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Cytokine Profile in JE and non JE Encephalitis

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Abstract: ***Background & Objectives: Encephalitis is parenchymal brain inflammation due to infectious or immune-mediated processes. Acute Encephalitis Syndrome due to unidentified causes has been an issue of concern in eastern Uttar Pradesh. This study was undertaken to study the cytokine response in JE and non JE AES. Methodology: This prospective study was performed in patients with acute encephalitis syndrome (AES) aged 1 to 15 years who had been admitted to a tertiary care teaching hospital. Patients who tested positive for typhoid, malaria, measles , mumps, herpes(HSV), Dengue were excluded . Serum and CSF samples of JE positive and non JE cases were further analysed for cytokine levels - Tumor necrosis factor -α, Interferon-γ, Interleukin-10 and chemokines interferon activated protein-10 and RANTES. Results: 12 were positive for JE IgM and 58 cases were from the Non JE category in which no aetiology was detected. Cytokines were tested in 45 patients, 12 were of JE and rest 33 of non JE. CSF level of IFN-γ, IL-10 and IP-10 were significantly higher in Non JE patients than in JE patients. IP-10 cut off levels in CSF of more than 1223.24 were helpful in differentiating non JE from JE with a sensitivity of 72.73% and specificity of 66.63%. Conclusion: This study highlights the differences in immunological response of the JE and non JE cases. Interferon gamma inducible protein-10 (IP-10), a chemokine was siginificantly increased in the non JE group and turned out to be a fairly sensitive test in differentiating JE from non JE.***

Keywords: AES, JE, non JE, cytokine, IP- 10.

1. Introduction

Acute Encephalitic syndrome is a surveillance definition [1]. In India, the Japanese encephalitis (JE) virus, [1] herpes simplex and enteroviruses [2] have been identified as major causative agents of encephalitis. In approx 70% cases etiology cannot be ascertained [3]. JE has been the cause of viral encephalitis in eastern Uttar Pradesh in India since 1978, but in recent years changing trends in the etiology have been reported. Data collected from this region over the years has shown, that despite a falling incidence of JE as a cause of encephalitis, the incidence of encephalitis still remains the same [4]. No confirmed viral aetiological agent could be isolated in these cases and they are commonly grouped as non-JE encephalitis. These patients also show distinctive clinical features which are different from those seen in classical JE infection [5].

Cytokines are integral part of immune response to any infection. The earliest host responses to viral infections are nonspecific and induce cytokines resulting in facilitation of the both arms of immunity [6],[7]. Many cytokines are known to play important roles in the development of host defense against viral infections. While cytokines such as IFN α, IFN-β and TNF-γ have the potential to trigger activation of intracellular antiviral pathways after they bind to specific receptors on the surface of the infected cells, other cytokines like IL-1 ,IL-2, IL-6, IL-12, IL-13 and IL-18 are believed to contribute to the antiviral response indirectly by modulating various aspects of the immune response [8]. It has also been shown that cytokines not only play a central role in modulating the immune responses and inflammatory reactions but some cytokines like IL-1, TNF-γ can also have direct cytotoxic effects [9]. Previous studies have attributed the neuronal injury in patients with viral encephalitis to a number of proinflammatory cytokines [10].

Since the etiology in most cases of encephalitis cannot be ascertained, differentiating between various etiologies can be on a clinical basis and the cytokine response of various viruses may also be different. Thus, the main aim of this study was to record differences, if any, in the and the immune response in confirmed JE and non-JE encephalitis cases.

2. Methodology

This cohort study was performed from July to October 2011. Consecutive patients with acute encephalitis syndrome (AES) aged 1 to 15 years who had been admitted to a tertiary care teaching hospital were enrolled for the study. Clinically, a case of AES is defined by the WHO, as a person of any age, at any time of year, with the acute onset of fever and a change in their mental status (including symptoms such as confusion, disorientation, coma or the inability to talk) and/or new onset seizures (excluding simple febrile seizures). Based on the CSF examination patients with bacterial meningitis (bacterial culture also) and tuberculous meningitis were excluded. Patients testing positive for malaria (MERISCREEN Malaria Pf/Pv Ab: Meril Diagnostics) and typhoid fever (Typhi Check Standard Diagnostic USA) were also excluded from the analysis. Cerebrospinal fluid (CSF) and serum samples were tested for: JE (anti JE IgM MAC ELISA), Dengue (Standard Diagnostic USA), Mumps and Measles (siemans antimeasles virus ELISA).

