Original Research Articl

Adenosine deaminase, an aid in the diagnosis of tubercular meningitis – hospital based study

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ABSTRACT

Introduction: Tubercular meningitis (TBM) is the most prevalent and most grave form of extrapulmonary tuberculosis. It is associated with high mortality rate. The confounding factor for this is the delay in diagnosing the disease and initiation of treatment. The present study aims to look for the diagnostic utility of ADA estimations in Tubercular meningitis patients.

Materials and Methods: For this study the patients with the signs and symptoms of meningitis and attending our hospital within a st ipulated period of one year approximately, were taken as subjects. As per the accepted criteria, the patients were segregated into two broad groups: tubercular meningitis cases and nontubercular meningitis cases. CSF specimens were collected and ADA levels were estimated on semiautoanalyser in the laboratory.

Results: Out of 31 patients of tubercular meningitis, 27 showed ADA levels above 10 U/L while 2 showed lower values. On the other hand 32 cases of non- tubercular meningitis showed ADA levels below 10 U/L and 4 showed higher values, thereby supporting the fact that ADA evaluation in CSF is of substantial value in diagnosing TBM. It also illustrious that if CSF ADA value of 10 U/L is taken as bench mark then the sensitivity for diagnosing TBM is 93.10% and specificity is 88.87% which is fairly good.

Conclusion: Therefore, just by evaluating CSF ADA levels we can diagnose the TBM cases with respectably good sensitivity and high specificity and consuming least time and resources. Therefore, ADA estimation is a valuable adjunct to routine investigations in diagnosis of TBM.

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1. Introduction

Tuberculosis, caused by Mycobacterium Tuberculae, is one of those diseases which is still spreading unrestrained in the most parts of the world. This disease has posed a serious threat to the well being and general health of the people. It is estimated that over one third of world's population has been wretched with the effects of this disease.[1] Lungs are one of the primary sites of disease affliction but there are many secondary as we ll as extrapulmonary targets. Among the many extrapulmonary forms of Tuberculosis, TBM ie Tuberculous Meningitis is said to be the main culprit behind maximum deaths reported . If we look at the figures, in the developing countries the incidence of TBM is approximately 7-12%.[2] TBM is usually associated with poorer outcome. About 20-25% cases present withdisease related sequaelae. Although it is very important to diagnose TBM as early as possible yet it is difficult and complicated. The reason behind is the overlapping signs and symptoms among patients of many chronic CNS disorders and other forms of meningitis.[3] The m ost dependable tests in the diagnosis of TBM are the cyto logical and biochemical analysis of CSF. The sensitivity and specificity of these age old methods is limited in differentiating TBM from non-TBM cases. Demonstration of Acid Fast Bacilli by the ZN stain and culture for TB bacilli are s till the gold standards. The sensitivities of ZN staining and culture are 10-40% and 8-49% respectively. In culture mycobacterium grows very slowly and it takes about 6 weeks to show growth.[4] PCR (Polymerase Chain Reaction) though considered as sensitive

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and specific tool to identify mycobacterial DNA, is costly and not widely available. [4,5]

Recently, ADA has been identified as surrogate biomarker of tubercular infection. ADA (Adenosine Deaminase) levels estimation in ascetic, peritoneal and pleural fluids has shown promising results in diagnosing extrapulmonary tuberculosis with rapidity and good sensitivity. For the conversion of Adenosine and Deoxyadenosine toionsine and Deoxyionsine respectively in the purine salvage pathway, the catalytic enzyme that comes into play is ADA. In the process ammonia is released. In the human body ADA is present in abundance in activated lymphocytes and it also some role in differentiation of these lymphoid cells. [5] It is released in excess when cell mediated response is generated against the tubercle bacilli. As a result, ADA acts as a bio marker of both T-Cell activation and Cell Mediated Immunity. [6,7] Much of the research work ha s already been published and many studies a re still on with the pretext to validate the utility of ADA estimations in distinguishing tubercular disease from non-tubercular.

In this study, with the prime aim to segregate the tubercular meningitis cases from nontubercular meningitis cases, we e valuated ADA levels in CSF of meningitis patients and tried to find out the role of ADA as a rapid, sensitive, reasonably accurate and affordable diagnostic tool.

2. Materials and Methods

This study was conducted from November 2016 to October 2017 for a period of one year at MMIMSR, Mullana, Ambala. A total of 65 clinically suspected cases of meningitis, those admitted in the medicine and pediatric wards of the hospital were selected. Out of which 31 cases were labeled with TBM and 34 cases with non- TBM (pyogenic or aseptic).

