STUDY OF CEREBROSPINAL FLUID NEOPTERIN IN PEDIATRIC NEUROINFLAMMATORY DISEASES

Alaa Mamdouh Mohamed Kamel[1], Nahla Mohamed Zakaria Yousef[1], Sahar Mohamed Ahmed Hassanein[2], Nesrine Aly Mohamed [1] and Walid Abdelhady Ahmed [1]

[1]Clinical Pathology Department, Faculty of Medicine, Ain Shams University.

[2]Pediatrics Department, Faculty of Medicine, Ain Shams University.

Corresponding author:
Alaa Mamdouh Mohamed Kamel
Tel: 01092579726
E-mail:
alaagaber203@gmail.com

Received: 17/10/2023
Accepted: 4/12/2023

Online ISSN: 2735-3540

ABSTRACT:

Background: *Acknowledgment of Neurological disorders in which irritation plays a key part have been expanded in children. Guidelines about diagnostic testing for pediatric patients with these suspected disorders are missing till presently.*

Aim of the work: *Our study aims to evaluate the diagnostic test accuracy of CSF neopterin in relation to standard chemical CSF biomarkers as total proteins and leucocytes in suspected pediatric patients with CNS inflammatory disorders.*

Patients and Methods: *Forty-eight pediatric patients without CNS diseases (group I) and 32 patients with CNS diseases (group II) were included. They were presented with disturbed conscious level, and / or fever and / or signs of acute neurological syndrome to the emergency room (ER) of Pediatrics Department of Ain Shams University hospitals. CSF chemical analysis, bacterial cultures, viral panel by PCR and CSF neopterin by ELISA were done in all patients.*

Results: *CSF neopterin levels showed high significance in patients with CNS diseases compared to those without CNS diseases and showed a significant positive correlation with CSF WBCs, protein and LDH. Logistic regression analysis revealed that CSF neopterin was the only independent parameter that proved to be a significant predictor for discrimination between the two studied patient groups.*

Conclusion: *CSF neopterin can be useful as a biomarker to differentiate between inflammatory CNS diseases and non-CNS diseases.*

Keywords: *CSF neopterin, Neuroinflammation, CNS diseases.*

1. INTRODUCTION:

Neuroinflammation is a condition which is characterized by production of proinflammatory mediators within the central nervous system (CNS). Neuroinflammation has become an important topic in neuroscience due to its association with neurodegenerative diseases. Although neuroinflammation is often associated with CNS damage, it also can occur in the absence of neurodegeneration, as in association with systemic infection (1).

Immune-mediated and inflammatory diseases of the CNS include different conditions that share the immunological dysregulation involvement of the CNS, in which the underlying mechanisms of dysregulation of the immune system are different. Different pathogenic mechanisms have been identified, such as cell-mediated and antibody-mediated, infection-triggered, paraneoplastic, and genetically defined mechanisms that can occur in previously healthy children and can cause different stages of the disease (2).

In pediatric neurology, cerebrospinal fluid (CSF) neopterin is reported as a portion of CSF neurotransmitter examinations, including dopamine and serotonin metabolites and pteridines. CSF neopterin is intrathecally produced and is increased only in CNS infectious illnesses not in infectious illnesses peripheral to the CNS. CSF neopterin, in this manner, shows up to be a particular marker of CNS immune activation [(3)].

It has been appeared that neopterin within the brain is independently produced, as there's no relation between the concentrations of neopterin within the plasma and CSF of patients with immune-inflammatory disorders. With respect to neopterin cellular sources within the brain, it has been recommended that microglia and astrocytes are the source of neopterin since these cells respond to IFN-γ [(4)].

Neopterin is one of the biochemical markers which are utilized to evaluate the intensity of the cell-mediated immune reaction. Neopterin concentrations not only permit identifying cell-mediated immune activation, but moreover permitting an assessment of oxidative stress because it gets to be apparent particularly during chronic illness states [(5)].

2. SUBJECTS AND METHODS:

2.1. Study design:

This was a diagnostic test accuracy cross-sectional study.