Patients testing positive for JE, either in serum or CSF were considered to be confirmed JE. Other cases that indicated a viral aetiology without any detectable aetiological agent, were grouped as non-JE patients. Clinical features and detailed investigation of JE cases were compared with the non-JE cases.

Serum and CSF samples of JE positive and non JE cases were further analysed for cytokine levels- Tumor necrosis factor -α, Interferon-γ, Interleukin-10 and chemokines interferon activated protein-10(IP-10) and RANTES by Duo

ELISA kit (R&D Systems) according to the manufacturers' instructions.

Statistical analysis

Data collected from study was entered into a master chart. Mean and standard deviations were calculated for all continuous variables. Student t-test was used to determine if the difference between the means was statistically significant. A p value <0·05 were regarded as significant. To test the efficacy of tumour markers as diagnostic test for discrimination between JE encephalitis and non-JE encephalitis, receiver operating curves (ROC) were plotted. All statistical analysis was done using graphpad Prism 5 statistical software and STATA-12.

3. Results

A total of 87 AES patients were included in the study. Seventeen patients were excluded from the study for the following reasons: 4 had pyogenic meningitis (CSF culture showed the following causative agents: Streptococcus pneumoniae); 5 patients were positive for malarial as tested by the rapid diagnostic test for malaria parasite and two were IgM typhoid. Measles IgM was positive in 5 cases, HSV IgM in 1 case; Mumps IgM and Dengue IgM was not positive in any. Twelve patients were classified as confirmed JE cases on the basis of positive IgM ELISA in serum or CSF. Fifty eight with probable viral encephalitis, where no virus was detected, were grouped under non-JE cases.

Cytokines were tested in 45 patients, 12 were positive for JE IgM and rest 33 cases were from the Non JE category. Serum and CSF levels of the TNF-α and RANTES in both groups were high but no significant difference in the rise between the groups was found. When comparing the Serum and CSF level of IFN-γ, IL-10 and IP-10 were significantly higher in Non JE patients than in JE patients (Fig 1a-1e & 2a-2e). ROC curves were drawn to test the diagnostic efficacy of TNF-α, Interferon-Y, RANTES, IL-10 and IP-10 to establish if any of these markers could help differentiating between JE and non-JE encephalitis. Only IP-10 with AUC of 0.8043 was a good choice for such differentiation. A cut-off value of IP-10 more than 1223.24 was helpful in differentiating non-JE from JE with a sensitivity of 72.73% and specificity of 66.63% (AUC = 0.8043, CI 0.67468-0.93391) (Fig-III). Interferon gamma and IL-10 levels were not found to be diagnostic. (figure 3 a-e).

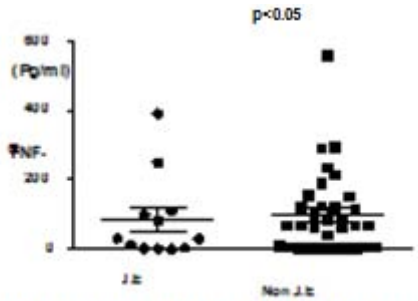
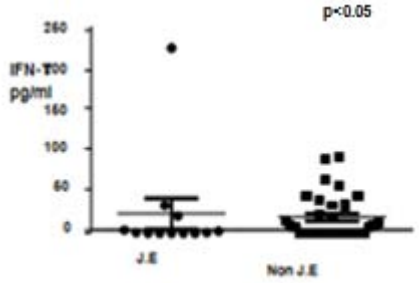