Diagnostic criteria used for TBM cases were Signs and Symptoms, presence of lymphocytic pleocytosis in CSF, raised protein levels and low glucose levels in CSF, Positive ZN stain / culture report and Positive response to anti-tubercular treatment for the duration of two months. Similarly, the diagnosis of Non TBM cases were based on more acute onset of signs of meningial irritation, specific biochemical estimation profile of CSF as well as negative results in ZN stain and negative cultures. The cases who have undergone prior treatment outside the hospital and the cases with CSF showing turbidity or hemorrhage were excluded from the study.

The CSF specimens were obtained by lumbar puncture. In each case a total of 3 ml CSF was collected and distributed in three vials and was subjected to various laboratory investigations including – biochemical, cytological and microbiological methods. Spectrophotometry method was used for ADA estimation. This principle of enzymatic analysis was first proposed by Guisti and Galanti. ADA

MTB diagnostic kit s from Avecon Healthcare Pvt. Ltd., Parwanoo, India, were used. And the steps followed according to manufactures instructions. A cut off reference value of >10 U/L CSF ADA was considered to be positive as per the guidelines provided in the test kit literature. Data from the study was analysed separately using statistical package from social sciences. Results are presented as Mean + SD (Standard Deviation).

3. Result

In this study a total of 65 patients were included out of which 31 patients were suffering from tuberculous meningitis and 34 patients were suffering from non-tuberculous meningitis, segregated on the basis of diagnostic criteria mentioned earlier. The Male: Female (M:F)ratio was 1.5:1 in TBM group and that was 1:1 in non TBM group (Table 1). In TBM cases the mean age (mean + SD) was 37 years + 20.83 years and the range was 1.00 – 72.00 years. The maximum patients were in age group of 16-30 years both in TBM and non- TBM group. In non-TBM cases the mean age was 33.38 + 21.18 with range of 1- 75 years (Table 2). The ADA CSF levels (mean + SD) in TBM and non-TBM were 15.47 + 8.72 U/L and 4.25 + 3.21 U/L respectively with ADA range lying between 10.20 – 41.10 U/L and 0.31 – 9.8 U/L respectively in the groups under study. This difference in the ADA values in the two patient groups studied was highly significant (P< 0.001). Taking CSF ADA cut off value as 10 U/L, it was found that out of 31 TBM cases, 21 had ADA values below 10 whereas among non TBM cases only 2 of the cases reflected ADA va lues above 10. The CSF sugar levels also did not produce any statistically significant difference. Whereas, it was coraborated that the mean CSF ADA levels tend to increase with the CSF protein levels and this relation was found statistically significant. Also CSF ADA values increased with the increase in the Lymphocyte count in CSF. This elaborate test procedure gave a sensitivity of 93.10% and specificity of 88.88% in diagnosing TBM, when cut off value of CSF ADA was set at 10 U/L. The positive predictive value and negative predictive value were 87.1% and 94.1% respectively (Tables 3 and **??**).

4. Discussion

India like most of the developing countries has a dubious distinction of having higher prevalence and incidence of Tuberculosis with lack of well equipped facilities to diagnose it. One of the greatest diagnostic dilemmas in clinical practice these days is to segregate patients of TBM from patients of Non-TBM because to its pleomorphic clinical presentation. [8] But the accurate diagnosis of TBM needs to be established urgently so that specific treatment is instituted at the earliest. In turn this will, not only produce substantial difference in the course of illness but at the same

time the development of CNS complication can be aborted at the very outset.[9]

