IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB)
ISSN: 2455-264X, Volume 3, Issue 6 (Nov.- Dec. 2017), PP 17-21
www.iosrjournals.org

Study of Free Sialic Acid and Aspartate Transaminase Levels In Cerebrospinal Fluid of Patients With Pyogenic Meningitis and Tuberculous Meningitis

Dr. Sandhya Rani Sadula [1], Dr. N.Vani[2] , Dr. S.Sailusha [3]
[1(Senior Resident, Department of Biochemistry, Osmania medical college, India)
[2](Professo and Head, Department of Biochemistry, Osmania medical college, India)
[3](Senior Resident, Department of Biochemistry, Osmania medical college, India)
Corresponding Author: Dr. Sandhya Rani Sadula

Abstract :
Objective*: Meningitis is an acute inflammation of meninges which more prevalent in worldwide. Sialic acid (SA) is a nine carbon sugar derived from mannosamine and pyruvate, bound to glycoproteins and glycolipids found in the brain tissue. The aim of the study is to evaluate the levels of CSF free sialic acid (FSA) in Pyogenic meningitis(PM) and Tuberculous meningitis(TBM) as a marker of Pyogenic Meningitis.*
Methods*: A case control study was done with 90 patients divided into 3 groups . Group 1- controls (n=30), group 2-Pyogenic Meningitis(n=30) and group 3- Tuberculous Meningitis(n=30)) with inclusion and exclusion criteria. CSF samples were analysed for Free Sialic acid, Aspartate transaminase, Glucose and Protein . Multiple comparisons between different groups were done using ANOVA test.*
Results*: In the present study CSF free SA levels were significantly high in PM (p<0.0001) as compared to TBM and controls. CSF AST levels were significantly high in both the meningitides. But,mean±SD of AST was slightly high in PM.*
Conclusions*: PM is associated with significantly increased FSA and AST. Thus, determination of free sialic acid levels and AST levels will be of help in differentiation between pyogenic and tuberculous meningitis.*
Keywords: *Aspartate Transaminase, Free Sialic Acid, Meningitis,*

Date of Submission: 02-11-2017 Date of acceptance: 16-11-2017

I. Introduction

Meningitis is an infection of the protective membrane that surround the brain and spinal cord. Meningitis is one of the most dreadful disease affecting the mankind at all ages. In India, the most common types of meningitis are pyogenic and tubercular. Clinical presentation of these forms of meningitis may not always be typical and hence may overlap[1].

Bacterial meningitis is a more prevalent worldwide life threatening illness. Bacterial meningitis remains a major cause of death and long term neurological disabilities despite advances in vaccine development and chemoprophylaxis[2].

Many different organisms can lead to inflammation of the meninges. Bacterial meningitis is more serious than viral meningitis which is though more common. Bacteria have a range of virulence factors that can overwhelm the host's defense mechanisms. Across all age groups, S. aureus, E. coli, K. pneumoniae, Pseudomonas aeruginosa, Streptococcus species and E. faecalis are mostcommon[2].

Of the various types of meningitis seen in adult neurological patients in India, a large percentage are diagnosed as tuberculous meningitis[3].

Mycobacterium tuberculosis causes Tuberculous meningitis. The physiopathology of this condition, in which disproportionate inflammatory phenomena rather than numbers of circulating bacteria play a role, hinders bacteriological diagnosis, and the available microbiological tests fail to attain the accuracy standards required[4].

1.1 Sialic Acid

The naturally occurring sialic acids are substituted neuraminic acid derivatives (Nacetyl, N-glycolyl, N,0-diacetylneuraminic acid). Neuraminic acid is a unsubstituted 9 carbonchain compound[5]. N-Acetylneuraminic acid (NANA) is a constituent of gangliosides and glycoproteins, present in significant quantity in brain[1].

The most commonly used investigation of differentiating tuberculous from pyogenic meningitis is by CSF culture, but tubercle bacilli take about six to eight weeks to grow,and, furtherrmore, the results of culture are usually negative in patients who have been partially treated with antibiotics before admission.

Several strains of microorganisms like pneumococci, H.influenza and tubercular bacilli etc. from the infected CSF elaborate neuraminidase which cleave the neuraminic acid and increase CSF sialic acid.

1.2 Aspartate Transaminase

AST is an intracellular aminotransferase which catalyzes the transfer of an amino group from an α-aminoacid to α ketoacid. CNS contains AST. Hence any injury or disease of CNS will increase the AST levels in CSF[1].

This study has been undertaken to evaluate the levels of CSF free Sialic acid in Pyogenic meningitis (PM) and Tubercular meningitis (TBM) as a marker of Pyogenic meningitis (PM) and to examine the diagnostic value of CSF Aspartate transaminase levels in Pyogenic meningitis.