Figure (1a)-serum TNF-α in JE and non JE patients



Figure(1b)-serum IFN-γ in JE and non JE patients

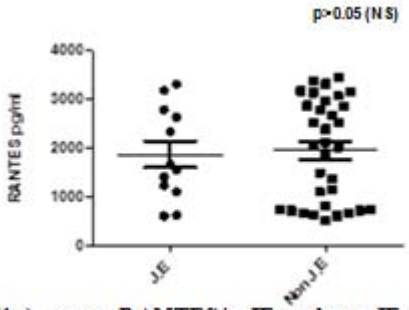


Figure (1c)- serum RANTES in JE and non JE patients

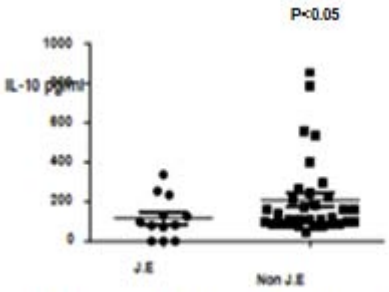


Figure (1d)- serum IL-10 in JE and non JE patients

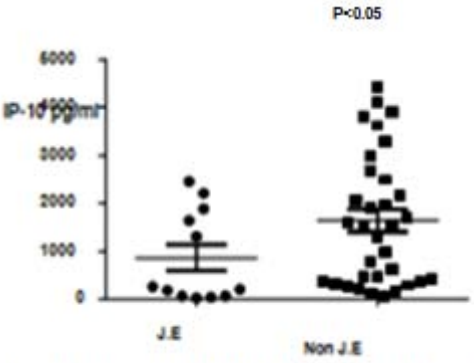


Figure (1e)- serum IP-10 in JE and non JE patients

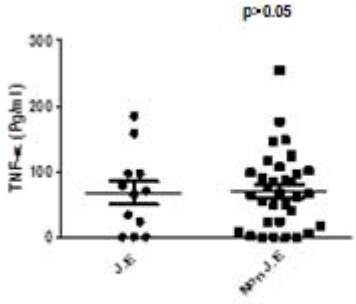


Figure (2a)–CSF TNF-α in JE and non JE patients

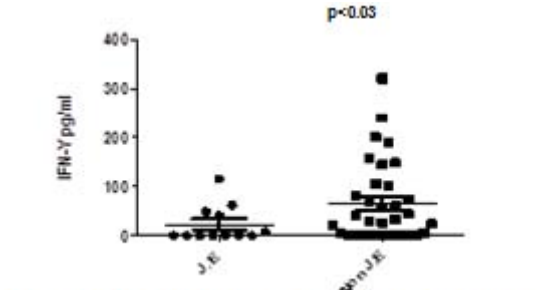


Figure (2b) CSF IFN-γ in JE and non JE patients

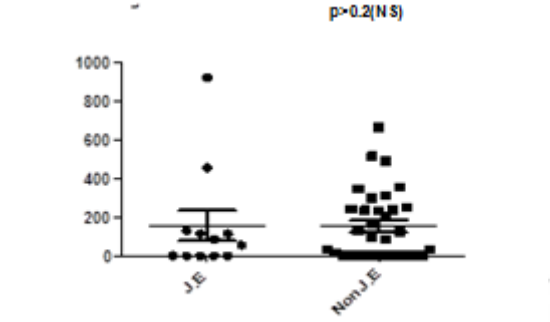


Figure (2c) CSF RANTES in JE and non JE patients

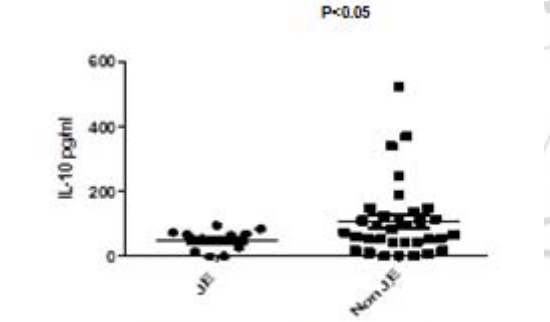


Figure (2d) CSF IL-10 in JE and non JE patients

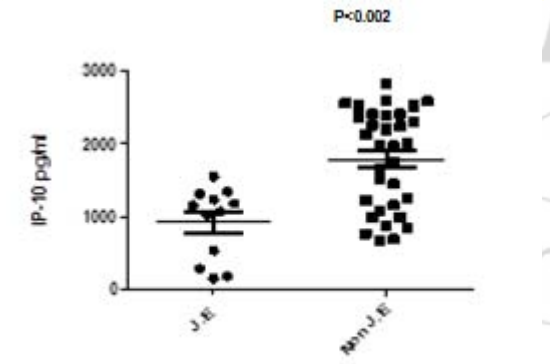


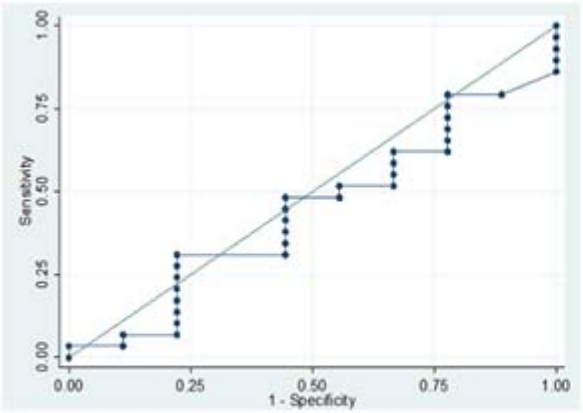
Figure (2e) CSF IP-10 in JE and non JE patients

4. Discussion

This study highlights a difference in immunological response of the JE and non JE cases. It appears that non JE infection initiates an inflammatory response with marked release of IP-10. Other inflammatory markers like IFN-γ, IL-10 and IP-10 are also significantly raised in Non JE patients as compared to JE. In our study group interferon gamma inducible protein (CXCL-10), a chemokine was significantly increased in the non JE group . It also turned out to be a fairly sensitive test in differentiating JE from non JE.

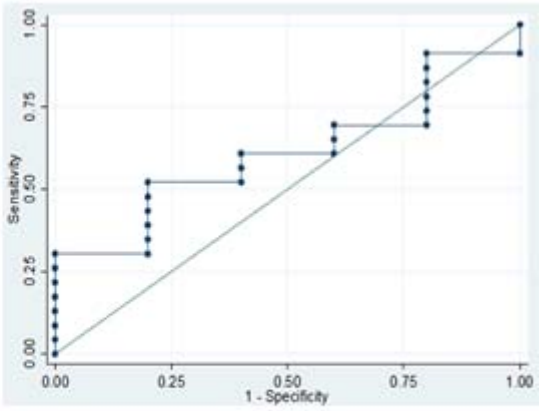
