DISCUSSION

Encephalitis is an inflammatory process that affects the parenchyma of the brain, usually present as a diffuse and, or focal neuropsychological dysfunction, indicates that the predominant clinical syndrome arises from infection and inflammatory reaction in the parenchyma of the brain rather than in the leptomeninges. When both the leptomeninges and brain parenchyma are involved, the term meningoencephalitis is used. [1]

Viral encephalitis is a medical emergency. The spectrum of brain involvement and the prognosis are dependent mainly on the specific pathogen and the immunological state of the host. Although specific therapy is limited to only several viral agents, correct immediate diagnosis and introduction of symptomatic and specific therapy has a dramatic influence upon survival and reduces the extent of permanent brain injury in survivors. [2]

Neopterin is a marker associated with the activity of monocytes, macrophages, which is produced and released into body fluids, under stimulation of T-lymphocytes. Neopterin is released into body fluids and participate in immune response and can be used to monitor activation of cell mediated immunity. [136, 137]

The specific nature of Neopterin biosynthesis in human monocyte-derived macrophages and dendritic cells signifies that neopterin is a marker for an activated Th1 mediated cellular immune system. [155]

Previous investigators have shown that CSF neopterin is a sensitive marker of active inflammation and is produced by monocytes secondary to interferon species stimulation (particularly interferon-gamma and to a lesser extent interferon-alpha). [155,188] CSF neopterin appears to normalize quickly once inflammation has resolved. [189]

Neopterin penetrates through the blood-brain-barrier, so that there usually exists a linear relationship between neopterin levels in blood and in cerebrospinal fluid (CSF). [190] Favourably neopterin levels in serum, plasma and other protein-containing body fluids not differ.

The CNS has unique immunological status there is increasing evidence that there is a vigorous innate immune response to viral infection of cells within it. [38]

Neuronal destruction most likely results from both, direct cytophatic influence of the virus, as well as inflammatory reaction mediated by released components from dying cells. Until now, few investigations on patients with herpes simplex virus encephalitis HSE, as well as on mouse model of the disease, revealed that cytokines and chemokines take part in development of defense mechanism against viral infection and injury of the brain tissue. [191-194]

Astrocytes are the most common cells in brain tissue and S-100B is synthesized in astroglial and Schwann cells. It is thought to be a mediator of neuronal-glial interactions in normal brain. [134] Extracellular S100B might participate in brain inflammation by activating astrocytes, microglia and neurons. [178]Damage to the astrocytes causes leakage of S-100B protein extracellularly into the CSF and a small proportion leaks into the circulation. [183]So, the identification of S 100 B protein for brain damage in viral encephalitis could be useful. [134]

The aim of our work was to study the clinical significance of serum S100 B protein and neopterin in patients with encephalitis.

Our study was done on twenty patients with clinical manifestations and positive CSF criteria of suspected viral encephalitis and twenty apparently healthy volunteers of matching age and sex.

In our study the diagnosis of suspected viral encephalitis was based on several elements, firstly clinical picture of patients that we examined (disturbed level of consciousness , abnormal behavior , vomiting , fever and exclusion of other causes of disturbed level of consciousness) , secondly predominance of lymphocytes in CSF and CSF cultures for bacterial infections were negative , additionally pictures of viral encephalitis appeared in some MRI brain that was done and finally by response of patients to acyclovir treatment with improvement of many of their symptoms.

In our study we found that the serum Neopterin level was significantly higher in patients with suspected viral encephalitis than in healthy volunteers.

Russell C Dale al, [195] reported that the encephalitis group all had elevated CSF neopterin, supporting the role of interferon-gamma in these disorders. Also children with other acute inflammatory CNS disorders commonly had elevated CSF Neopterin as cerebral vasculitis (that had large-vessel vasculitis). [195]

Recent studies in brain trauma have shown elevated serum neopterin (both acutely and >1y after injury), suggesting that ongoing oxidative stress and inflammation is occurring, which may contribute to continuing brain dysfunction. [196,197] There is also an increasing literature devoted to inflammatory mechanisms operating in neonatal hypoxic–ischaemic encephalopathy. [198]