2.2. Subjects:

This study was conducted on 80 patients who were presented with disturbed conscious level, and/or fever and/or signs of acute neurological syndrome to the emergency room (ER) of Pediatrics Department of Ain Shams University Hospitals, from June 2021 to February 2022. **Subjects were classified according to final diagnosis into two groups: Group (I)** included 48 patients without CNS disease. **Group (II)** included 32 patients with inflammatory CNS disease.

2.3. Sample collection:

Five ml of CSF were withdrawn from each patient aseptically into a sterile disposable syringe and collected in plain tube and was preserved at -80°C until used for detection of neopterin.

2.4. Neopterin measurement in CSF:

Neopterin levels (ng/mL) were measured by using a competitive enzyme linked immunosorbent assay (ELISA) kit (Cat. # 430206; Wuhan Fine Biotech Co., Ltd., Wuhan, Hubei, China) according to the instructions of the manufacturer. Briefly, CSF was added to plates coated with a monoclonal antibody against neopterin and incubated. During the reaction, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the biotinylated detection antibody specific to target. Excess conjugate and unbound sample or standard were washed from the plate, and horseradish peroxidase-streptavidin was added to each microplate well and incubated. Then tetramethylbenzidine substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and the color change were measured spectrophotometrically at a wavelength of 450nm using a Stat Fax 2100 microplate reader (Awareness Technology, Inc., Palm City, FL), with the lower end of sensitivity of the assay being 0.094 ng/mL.

2.5. Statistical methods:

The collected data was revised, coded, tabulated and introduced to a PC using Statistical Package for Social Science (SPSS 25) (IBM corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). Data was presented, and suitable analysis was done according to the type of data obtained for each parameter. Median and 25th-75th percentile range were the tools to express

non-parametric numerical data. Mann Whitney Test (U test) was used to assess the statistically significant difference of a non-parametric variable between the two studied groups. Correlation analysis using Spearman's rho method was used to assess the strength of association between two quantitative variables. The ROC Curve (Receiver Operating Characteristic) was used to evaluate sensitivity and specificity for quantitative diagnostic measure that classifies cases into one of two groups. A statistical significance was considered at $P < 0.05$.

3. ETHICAL CONSIDERATION:

The study was approved by the Faculty of Medicine, Ain Shams University Research Ethical Committee (FMASU MS 295/2021).

4. RESULTS:

Eighty pediatric patients with suspected CNS inflammatory disorders were included in the study. The comparative statistics between the two groups as regards CSF neopterin and WBCs count showed significant difference. As regards CSF proteins, the statistical comparison showed no significant difference as shown in table 1 and diagrams 1,2 and 3.

Table (1): Comparison between the two studied groups regarding CSF laboratory investigations.

Parameter	Group (I) Non-CNS diseases (n=48)	Group (II) Inflammatory CNS diseases (n=32)	Mann-Whitney (U) test		
	Median (25^{th} -75^{th} percentile)	Median (25^{th} -75^{th} percentile)	z	p	Sig.
Neopterin (ng/ml)	0.3 (0.09 - 0.33)	0.3 (0.15 - 0.6)	-2.015	0.044	S
WBCs (xmm^3)	20 (7 - 25)	57.5 (28 - 256.5)	-2.771	0.006	HS
Protein (mg/dl)	55.35 (30.8 - 127.5)	71.05 (36 - 221.8)	-1.203	0.229	NS