Cytokines play a significant role in the development of an acute or chronic inflammatory response, and many chemokines exhibit redundant and pleiotropic effects that work together and contribute to the inflammatory response [11]. IP-10 is a CXC chemokine secreted by several cell types, including macrophages, microglia and astrocytes in response to stimuli, such as interferon (IFN)-α, IFN-β, IFN-γ or viruses. [12]-[15]. It is a potent chemoattractant for NK cells and particularly T cells during viral infection [16]-[18]. It is also a fascinating chemokine because of its controversial roles in viral infection. It has been implicated to aggravate diseases in mice infected with mouse hepatitis virus, herpes simplex virus or lymphocytic choriomeningitis virus, and in humans infected with human immunodeficiency virus, hepatitis C virus or severe acute respiratory syndrome coronavirus [19]-[24]. However, endogenous IP-10 has been shown to protect mice infected with coxsackievirus B3, dengue virus, herpes simplex virus or mouse hepatitis virus by promoting viral clearance in tissues [16],[18],[25],[26] . IP-10 can suppress viral infection by recruiting mainly leukocytes [16],[17],[26],[27] or directly by inhibiting viral replication [25],[28]. Moreover, leukocyte recruitment mediated by IP-10 varies with the infecting virus and infected organ [16],[17],[26],[27]. Several independent studies showed that EV71– infected patients with BE (brain stem encephalitis) and PE (pulmonary edema) exhibit various elevated chemokines and cytokines, including interferon (IFN)-γ (a Th1 cytokine), IL-6 (a pleiotropic cytokine), IL-1β (a pro-inflammatory cytokine , IL-10 (an immunoregulatory cytokine), IL-13 (a Th2 cytokine), and the chemokines IL-8) and IP-10 [29],[30] among others. Thus, IP10 is being increasingly used in the pathogenesis, assessing the severity and prognosis for many viral illnesses and non viral illnesses.