Other acute disorders with an elevated CSF neopterin included acute leukoencephalopathy of unknown origin, an acute basal-ganglia 'encephalitic attack' during a glutaric aciduria type 1 decompensation, and acute hypoxic–ischaemic injury. These disorders are clearly heterogeneous but are all associated with rapid and severe cell injury. [195]

Reder A.T et al, [199] reported that elevated serum neopterin levels from patients with diseases involving a cellular immune response as in multiple sclerosis MS as well as aseptic meningo-encephalitis (AME), systemic as well as intrathecal cellular immune mechanisms are generally considered to be involved in the pathogenesis of these diseases. [199]

In consistent with our results Monika Bociaga-Jasik et al, [200] reported that a significant increase of neopterin concentration in CSF of patients with herpes simplex encephalitis (HSE) was observed in comparison to the control group, which suggests their role in pathogenesis of CNS during the course of HSV infection. neopterin levels was higher in cases with severe course of HSE. [200]

Also Aurelius et al, [201] found an increased level of neopterin in CSF of patients with an activated immune system during the course of HSE. [201]

In line with our results Azumagawa K et al, [202] reported the role of neopterin during different CNS diseases in children and showed its increased concentration also in encephalitis. [202]

In our study we found that the neopterin level had no statistical significant difference between males and females.

Our results were similar to Diamondstone et al, [146] They reported that neopterin levels did not correlate significantly with gender of patients. [146]

Against our results, Xavier Garcia-Moll et al, [138] reported that women had significantly higher neopterin levels than men as neopterin was a marker of risk in women with coronary artery disease. [138]

In our study regarding to serum neopterin level there were no statistical significant difference between patients with convulsion and without convulsion.

Kawakami Y et al, [203] reported that the CSF neopterin levels were significantly higher in patients with typical febrile convulsions (FCs) than in those with pyrexia without convulsions or convulsions without pyrexia, namely, epilepsy. The CSF neopterin/serum neopterin ratio (C/S ratio) was also higher in patients with typical FCs than in those with pyrexia without convulsions or convulsions without pyrexia. Patients with prolonged FCs tended to have higher CSF neopterin levels than those with typical FCs. The results of this study suggested that some immune activation in the central nervous system (CNS) compartment may be related to the mechanisms of FCs. [203] And these were against our result as our study was done on small sample of patients (20 patientts) and only 14 patients only had convulsion but these study was done on larger sample of patients (158 patients).

In our results regarding to conscious level serum neopterin level had no statistical significant difference between patients were drowsy, comatosed, semi-conscious and consious. Also in our study serum Neopterin level had no statistical significant difference with CRP.

Against our results Samia Boseila et al, [204] reported that serum neopterin level correlated positively with CRP level which is a laboratory marker of neonatal sepsis pointing to their usefulness as additional markers of sepsis, this is in agreement with the study of Czyewska et al, 2005. [205] The combination of serum neopterin level and CRP is a reliable test for the diagnosis of early onset bacterial infection and may be helpful in establishing antibiotic therapy in newborn. [206] These difference with our results because it was based on bacterial infections and sepsis and CRP had its role in bacterial infection but our study was done on viral infection and CRP had limited role in viral infection.

In our results regarding to severity and complications of the patients with encephalitis serum neopterin level showed increased statistical significance regarding to complications as eye symptoms, abnormal gait and delayed speech. It can be explained by the increased production of neopterin, which leads to prolonged oxidative stress and enhanced cytotoxic effect of reactive oxygen species (ROS). Nitric oxide acts as an immune mediator that leads to neuronal damage. [207] The last published studies conducted on mouse model of HSE confirm the role of oxidative stress as the response to viral infection in neuron injury. [208, 209]

Monika Bociaga-Jasik et al, [200] also revealed the role of inflammatory response in the brain damage during the course of HSE. HSV infection causes microglia activation and production of immune mediators, which are responsible for virus elimination, but also can lead to the CNS injury. [10,192,210] Neopterin production cause ongoing oxidative stress. Neopterin biosynthesis can be connected with nitric oxide synthase (iNOS) activity, and accumulation of nitric oxide.[154,211] All these processes lead to the neurodegeneration. The results of the study clearly indicate the role of neopterin in the injury of CNS during the course of HSE. The levels above which the risk of severe complications increase can be useful to make decision about introduction of steroids to the therapy, which, according to recent publications, can be beneficial for the patients, and improve their prognosis. [212, 213]

Azumagawa K et al, [202] reported that serum neopterin concentrations were significantly elevated in encephalitis patients, especially in two cases with serious neurological sequelae as mental retardation. [202]

In our study there was higher level of neopterin in non survivors and there was positive statistical significance between neopterin and death in patient with encephalitis.

