



## Original Article

## Reference Interval of Cerebrospinal Fluid-Adenosine Deaminase Enzyme in Apparently Healthy Subjects A Retrospective Study in India

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### ABSTRACT

#### Article history

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#### Key words

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**Background and Aims:** The aim of the present study was to re-evaluate the upper limit of normal range (ULN) for adenosine deaminase (ADA) level in cerebrospinal fluid (CSF) of healthy subjects in Indian population.

**Materials and Methods:** Posteriori strategy was used for reference population selection. Individuals whose CSF samples had come to the laboratory of Bhatia Hospital, Mumbai (India) for ADA estimation during the period of January 2015-October 2016. After database search, 130 subjects were selected. Hexokinase method for CSF-glucose, turbidometric method for total protein and enzymatic method using the ADA assay kit BSBE for CSF-ADA was used. Non-parametric methodology recommended by IFCC/CLSI for reference intervals calculation was adopted. The 2.5 and 97.5 percentile formed the lower and upper limits of Reference Interval.

**Results:** ULN range of CSF-ADA for the total study cohort was- 10.7 U/L, which was quite higher compared to the ULN provided in the reagent kit insert (5.0 U/L). Upon gender wise comparison, female showed higher median CSF-ADA activity compared to male (Male-7.2; Female-8.2  $p=0.01$ ), but upon application of robust methods, ULN of CSF-ADA did not show significant differences (ULN in Male-12.3 and in Female-12.5). Age wise analysis revealed that with increasing age; ULN of CSF-ADA showed increasing till the age of 50 years and then showed decreasing trend with further increase in age.

**Conclusions:** ADA level of 10.7 U/L in CSF should be adopted as the upper limit of normal range and gender based partitioning is not recommended. Age should be considered during the interpretation of CSF-ADA results for making this diagnostic test more clinically relevant.

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## Introduction

Reference Intervals (RIs) denote normative values related to laboratory parameters/ analytes used by diagnostic centers for clinical diagnosis [1]. The clinical chemistry reference interval is one of the most important decision making tools used to differentiate between healthy and diseased individuals. RIs should be derived from healthy subjects and should be representative of the local population. It is a major responsibility of clinical laboratories to provide correct RIs; because the quality of RIs is crucial for the interpretation of the results [2].

In India, clinical laboratories use the reference values of commonly used analytes supplied by diagnostic reagent and equipment manufacturers in their instructions without giving details of the original source of the data [1]. Reference intervals can vary with variations in diet, lifestyle, environmental factors, and race/ethnicity. Many pre analytical factors (i.e. specimen collection and handling procedures, etc.), analytical procedures, test performance and statistical processes. The information about these factors is not generally provided by the manufacturers, thus it is difficult to assess the suitability of the supplied interval for local use.

In Australia, the guidelines presented in national association of testing authority (NATA) summary of ISO/IEC 17025 documents suggest that laboratories should either establish their own detailed RIs or must validate reference intervals published elsewhere for their own methods and population. ISO 15189 also requires the laboratory to review the biological reference interval from time to time [3].

Laboratory analysis of cerebrospinal fluid (CSF) is generally requested to aid in patient management; e.g., infectious diseases, neoplastic processes, infraction, trauma, autoimmunity, and degeneration of central nervous system. The adenosine deaminase (ADA) is an enzyme involved in the metabolism of purine and catalyses the hydrolytic deamination of adenosine to inosine and ammonia. It is abundant in leukocytes; plays an important role in the maturation of monocytes, macrophages and T lymphocytes. Increased concentration of the ADA indicates increased inflammatory activity as a sequel to septic central nervous system infection [4]. There has been interest in ADA as an inflammatory marker in the diagnosis and management of cerebral diseases such as tuberculosis as well as other bacterial infections in which the cellular immunity response is actively involved [5].

It was observed in our laboratory that CSF-ADA tests results have shown higher values in the samples with normal biochemical and CSF routine examination results as compared to the upper limit of normal range (ULN) provided in the reagent kit insert. So, it was decided to evaluate RIs of CSF-ADA for our population and with the method and instrumentation used in our laboratory.

The present study was designed to establish in-house RIs of cerebrospinal fluid-Adenosine deaminase enzyme (CSF-ADA) in healthy subjects using posteriori approach. Difficulty in obtaining CSF samples from healthy

subjects had guided the study towards posteriori approach of sample collection.

## Material and methods

Present observational study was performed at the departments of biochemistry and pathology, Bhatia Hospital, Mumbai. The posteriori (retrospective) strategy was used for selection of reference population in which database containing both analysis results and information on a large number of individuals was searched. Values of individuals fulfilling defined inclusion-exclusion criteria were selected as sample [6]. The study was approved by the Institutional Ethics Committee.