JE infection is on the decline in this region [6] and with the changing landscape it can be concluded, that IP-10 may be a useful clinical marker to identify non JE from JE. Its usefulness could be further studied for determining the pathogenesis of non JE AES, the etiology of which remains undetermined and may be a explored as a potential therapeutic target for the future.



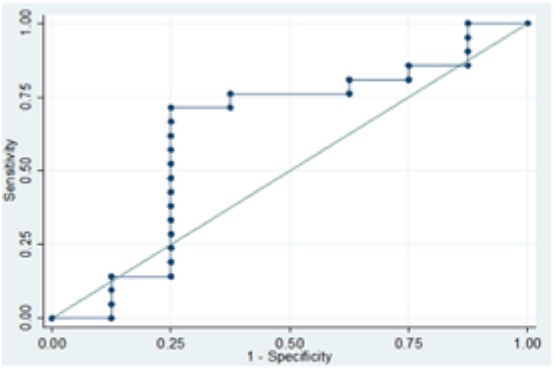
Area under ROC curve = 0.4406

Figure (3a): ROC for TNF-α in diagnosing Non-JE encephalitis in csf



Area under ROC curve = 0.6087

Figure (3b): ROC for Interferon-Y in diagnosing Non-JE encephalitis in csf



Area under ROC curve = 0.6310

Figure (3c): ROC for RANTES in diagnosing Non-JE encephalitis

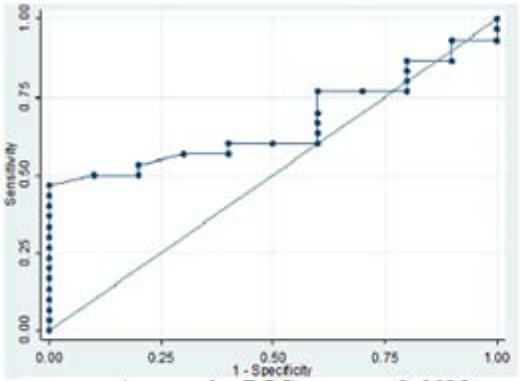
AUROCC*	Interpretation
1.0	Perfect test
0.9 to 0.99	Excellent test
0.8 to 0.89	Good test
0.7 to 0.79	Fair test
0.51 to 0.69	Poor test

0.5 – Negative predictor

≤0.5 – Worthless test

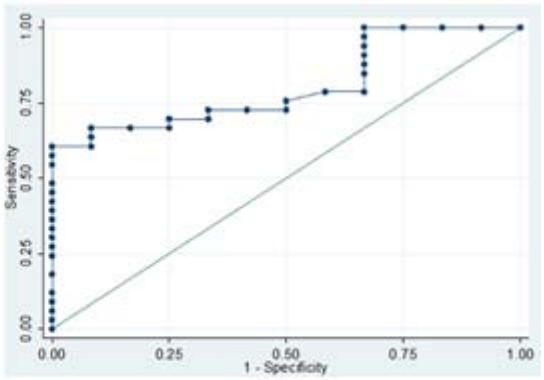
0.5 to 1.0 – Positive predictor

***Area under ROC curve**



Area under ROC curve = 0.6633

Figure (3d) ROC for Interleukin 10 in diagnosing Non-JE encephalitis in csf



Area under ROC curve = 0.8043

Figure (3e) ROC for IP 10 in diagnosing Non-JE encephalitis in csf

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