In consistent with our results, Samia Boseila et al, [204] reported that a highly positive significant correlation was detected between the outcome of the infected neonates and serum neopterin level. This positive significant predictor of mortality in the studied patients is similar to the results of Murr et al, 2001[206] and Ruokonen et al,2002,[214] who reported an increase of serum neopterin level with the severity of infection and a higher level in non-survivors.

In line with our results Schachtele SJ et al, [211] based on the conducted analysis revealed that concentration of neopterin was not only significantly increased, but also correlated with severity of the disease, and a high concentration was connected with the risk of death. [211]

MRI is an expensive and time-consuming test. Therefore, the ability to measure biological markers in body fluids correlating with MRI disease activity is of great importance. In our study we found the mean value of serum neopetrin was significantly higher in those patients with remarkable criteria of MRI than patients with unremarkable criteria of MRI.

Murata R et al, [215] reported that increased CSF Neopterin level and they reported the high-intensity area in MRI might be evidence of local inflammation and the resulting cell damage. neopterin seemed to be biochemical markers in patients with subacute sclerosing panencephalitis (SSPE) for detection of the extent of lesions, and their measurement may provide information useful for evaluation of the therapeutic response. [215]

S100B is a calcium-binding protein primarily present in nervous tissue.[169,216] Increased S100B in biological fluids has been shown to be a marker of brain damage both in adults and during the antenatal and postnatal periods.[166,167]

Brain injury results in leakage of S100B into the CSF and passage into the blood stream through transient disruption of the BBB or via CSF circulation [217] S100B is metabolized by the kidneys and excreted in the urine. Concentrations usually normalize

within 24 h after an acute injury; therefore, persistent increases in S100B may reflect ongoing or secondary cellular injury. [218, 219]

At increased concentrations, S100B may have a neurotoxic effect by inducing apoptosis, causing the release of proinflammatory cytokines as well as nitric oxide from astroglial cells, and contributing to oxidative stress. [220-222] Therefore, increased concentrations not only reflect tissue damage but may also exacerbate it. [222]

S-100β protein concentrations are more specific to the brain than to any other organ. Given that 80-90% of the total amount of S-100β is found in cerebral tissue, [180] and that serum concentrations of S-100β protein have been correlated with the extent of brain damage in traumatic brain injury on computed tomography [223] and in patients with ischaemic stroke, [224] the attributable concentrations and influence of extracerebral sources of S-100β is thus likely to be minimal. [225]

In our study the mean serum S100 B protein was significantly higher in patients with suspected viral encephalitis than in control group.

In consistent with our results a retrospective pilot study analysed serum S100 B in patients with various infectious diseases of CNS and non-CNS origin. Generally, infections affecting the brain showed higher S100B levels than those of extracerebral nature and found that patients with viral encephalitis displayed the highest S100 B levels. This is not surprising since these infections (especially HSV infections) are known to cause cellular damage to the cerebrum. These data seem to support the idea that S100 B is a marker for brain damage. [226]

In line with our results, M. Studahl et al, [227] reported that Herpes simplex virus with its cytotoxic effect both on glial and neuronal cells generates extremely high levels of S-100B in the CSF in the acute stage of HSE and the protein leaks out in the circulation and can be measured in serum. CSF S-100B concentrations reached markedly high levels in patients with HSE encephalitis indicating severe astroglial destruction. And there is no correlation between levels of serum S-100B in HSE patients in acute stage and outcome. As it is not certain that the amount of brain damage measured by biochemical markers is correlated to more severe sequelae since small damage to specific brain cells may give severe neurological deficits, while larger damage in a ”silent” area of the brain may give rise to only minor neurological sequelae. [227]

Diego Gazzolo et al, [228] reported that CSF S100B concentrations were significantly higher in the infants of the bacterial meningitis with encephalitis (BME) than in the bacterial meningitis only (BMO) and the controls.Multiple logistic regression analysis showed a positive correlation only between CSF S100B and the occurrence of BME. ROC curve analysis showed that higher S100B values were diagnostic for early BME detection, with a S100B cutoff of 1.0 μg/L, a sensitivity of 91% (95% confidence interval, 71–98.6%), a specificity of 82% (95% confidence interval, 70–91.4%), and an area under the curve of 0.918. [228]