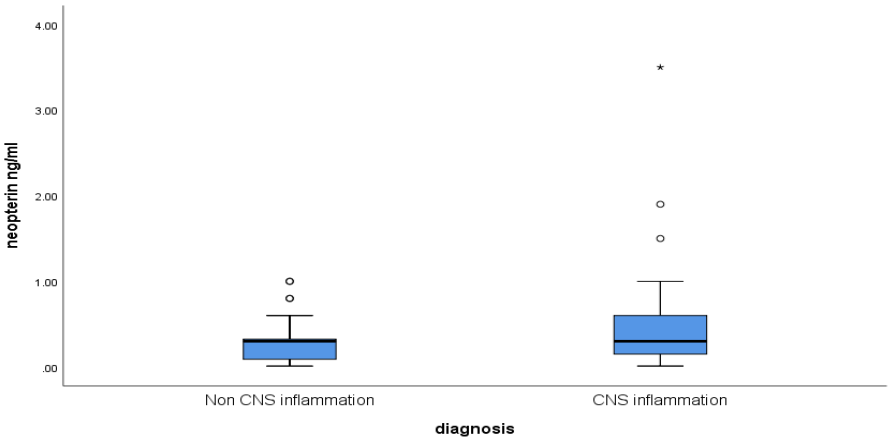

Diagram (1): Median values of CSF neopterin in group I (non-CNS diseases) and group II (inflammatory CNS diseases).

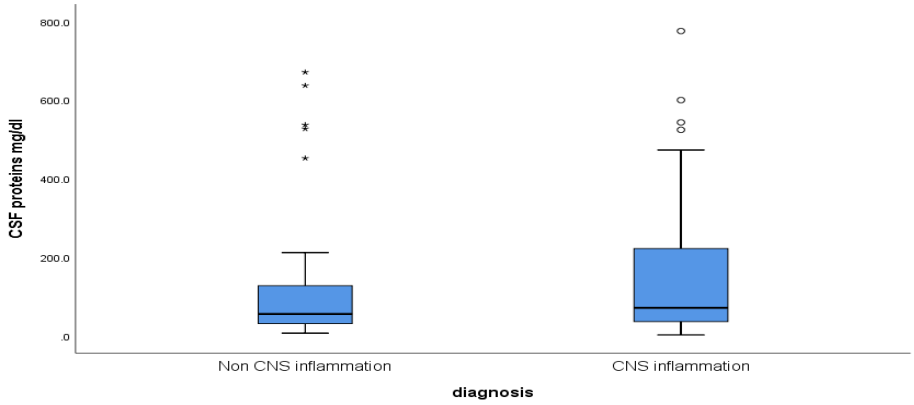

Diagram (2): Median values of CSF protein in group I (non-CNS diseases) and group II (inflammatory CNS diseases).

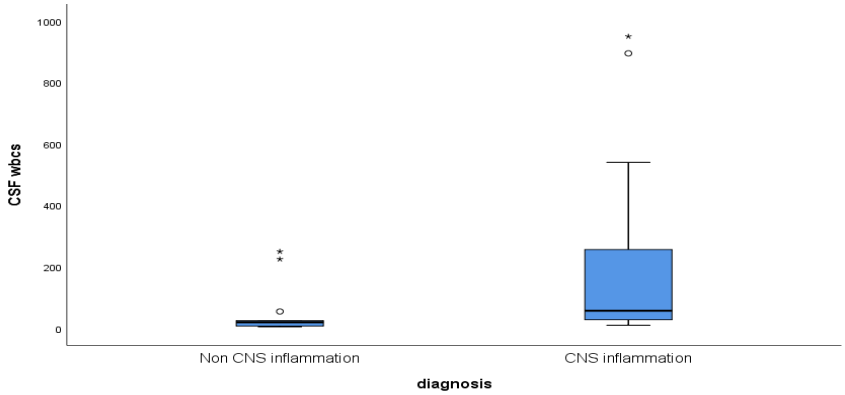

Diagram (3): Median values of CSF WBCs in group I (non-CNS diseases) and group II (inflammatory CNS diseases).

The Roc curve for CSF neopterin as shown in table 2 and diagram 4. estimated our cut-off value to be >0.35ng/ml.

Table (2): The diagnostic characteristics of CSF neopterin.

Cut-off value	AUC	95% CI	P	Sensitivity	Specificity	PPV	NPV	+LR	-LR
>0.35 ng/ml	0.632	0.517-0.737	0.044	46.88%	77.08%	57.7%	68.5%	2.05	0.69

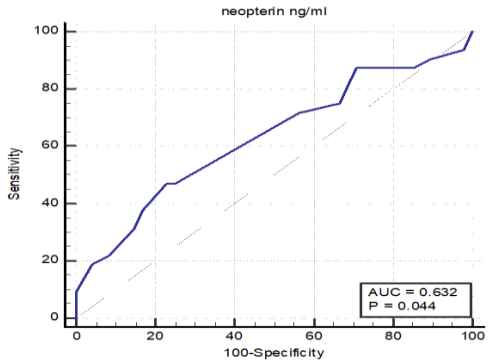

Diagram (4): Roc curve for CSF neopterin.