II. Materials And Methods

A case control study of 90 subjects was conducted in the Department of Biochemistry, Osmania General Hospital, Hyderabad. The study subjects are divided equally into 3 groups.

Group 1 - Non meningitis controls

Group 2 - Pyogenic meningitis

Group 3 – Tuberculous meningitis

All the subjects were in the age group upto 55 years and of either sex. Informed oral consent was taken from all individuals who took part in the study.

2.1 Inclusion criteria

- Group 1 included Non meningitis controls include cases of CNS trauma, Guillain Barre syndrome, Multiple sclerosis,CNS tumors and Sarcoidosis that were matched for age and sex.
- Group 2 included Clinically diagnosed Pyogenic meningitis
- Group 3 included Clinically diagnosed Tubercular meningitis

2.2 Exclusion criteria

- Viral,fungal,parasitic meningitis cases were excluded.
- Xanthochromic csf samples were excluded.

2.3 Collection of sample

CSF samples were collected under aseptic conditions from all groups. Samples were analysed for free sialic acid, AST, glucose and protein.

2.4 Methods

Sialic acid was analysed by modified thiobarbturic acid assay of warren, AST was analysed by modified IFCC method, glucose was analysed by Trinder's Method (GOD-POD) and proteins were analysed by Turbidimetric method by 3%sulfosalicylic acid.

2.5 Statistics

The data was analyzed using Graph Pad Prism Demo and software version 6 and the results were expressed as Mean and Standard deviation of various parameters in different groups. Multiple comparisons ANOVA was done to assess the significance of difference of mean values of different parameters in between the groups and represented by p values (p value < 0.05 is considered as significant).

III. Results

Table 1: Mean ± SD and P-value of various parameters in all groups

Parameter	Mean±S.D of controls	Mean±S.D of pyogenic meningitis	Mean±S.D of tubercular meningitis	P-value in pyogenic meningitis	P-value in tubercular meningitis
Free sialic acid	31.47 ±12.24	92.27 ± 32.19	38.93 ± 17.96	0.0001(HS)	>0.05(NS)
AST	8.30 ± 2.74	20.40 ± 5.96	12.83 ± 3.17	0.0001(HS)	<0.001(S)
CSF Glucose	74.57 ± 15.21	33.20 ± 17.02	36.60 ± 17.27	0.0001(HS)	0.0001(HS)
CSF Protein	36.93 ± 11.05	121.3 ± 74.90	101.4 ± 32.58	0.0001(HS)	0.0001(HS)

(HS- Highly significant; NS- not significant: S -significant)

As reported in Table 1 Mean± S.D of FSA, AST were significantly increased in PM compared to TBM and controls. The Mean± S.D of csf glucose were low in PM and TBM compared to controls and statistically significant. The Mean± S.D of csf protein were high in PM and TBM compared to controls and statistically significant.

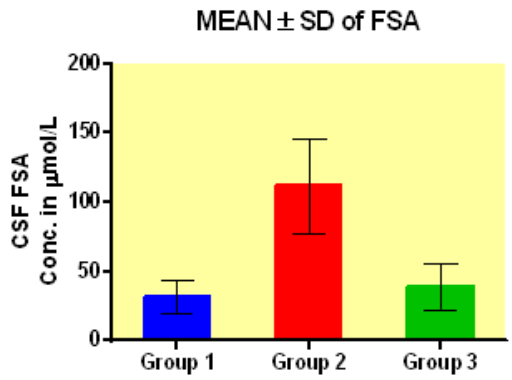

Figure 1: Graphical representation of Mean ± SD of Free sialic acid in three groups