Increased S100B concentrations in CSF are a reasonably direct manifestation of damage to the central nervous system, which is known to contain protein at this stage. Higher S100B concentrations may reflect a bacterial infection because such infections are known to induce a cascade of events, including activation of proinflammatory cytokines,

which in the brain trigger exaggerated activation of glial cells, which produce S100B.[229] High extracellular concentrations of S100B have been shown to be neurotoxic, leading to apoptosis and neuronal death via a nitric oxide-mediated pathway.[166,230] And it is possible that at least part of the S100B measured in the CSF of infants with encephalitis derives from this process and participates in the pathologic events accompanying parenchymal damage.

In our results we found that the age and gender had no statistical significant difference correlation with serum S 100 B protein. And our explanation that we have no extremes of age in our studied groups.

Against our results Portela et al, [231] reported that age had a significant negative correlation with S100B, with the highest values found in neonates. Age-related variations in S100B concentrations are, therefore, probably due to the role of S100B in the maturation of glial cells, formation of synapses, and general brain morphogenesis, which occur most frequently during early childhood. [231] After the age of 20 years, the baseline concentrations seemed to stabilize. [231]

In our study there was no statistical significant difference between males and females regarding to serum S 100 B protein.

In line with our results Portela et al, [231] reported that no differences based on sex regarding to S100 B protein.

Against our results Gazzolo et al, [232] reported that S100B concentrations in serum were associated with age in a detailed study of children between the ages of 0 and 15 years. Highest values were recorded during the first year of life and then again between 7 and 13 years. The authors hypothesize that these epochs correspond to the greatest spurts of growth and maturation; infancy and early adolescence. Different peaks of S100B based on sex were also found, again a possible manifestation of the growth phenomenon, which varies between the sexes.

Bouvier et al, [233] found that serum S100B concentrations decreased with age. Their results in an exclusively pediatric cohort showed that the highest concentrations of S100B were recorded before the age of 2 years. The permeability of the BBB, higher protein turnover in neuronal cells, and low renal secretion of S100B, as well as dynamic CNS development in children under 2 years old are possible explanations proposed for this finding.

However, Spinella et al, [222] found a positive correlation between age and CSF S100B in their study on pediatric patients ranging in age from 3 days to 17.8 years.

Nygaard et al, [234] reported in a study of adults found that S100B in the CSF increased with age, and also significantly higher in men. S100B could not be detected in serum samples in this study.

In line with our results Wiesmann et al, [235] reported that no significant age- or sex-related differences were found in this study by who selected a healthy blood donors whom they divided equally in 5 age bands each of approximately 9 years, spanning 18 to 65 years.

In our study we found that there was no statistical significant difference between encephalitic patients with or without convulsion regarding to S 100 B protein.

Also we found that there was no statistical significant difference between encephalitic patients with or without complications like (delayed speech, eye symptoms and abnormal gait) regarding to serum S 100 B protein.

Against our results Andreas Raabeet al, [236] reported that 13% of patients had complication with neurological deterioration occurred. All patients showed pathologically increased serum S-100B values. 85 % of these patients showed S-100B increases > 0.5 µg/l. In (16%), the increase in S-100B was the first sign of neurological complication and prompted emergency computed tomography scanning. [236]

Also Sherifa A Hamed et al, [220] reported that S-100B were higher in bacterial meningitis patients with neurological complicationscompared to patients without neurological complications. [220]

In a meta-analysis study identified a significant association between S-100β protein serum concentrations and short (less than three months), mid (three to six months) or long term (six months and above) prognosis in patients with moderate or severe traumatic brain injury. The concentrations were significantly correlated with unfavourable prognosis, as defined by mortality or Glasgow outcome score ≤3, irrespective of concomitant traumatic injuries. [237]

Hirokazu Tsukahara et al, [237] were consistent with those from two previous systematic reviews conducted in patients with stroke and cardiac arrest.[238,239] The first review found an association between S-100β protein concentrations and prognostic features (infarct volume and stroke severity), [239] while the second review showed that S-100β protein might be a better outcome predictor than the neurone specific enolase after cardiac arrest.[238] Their results were also consistent with a large observational study performed in unselected neurocritically ill patients that found that S-100β was associated with neurological deterioration or complications. [236]

In our study we found that there was no statistical significant difference between encephalitic patients who were drowsy, comatosed or semi-consious regarding to serum S 100 B protein.