CSF neopterin showed positive significant correlation with CSF WBCs, CSF protein and CSF LDH. It also showed negative significant correlation with CSF glucose as in table 3.

Table (3): Correlation between CSF neopterin and the different studied CSF chemical parameters in the whole group of patients (n = 80).

CSF neopterin (ng/ml)	CSF WBCs	CSF proteins	CSF glucose	CSF LDH
R_s*	0.479	0.453	-0.234	0.495
P	0.006	<0.001	0.037	<0.001
Sig.	HS	HS	S	HS

* R_s = Spearman's Rho correlation test.

Logistic multiple regression analysis was done to study the significance of CSF neopterin, CSF WBCs, CSF proteins, CSF glucose, CSF LDH as predictor variables upon discrimination between non-CNS diseases and inflammatory CNS diseases (as dependent variable) in the whole patients. CSF neopterin was the only independent parameter that proved to be a significant predictor for discrimination between the two studied groups as shown in table 4.

Table (4): Logistic regression analysis to study the effect of CSF neopterin as an independent predictor factor to discriminate between group I and II patients.

	Odd's ratio (OR)	p	OR 95% CI	
			Lower	Upper
CSF neopterin (ng/ml)	4.893	0.034 (S)	1.129	21.209

5. DISCUSSION:

Neuroinflammation is an inflammatory reaction inside the central nervous system (CNS). Microglial cells and astrocytes are the main source of inflammatory responses within the CNS. Microglial cells have a vital part in neurogenesis, neuronal plasticity, and regeneration. They are the primary line of the body's immune defense against any brain harm, performing the functions of phagocytosis of harmful substances and discharge of cytotoxic components. Being active, they discharge cytokines and neurotoxic agents, which cause more harm to the nervous tissue. Astrocytes are specialized glial cells that give a supporting function for the nervous system [6].

It has been shown that neopterin in the brain is independently produced, as no correlation could be found between the concentrations of neopterin in the plasma and CSF of patients with immune-inflammatory disorders. Regarding neopterin cellular sources in the brain, it has been suggested that CSF neopterin is produced by microglia and astrocytes since these cells respond to IFN-γ [4].

The aim of our study was to evaluate the diagnostic test accuracy of CSF neopterin in relation to standard chemical CSF biomarkers as total proteins and leucocytes in suspected pediatric patients with CNS inflammatory disorders. This diagnostic test accuracy cross-sectional study included 80 patients aged from 3 days up to 18 years old presented to

the pediatrics ER with disturbed conscious level, and/or fever and/or signs of acute neurological syndrome.

In our study, the 80 patients who were clinically suspected to have CNS inflammatory disease underwent brain MRI and lumbar puncture where CSF proteins, total leucocytes, glucose and LDH were measured as well as CSF bacterial culture and viral panel examination using real-time PCR were done to establish the diagnosis. Then they were classified into two groups: group I comprised 48 patients who proved to have diseases other than CNS diseases and group II that comprised 32 patients with confirmed inflammatory CNS diseases. In all included subjects in the two groups, we measured CSF neopterin by ELISA and the results showed significant difference between the two groups. A ROC curve was created for neopterin, that estimated our cut-off value to be >0.35ng/ml (p=0.044) with AUC=0.632, specificity = 77.08%, sensitivity = 46.88%, positive predictive value = 57.7% and negative predictive value = 68.5%.

Our results regarding CSF neopterin were similar to a study done by Russell et al. (2009) who aimed to test the efficacy of elevated CSF neopterin as a biological marker of CNS inflammation. They retrospectively reviewed CSF neopterin in 158 children. They reported that the acute group had statistically significant elevated CSF neopterin compared with the chronic static group with p-value <0.001.