Most of our laboratories take the demonstration of AFB (Acid Fast Bacilli) in the CSF smears and positivity in the culture as the sole criterion for the diagnosis of TBM. But inspite of best of efforts smear methods (are often negative) are unreliable and unpredictable. Culture though taken as Gold standard consumes lot of time to show growth i.e. about 4-6 weeks.[10] So, in countries which are resource limited and have high prevalence it is of great relevance to look for newer methods which are cost effective, rapid, reasonably sensitive and specific.[11] ADA being a marker of cell mediated immunity serves the purpose in aiding the presumptive diagnosis of TBM. Many studies have been carried out in the recent past which inferred that ADA estimations in body fluids like pleural and peritoneal fluids have diagnostic significance in extrapulmonary tuberculosis.[12] British Infection Society for the diagnosis and treatment of TB particularly of the Central Nervous System in adults as well as children is one of the few societies which has recommended the ADA assays should be carried out in CSF samples of TBM patients because it has high diagnostic utility. They also endorsed that ADA activity is quite high in TBM patients.[13] The same was reflected in present study, as we were able to distinguish clearly between tuberculous meningitis patients from Non-TBM cases simply by taking ADA CSF value of 10 U/L as a watershed point. The differences in the ADA values in CSF between the TBM and non TBM cases were highly statistically significant i.e. $p<0.001$. Results of our study indicate that ADA levels in CSF exhibit fairly high accuracy in diagnosing TBM and showed a sensitivity of 93.10% and specificity of 88.88%. In addition the positive predictive value of test came out to be 87.1%. Using similar cut off values a number of workers have earlier authenticated their dependency on CSF ADA activity in diagnosing TBM cases. Gupta et al in his study, while again taking 10 U/L as cut off value of CSF ADA levels, reported a sensitivity of 94.73% and specificity of 90.47% in differentiating TBM patients from non TBM patients. His findings were in support of our findings.[14] Similarly after reviewing and comparing the literature, Agarwal S also by using same limiting value described a sensitivity of 99.90% and specificity 87.50%. Whereas, Belagavi and Shalini in their study were able to find a lower sensitivity of 73.9% and a comparable specificity of 92.6%.[15,16]

The value of ADA that is taken as fence /limiting border/cut off point holds enormous significance in the calculation of the sensitivity and specificity of CSF ADA test.[17] In one of the original research work carried out in Gujarat, a sensitivity and specificity values of 98.46% and 97.65% respectively was found when ADA cut off value of 9 U/L was taken in CSF.[18] In order to improve the reproducibility of the test, Xu et al pooled various

studies with different ADA cut off points but still reported a sensitivity of 79% and a specificity of 91%.[19] Studies following these meta analysis by Gupta BK et al, Sun Q et al also vehemently supported the fact that the clinical utility of ADA for the diagnosis of TBM has noteworthy.[20] The standardised or exact ADA value that is considered as the diagnostic for TBM ha s yet to be established. T he different values of ADA used in different studies ranged from >5.0 to <15.0 U/L.[21] Peruvian guidelines are amongst the few existing ones that explicitly advocate the evaluation of ADA in CSF to diagnose TBM.[22]

Table 1: Showing sex distribution of cases

Type	Male	Female	Ratio(M:F)	Total
TBM	19	12	1.5:1	31
Non TBM	17	17	1:1	34

Table 2: Showing age distribution of cases

Age in years	TBM	Non TBM	Total	% age
< 1 yr	3	1	4	6.15
1-15	2	5	7	10.77
16-30	10	13	23	35.38
31-45	5	3	8	12.31
46-60	7	7	14	21.54
61-75	3	5	8	12.31
>75	1	0	1	1.53
Total	**31**	**34**	**65**	

Table 3: CSF ADA values in U/L

Group	No. of cases	Range	Mean	SD
TBM	31	10.20-41.10	37	20.83
Non- TBM	34	0.31-9.81	4.25	3.21

TBM Vs Non- TBM 't' test value- 6.745
$P<0.001$

The algorithm that is followed in establishing the diagnosis of TBM cases is the multidisciplinary approach ie by using clinical, radiological, cytological, biochemical and even microbiological approaches. But in recent years, newer diagnostic techniques have sprouted up. The assays in particular molecular methods like Gene Xpert MTB/RIF have been developed and these could contribute to the diagnosis of extrapulmonary forms of tuberculosis. However, rolling out these tests for routine diagnosis will take time; as such tests are costly and consume considerable resources for establishment. Their foolproof clinical utility needs further assessment.[23] The methods to measure ADA levels in our laboratories are very simple to perform and are starkly inexpensive if we compare them with molecular methods. Also these can be easily adapted to an auto-analyser. That is why these can be used in laboratories with limited resources particularly in under developed and developing countries. In India, the incidence of

Table 4: CSF Mean ADA, Sugar, Protein and Cell counts in the two groups

Group	ADA mean	Mean Sugar	Mean Protein	Mean Cell count	Lymphocyte %
TBM	37.00	28.81	96.95	130	91
Non-TBM	4.25	60.01	24.81	56	27

Tuberculosis is very high and w despite extensive work on pulmonary and extrapulmonary tuberculosis only a few diagnostic kits are available for diagnosing TBM.

In conclusion, our results suggest that CSF ADA levels are elevated in TBM patients as compared to non-TBM patients and thus estimating CSF ADA levels is a useful marker for clinicians to make an early diagnosis of TBM.

5. Source of funding

None.

Conflicts of interest

None.

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