In our study we found that there was no statistical significant difference between encephalitic patients who had unremarkable criteria of MRI and remarkable criteria of MRI regarding to serum S 100 B protein.

Manfred Herrmann et al, [240] reported that patients with and without visible intracerebral pathology in CT scans exhibited elevated concentrations of S-100B after traumatic brain injuiry (TBI) and a significant decrease in the follow-up blood samples. Release patterns of S-100B differed in patients with primary cortical contusions, diffuse axonal injury (DAI), and signs of cerebral edema (ICP) without focal mass lesions. All serum concentrations of S-100B were significantly correlated with the volume of contusions. Thisindicate that the early release patterns of S-100 may mirror different pathophysiological consequences of traumatic brain injury. [240]

In our study we also found that the mean value of serum S100 B protein was significantly higher in those encephalitis patients who had been ventilated than patients who had not been ventilated.

In our study we found that there was no statistical significant difference between survivors and non survivors of encephalitic patients regarding to serum S 100 B protein level.

Against our results Hirokazu Tsukaharaet al, [237] reported that S 100B was significantly higher in non-survivors than in survivors, poor outcomes in children with acute encephalitis/encephalopathy (AEE) were 91% for S100B. [237]

In line with our results Marie Studahl et al, [227] reported there was no correlation between levels of serum S-100B in herpes simplex encephalitis HSE patients in acute stage and outcome after 6 months. [227]

Also Aspazija Sofijanova et al, [241] reported that S100B protein is higher at asphyxiated term neonates with high risk for hypoxic ischemic encephalopathy, and S100B protein is a excellent marker for acute brain injury. [241]

The S100B protein is produced and released primarily by astrocytes in the CNS where it has trophic functions on neurons and the glia. [242] Several studies have already shown its role as a peripheral biochemical marker in cases of brain impairment, such as head trauma, stroke, CNS tumors, Alzheimer disease, schizophrenia, systemic lupus erythematosus. [243-247] However, according to Wijnberger et al, due to the short half-life of S100B (25 min-2 hours) no direct causal relationship can be established with brain injury, except for acute events. [248]

Spinella et al, [222] also compared CSF S100B between healthy children and those with established meningitis and found the latter had significantly higher concentrations.

Hamed et al, [220] examined concentrations of S100B and markers of oxidative stress and antioxidative activity in children with bacterial meningitis. S100B was increased in both serum and CSF samples. These investigators contended that brain injury resulting from bacterial meningitis is evidenced by the increased intrathecal production of S100B and markers of oxidative activity, and that the concentrations of these markers are related to injury severity.

A study of infants with bacterial meningitis [228] found increases in CSF S100B, with the highest values recorded in infants who developed encephalitis in addition to their meningitis. An ROC indicated that a CSF S100B concentration above 1.0 µg/L was diagnostic for the early detection of bacterial meningitis with encephalitis, with a sensitivity of 91% and a specificity of 82%, with an area under the curve of 0.92. S100B also surpassed standard monitoring techniques in identifying the development of encephalitis. [228]

Mokuno et al, [249] examined CSF S100B in patients with a rangeof neurological pathologies, including encephalitis,meningitis, cerebral infarction, Parkinson disease, sclerosis,and others. S100B were increased inseveral of these conditions, but not always simultaneously, suggesting that their presence may reflect the kind of tissue damage, i.e.,

glial vs neuronal. Concentrationswere highest in encephalitis and cerebral infarction, and S100B concentrations normalized early.

In our study a (ROC) curve indicated that a serum neopetrin concentration above 367.59 (nmol/l) was diagnostic for the early detection of complications of encephalitis with positive predictive value (PPV) 90.0 and negative predictive value 90.0 and accuracy 90.0 and a sensitivity of 90% and a specificity of 90%, with an area under the curve of 0.915.