Also, our results were similar to Molero-Luis et al. (2020) who conducted an observational retrospective and case-control study to analyze the CSF neopterin, total proteins, and leukocytes in pediatric patients with neuroinflammatory disorders. CSF samples from 277 subjects were included and classified into four groups: viral meningoencephalitis, bacterial meningitis, acquired immune mediated disorders, and patients with non-immune diseases (control group). They reported that regarding neopterin, the highest values were observed in the bacterial and viral meningitis groups, while the lowest were found in the acquired autoimmune disease and control groups.

In a study done by Molero-Luis et al. (2013) who aimed to establish new CSF neopterin cut-off value, they studied two groups of patients: Group 1 comprised 68 patients with meningoencephalitis, and Group 2 comprised 52 children with a confirmed peripheral infection and no CNS involvement. They reported that significant differences were observed between groups 1 and 2 with p-value < 0.001 for CSF neopterin. A ROC curve was established, and they estimated the cut-off value to be 24.4ng/ml with the AUC= 0.934, specificity= 88.5% and sensitivity= 91.3%.

Our results regarding CSF leucocytes (WBCs) count showed significance between the two groups and this was similar to the study by Molero-Luis et al. (2020) who reported that considering CSF leucocytes, bacterial followed by viral meningoencephalitis displayed the highest values and showed significant differences when compared with the other groups. According to Molero-Luis et al. (2013), they again reported that significant differences of CSF WBCs were observed between groups 1 (patients with meningoencephalitis) and 2 (patients with a confirmed peripheral infection and no CNS involvement) with p-value < 0.001.

Our results regarding CSF proteins showed no significance between the two groups and this is unlike the study by Molero-Luis et al. (2013) who reported that significant differences of CSF proteins were observed between groups 1 (patients with meningoencephalitis) and 2 (patients with a confirmed peripheral infection and no CNS involvement) with p-value < 0.001. According to Molero-Luis et al. (2020), they reported that for CSF total proteins, the highest values were detected in the bacterial infection group, which showed significant

differences when compared with the other groups (viral meningoencephalitis, acquired immune mediated disorders, and patients with no-immune diseases (control group). The discrepancy between our results and others could be attributed to the difference in the clinical characteristics and the number of patients included in the studies.

A positive significant correlation was observed between CSF neopterin and CSF WBCs ($r = 0.479$, $p = 0.006$), CSF proteins in mg/dl ($r = 0.453$, $p < 0.001$), and CSF LDH in U/L ($r = 0.495$, $p < 0.001$). These results were similar to Molero-Luis et al. (2013). They reported that a significant positive correlation was observed for CSF neopterin with CSF proteins ($r = 0.178$, $p < 0.001$) and leukocytes ($r = 0.148$, $p < 0.001$).

Our results also showed a negative significant correlation between CSF neopterin and CSF glucose in mg/dl ($r = -0.234$, $p = 0.037$). This was unlike Molero-Luis et al. (2013) who reported that there was no significance between CSF neopterin and CSF glucose. Again, this disagreement could be due to different clinical characteristics of patients included in both studies.

6. Conclusion:

Although there were some limitations for our study such as the small sample size and the difficulty in collecting CSF samples from pediatric patients as it is an invasive diagnostic procedure, however, our study could establish the significance of CSF neopterin as a biomarker to differentiate between inflammatory CNS diseases and non-CNS diseases.

7. Conflict of Interest:

No conflict of interest.