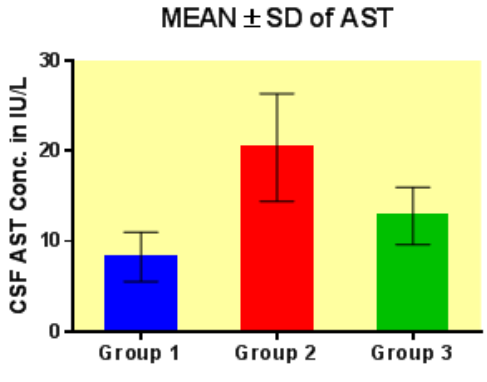

Figure 2: Graphical representation of Mean ± SD of AST in three groups

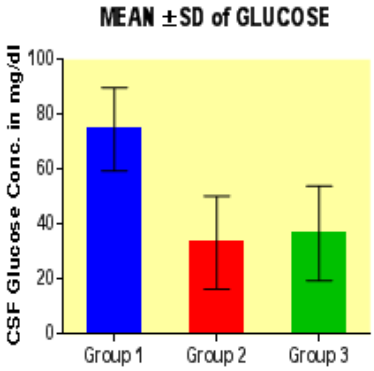

Figure 3: Graphical representation of Mean ± SD of Glucose in three groups

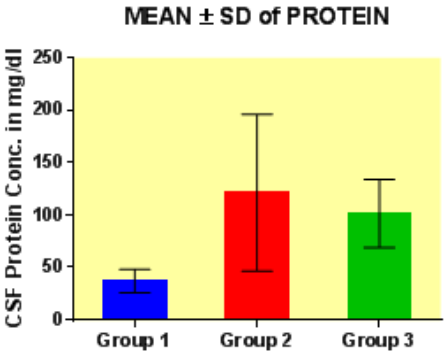

Figure 4: Graphical representation of Mean ± SD of Protein in three groups

Pearsons Correlation between different parameters in Pyogenic Meningitis group showed positively correlation of FSA with AST and protein, statistically not significant. Pearsons Correlation between different parameters in Tubercular Meningitis group showed positively correlation of FSA with AST and glucose, statistically not significant.

IV. Discussion

Meningitis is typically caused by an infectious microorganisms. Most infections are due to bacteria,viruses, fungi, and protozoa being the next most common causes[6]. In bacterial meningitis, bacteria reach the meninges by one of two main routes: through the bloodstream or through direct contact between the meninges and either the nasal cavity or the skin[7].

Sialic acid (N-acetyl neuraminic acid) is an important nine carbon sugar bound to glycoproteins and glycolipids found in the brain tissue. The predominant form of sialic acid in brain is N-Acetylneuraminic acid (Neu5Ac). It exists in 2 forms - a) nondialysable form, which is released from serum proteins and b) a dialysable free form derived probably from metabolic activity in CNS[1]. Since sialic acids are major constituents of glycoproteins and glycolipids bearing neurons and CNS contains more AST this study has been concerned with FSA and AST levels.

In the present study significantly ($P<0.0001$) increased free SA concentrations of CSF in patients with PM in comparison to matched controls. The exact mechanism for increased free SA in PM is not known. However, it could be due to: glycoprotein and glycolipid bearing neurons which are rich in sialic acid and the enzyme neuraminidase elaborated by pyogenic organisms acted upon neuraminic acid which cleaves terminal N-acetyl neuraminic acid from the adjacent sugar of glycoprotein or glycolipids and releases into CSF[1].

In the present study CSF FSA slightly increased but statistically not significant in subjects with TBM in comparison to controls. This could be explained on the basis of lack of neuraminidase enzyme in tubercle bacilli[1].

In a study conducted by Ahmed *et al*., have shown that slightly increased but statistically not significant CSF free SA levels in subjects with TBM in comparison to controls[8].

In other studies the increase in free sialic acid levels are supported by O'Toole et al[7].,Carpenter et al.[9],Balasubramanian et al and Darbari et al.[10] CSF free SA levels are significantly increased in pyogenic meningitis.

Balasubramanian et al., observed raised free sialic acid values in the CSF of patients with pyogenic meningitis as compared with patients suffering from nonpyogenic chronic meningitis of various types and other neurological disorders.They also found that even in cases of pyogenic meningitis where partial clinical treatment had been given, the increased free sialic acid level in CSF was found to persist[11].

AST is an intracellular aminotransferase which catalyzes the transfer of an amino group from an α-aminoacid to α-ketoacid, concentrated more in CNS than hepatic tissue and almost as much as cardiac tissue. Hence any disease or injury of CNS will increase the AST levels in CSF[1].

In the present study significantly (p value <0.0001) increased concentrations of CSF AST in both PM and TBM. But the CSF AST was raised slightly more in PM compared to TBM. The increased CSF AST levels in PM could be due to (a) increased outflow of enzymes from the destroyed cells of CNS and (b) impairment of blood brain and blood CSF barriers due to hypoxia, leading to decreased elimination of this enzyme from CSF[12].

The decreased CSF AST activity in TBM when compared to PM was attributed to subacute nature of the disease and delay in early diagnosis leading to regression of enzyme levels. These findings are in agreement with the results of other studies Praharaj et al., Agarwal et al.,and Shirole et al., showed that there was a decreased CSF AST activity in TBM when compared to PM[13]-[15].

In the present study significantly (p value <0.001) decreased concentrations of CSF glucose in both PM and TBM. The decrease in glucose levels in meningitis is not caused by an increased glucose consumption by leukocytes and bacteria in the CNS, but is thought to be the result of altered glucose transport through the blood-brain barrier[16].