In our study a (ROC) curve indicated that a serum neopetrin concentration above 379.45 (nmol/l) was diagnostic to detect outcome of patients who had encephalitis with positive predictive value (PPV) 44.44 and negative predictive value 100.0 and accuracy 75.0 and a sensitivity of 100% and a specificity of 68.75 %, with an area under the curve of 0.875.

In our study a (ROC) curve indicated that a serum S 100 B protein concentrations above 1.0 (µg/l) was diagnostic to detect patients who had encephalitis were ventilated with positive predictive value (PPV) 44.44 and negative predictive value 100.0 and accuracy 75.0 and a sensitivity of 100% and a specificity of 68.75 %, with an area under the curve of 0.875.

SUMMARY

Viral encephalitis is a medical emergency. The spectrum of brain involvement and the prognosis are dependent mainly on the specific pathogen and the immunological state of the host. Although specific therapy is limited to only several viral agents, correct immediate diagnosis and introduction of symptomatic and specific therapy has a dramatic influence upon survival and reduces the extent of permanent brain injury in survivors.[(2)]

Multiple studies were done to detect new markers for follow up disease progression and its prognosis. One of these markers is Neopterin which is produced by monocytes/macrophages when activated by interferon gamma secreted from T lymphocytes as a part of body immune response and assessment of neopterin concentration allows estimating the level of cellular immunity activation, as well as monitoring and predicting the progression of the disease. [(206)]

Astrocytes are the most common cells in brain tissue and S-100B protein is synthesized in astroglial and Schwann cells. It is an acidic calcium-binding protein and is thought to be a mediator of neuronal-glial interactions in normal brain. [(134)] Damage to the astrocytes causes leakage of S-100B protein extracellularly into the CSF and a small proportion leaks into the circulation. [(183)] So the identification of S 100 B protein for brain damage in viral encephalitis could be useful. [(134)]

The aim of this work was to study the clinical significance of S100 B protein and neopterin in patients with encephalitis.

The study was conducted on 40 subjects who divided into two groups.

- Group I: consisted of twenty patients with clinical manifestations and positive CSF criteria of suspected viral encephalitis.
- Group II: consisted of twenty apparently healthy volunteers of matching age and sex.

Patients were selected from those attending Alexandria Fever Hospital that were diagnosed as suspected viral encephalitis.

All patients and controls were subjected to detailed history taking and thorough clinical examination focusing on neurological manifestations, CBC, RBS and CRP. Only patients were subjected to lumbar puncture for CSF analysis, CSF culture for bacterial infection, MRI brain and CT brain.

Estimation of serum S100 B protein and neopterin by ELISA was done.

In our study we found that the serum Neopterin level was significantly higher in patients with suspected viral encephalitis than in healthy volunteers.

Also serum neopterin level showed increased statistical significance regarding to severity and complications as eye symptoms, abnormal gait and delayed speech.

We found that higher level of serum neopterin in non survivors and there was positive statistical significance between neopterin and death in patient with encephalitis.

We found the mean value of serum neopetrin was significantly higher in those patients with remarkable criteria of MRI than patients with unremarkable criteria of MRI.

In our study the mean serum S100 B protein was significantly higher in patients with suspected viral encephalitis than in control group.

Also we found that the mean value of serum S100 B protein was significantly higher in those encephalitic patients who had been ventilated than patients who had not been ventilated.

CONCLUSIONS

- Overall, these data suggest that serum Neopterin level is significantly higher in patients with suspected viral encephalitis than in healthy volunteers and it is a good marker for encepalitis.
- The measurement of serum Neopterin could be a useful parameter in predicting the severity and complications of patients with suspected viral encephalitis, together with other conventional clinical manifestations and CSF parameters.
- Serum neopterin is a promising biological marker in body fluids correlating with MRI brain crietaria.
- Higher level of serum neopterin in non survivors suggest that it is used as a good predictor for bad outcome in patients with encephalitis.
- The mean value of serum S100 B protein is significantly higher in patients with suspected viral encephalitis than in healthy group. So Increased S100B protein in biological fluids has been shown to be a marker of brain inflammation in encephalitic patients.
- The mean value of serum S100 B protein was significantly higher in those encephalitic patients who had been ventilated than patients who had not been ventilated. Therefore, increased concentrations reflect tissue damage and outcome of patients with encephalitis.