8. REFERENCES:

1. **James P & Diane B (2019):** Neuroinflammation disorders exacerbated by environmental stressors. Metabolism; 100: 153951.
2. **Wells E, Hacohen Y, Waldman A, illema JM, Soldatos A ….. Banwell B (2018):** Neuroimmune disorders of the central nervous system in children in the molecular era. Nature reviews neurology; 14: 433-45.
3. **Russell C, Fabienne B, Elizabeth F & John E (2009):** Cerebrospinal fluid neopterin in pediatric neurology: a marker of active central nervous system inflammation. Developmental Medicine and Child Neurology; 51: 317-323.
4. **Molero-Luis M, Casas A, Orellana G, Ormazabal A, Sierra C ….. Artuch R (2020):** Cerebrospinal fluid neopterin as a biomarker of neuroinflammatory diseases. Scientific reports; 10: 18291.
5. **Fuchs D, Jaeger M, Widner B, Wirleitner B, Artner-Dworzak E & Leblhuber F (2001):** Is hyperhomocysteinemia due to oxidative depletion of folate rather than insufficient dietary intake. Clinical Chemistry and Laboratory Medicine; 39: 691-694.
6. **Grigoryan G (2022):** Neuroinflammation and Reconsolidation of Memory. Neurochemical Journal; 39 (2): 99-112.
7. **Molero-Luis M, Fernández-Ureña S, Jordán I, Serrano M, Ormazábal A, Garcia-Cazorla À & Artuch R (2013):** Cerebrospinal Fluid Neopterin Analysis in Neuropediatric Patients: Establishment of a New Cut Off-Value for the Identification of Inflammatory-Immune Mediated Processes. PLOS ONE; 8(12): e83237.

دراسة النيوبترين فى السائل النخاعي في أمراض التهاب الجهاز العصبى لدى الأطفال

آلاء ممدوح محمد كامل[1]، نهلة محمد زكريا يوسف[1]، سحر محمد أحمد حسنين[2]، نسرين على محمد[1]، وليد عبدالهادى أحمد[1]

قسم الباثولوجيا الإكلينيكيه - كلية الطب - جامعة عين شمس[1]
قسم طب الأطفال - كلية الطب - جامعة عين شمس[2]

نبذة مختصرة : تم التعرف بشكل متزايد على الاضطرابات العصبية التي يلعب فيها الالتهاب دورًا رئيسيًا لدى الأطفال. لا توجد إرشادات كافية بشأن الاختبارات التشخيصية لمرضى الأطفال المصابين بهذه الاضطرابات المشتبه بها.

الهدف من العمل : تقييم دقة الاختبار التشخيصي لـ CSF neopterin فيما يتعلق بالعلامات الحيوية القياسية للـ CSF كبروتينات وخلايا بيضاء في مرضى الأطفال المشتبه بهم الذين يعانون من اضطرابات التهابية في الجهاز العصبي المركزي.

المرضى و الطرق: تم إجراء هذه الدراسة على 48 مريضًا من الأطفال غير المصابين بأمراض الجهاز العصبي المركزي (المجموعة الأولى) و32 مريضًا يعانون من أمراض الجهاز العصبي المركزي (المجموعة الثانية). تم استقبالهم بمستوى وعي مضطرب و / أو حمى و/ أوعلامات متلازمة عصبية حادة إلى غرفة الطوارئ في قسم طب الأطفال في مستشفيات جامعة عين شمس. تم إجراء التحليل الكيميائي للسائل النخاعي ، والزراعات البكتيرية ، واللوحة الفيروسية بواسطة تفاعل البوليميراز المتسلسل (PCR) والنيوبترين فى السائل النخاعى بواسطة ELISA في جميع المرضى.

النتائج: كانت مستويات نيوبترين السائل الدماغي النخاعي أعلى بشكل ملحوظ في مرضى الجهاز العصبي المركزي مقارنة بأولئك الذين لا يعانون من أمراض الجهاز العصبي المركزي وأظهرت ارتباطًا إيجابيًا كبيرًا مع خلايا الدم البيضاء في السائل النخاعي والبروتين و LDH. كشف تحليل الانحدار اللوجستي أن نيوبترين السائل الدماغي النخاعي كان العامل المستقل الوحيد الذي أثبت أنه مؤشر مهم للتمييز بين مجموعتي المرضى الخاضعين للدراسة.

الاستنتاج: يمكن أن يكون نيوبترين السائل الدماغي النخاعي مفيدًا كعلامة بيولوجية للتمييز بين أمراض الجهاز العصبي المركزي الالتهابية والأمراض غير المرتبطة بالجهاز العصبي المركزي.