In the present study significantly (p value <0.001) increased concentrations of CSF protein in both PM and TBM. The increase in csf protein in PM and TBM, due to an increase in the presence of the replicating bacteria, which have a high composition of protein, and an increase in the number of cells that fight infection and inflammation, which are also composed of protein.

V. Conclusion

In the present study, significantly increased concentrations of CSF free SA in patients with PM and slightly increased but statistically non significant CSF free SA levels in subjects with TBM in comparison to controls were observed.

To conclude, results of this study indicate thata very high CSF free SA and AST were found to be characteristic of PM. These results do indicate that there is a significant increase in FSA in PM, suggesting that thisparameter may be useful to differentiate PM from TBM as a marker of PM.

Limitations of the study 1) Study should have been carried out on a larger number of cases. 2)There were two disadvantages. One is that, in meningitis caused by several strains of meningococci or H. influenzae which do not elaborate the enzyme neuraminidase the high free sialic acid levels in CSF cannot be expected. Another disadvantage is that in mixed infecion of both pyogenic and tuberculous organisms free CSF NANA will indicate only the pyogenic infection.

References

[1] S.P. Kulkarni, Dr. C.R. Mallikarjuna, Dr. D.S. Jayaprakash Murthy. Cerebrospinal fluid free sialic acid and aspartate transaminase levels in meningitis. Indian Journal of Clinical Biochemistry, 2006, 21 (1) 185-188.
[2] Fatima Khan, Meher Rizvi, Nazish Fatima. Bacterial meningitis in North India: Trends over a period of eight years. Neurology Asia 2011; 16(1) : 47 – 56.
[3] Tausif alam, Rebecca cherian, P. T. Raman, and A. S. Balasubramanian; Free sialic acid levels in the cerebrospinal fluid of patients with meningitis. Journal of Neurology, Neurosurgery, and Psychiatry, 1976, 39, 1201-1203
[4] Lely Solaria,b, Alonso Soto , Juan Carlos Agapito , Vilma Acurio , Dante Vargas , Tulia Battaglioli , Roberto Alfonso Accinelli , Eduardo Gotuzzo , Patrick van der Stuyft .The validity of cerebrospinal fluid parameters for the diagnosis of tuberculous meningitis. International Journal of Infectious Diseases.
[5] Warren, L. (1959). The thiobarbituric acid assay of sialic acids. J. of Biological Chemistry, 234 (8):197175.
[6] Tunkel AR, Hartman BJ, Kaplan SL et al. (November 2004). "Practice guidelines for the management of bacterial meningitis". Clinical Infectious Diseases 39 (9): 1267–84.doi:10.1086/425368. PMID 15494903
[7] Ahmad, P. et al. (1985). Cerebrospinal fluidsialic acid in tubercular meningitis. IndianPaediatrics 22, 191-193.
[8] O'Toole, R.D. et al.(1971). Neuraminidase activity in bacterial meningitis. J. Clin. Invest. 50, 979-985.
[9] Carpenter, R. R., and R. G. Petersdorf. 1962. The clinicalspectrum of bacterial meningitis. Amter. J. Med. 33:262.
[10] A R Tunkel and W M Scheld. Pathogenesis and pathophysiology of bacterial meningitis. ClinMicrobiol Rev. 1993 Apr; 6(2): 118-136. PMCID: PMC358273.
[11] Balasubramanian, A. S., Raman, P. T., and Taori, G. M.(1974). Free and bound N-acetylneuraminic acid in thecerebrospinal fluid in various neurological disorders. Indian Journal of Medical Research, 62, 781- 787.

[12] Richiard d. O'toole, Louise goode, and Calderon Howe; Neuraminidase Activity in Bacterial Meningitis
[13] Praharaj, S.C. (1979). CSF glutamictransaminase level in tubercular and pyogenicmeningitis in children. Indian Paediatrics 16 (8),673-678.
[14] Agarwal, M. (1989). CSF GOT levels in CNSinfections. Indian Paediatrics 26, 1245-1248.
[15] Shirole, D.B. and Chaya Nair. (1974). Cerebrospinal fluid transaminases in neurologicaldisorders. Indian Paediatrics Vol. XI, No. 8, 539-543.
[16] Seehusen DA, Reeves MM, Fomin DA (September 2003). "Cerebrospinal fluid analysis". Am Fam Physician 68 (6): 1103–8. PMID 14524396

IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) is UGC approved Journal with Sl. No. 4033, Journal no. 44202.

Dr. Sandhya Rani Sadula "Study of Free Sialic Acid and Aspartate Transaminase Levels In Cerebrospinal Fluid of Patients With Pyogenic Meningitis and Tuberculous Meningitis." IOSR Journal of Biotechnology and Biochemistry (IOSR-JBB) , vol. 3, no. 6, 2017, pp. 17-21