither of IVMTX cumulative dose nor cumulative dose of vincristine.

The studied ALL patients had delayed SSEPs. Similarly Vainionpaa *et al.* (1997) argued that intrathecal methotrexate therapy is responsible for the spinal conduction delay. Also, Bay *et al.* (2005) found that SEP abnormalities developed after the first dose of chemotherapy. Because of that, despite the possibility of axonal neuropathy related to vincristine, they thought that the demyelinating toxicity of intrathecal methotrexate treatment was the main causative factor of SEP abnormalities. Present study points to the importance of early detection of this spinal cord dysfunction which may occur early after the induction phase as there are limited treatment options for myelopathy and arachnoiditis. Koh *et al.* (1999) administrated intravenous methylprednisolone (30 mg/kg/day) for 3 days. Drachtman *et al.* (2002) reported that dextromethorphan was effective in the treatment.

In conclusion, ALL patients have prolonged latency of SSEP at both cervical spinal cord and cortical levels compared to controls. Somatosensory evoked potential latency is increased after IMTX therapy although they do not correlate with clinical neurological findings. The SSEP could be an early diagnostic tool for occurrance of subclinical neuropathy, in ALL under therapy before detection by clinical neurological examination.

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