### Selection criteria and analytical procedures

The reference population chosen for this study comprised of individuals whose CSF samples had come to the laboratory between the periods of January 2015 to October 2016. Individuals having CSF-protein > 80 mg/dl, along with glucose > 250 mg/dl and CSF-Total cell count > 20 cells/column were excluded from the study. CSF glucose and protein levels were measured on COBAS 400+ Integra analyser (Roche Diagnostics). CSF-ADA level was measured on CHEM-7 instrument of ERBA. Total cell count of CSF was done on SYSMEX-XT 2000I. Measurement of glucose and protein in CSF was done using GLUC3 and TPUC3 reagents of Roche Diagnostics GmbH, Mannheim during the whole study period. CSF-glucose was measured using the hexokinase method, CSF total protein was measured using turbidometric method and CSF-ADA was measured by enzymatic method using the

ADA assay kit BSBE, Beijing Strong Biotechnologies, China [7].

Prior to biochemical analysis, instruments were calibrated as recommended by the manufacturer. Control materials used were – PN-PUC, PP-PUC (from Roche Diagnostics) for CSF protein, PCC1 and PCC2 (from Roche Diagnostics) for CSF glucose and LEVEL1 and LEVEL-2 controls (from Gcell; BSBE, China) for ADA. These 2 level controls were run every day as a part of internal quality control. After database search, total 125 subjects were included in the reference group after applying above mentioned exclusion criteria and used for RI establishment.

### Statistical analysis

After filtering data according to demographics, it was subjected to statistical analysis. Qualitative data were presented as a percentage (%) and quantitative data were presented as median and interquartile range (IQR). Quantitative biochemical parameters between the 2 subgroups (male and female) were compared using Mann-Whitney test. The P value of <0.05 was considered statistically significant.

The CSF-ADA enzyme revealed non-Gaussian distribution as tested by D'Agostino-Pearson test (Fig. 1). All outliers were removed using Tukey's method before calculating RIs. Reference interval was calculated using a "non-parametric method" for the total population. For male and female subgroups, robust method of RI calculation (for small sample size) was used as described in the CLSI/IFCC guidelines C28-A3 [8]. Median, central 95 percentile and 90% confidence interval (CI) for lower and upper limit were calculated. The 2.5 percentile and

97.5 percentile formed the lower and upper limits of reference intervals. The MedCalc

Statistical Software Version-12 was used for all statistical analysis.

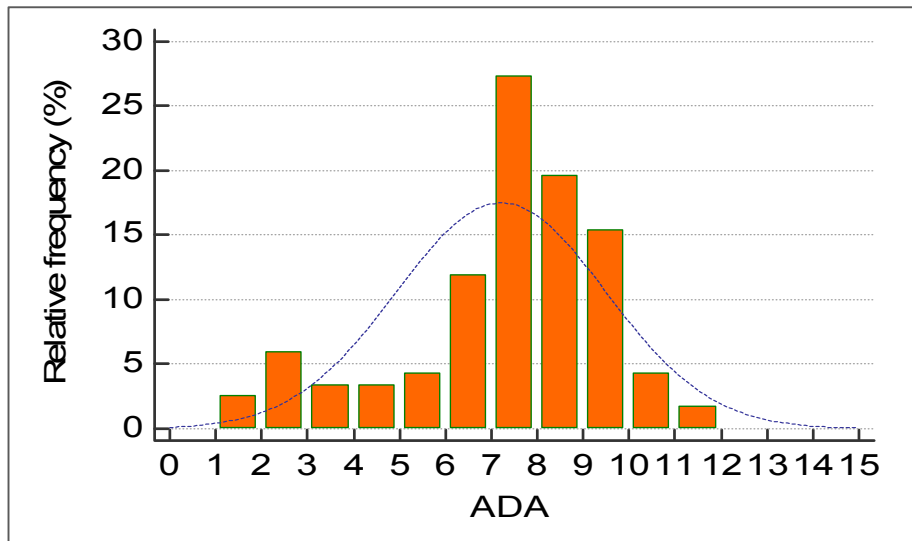


Fig. 1. Distribution curve/Frequency histogram of CSF-ADA enzyme

## Results

### Demographic characteristics of study population

The reference population comprised of 125 subjects out of which 4 subjects were excluded after applying Tukey's outlier test. Finally 121 subjects were included in the study. Out of total subjects, 62 (51.2 %) were male and 59 (48.8 %) were female. Our study population covered broad age range of study subjects. The mean age of the cohort was 42.17 (1-90 years). The age distribution of the study cohort was- 1-10 yrs (2.5%); 11-20 yrs (18.2%); 21-30 yrs (15.7); 31-40 yrs- (13.2%); 41-50 yrs- (14.0%); 51-60 yrs- (12.4%); 61-70 yrs- (13.2%); 71-80 yrs- (5.8%); 81-90 yrs- (4.1%). Table 1 depicts the demographic and biochemical characteristics of reference population and their gender wise comparison. The ADA level in CSF samples was significantly higher in the female group as

compared to the male group ( $p < 0.05$ ). No significant difference could be observed in CSF glucose, protein and cell count between these two groups.

Table 2 shows the RIs of CSF-ADA in total, male and female population. In the total population, the sample size was more than 121; so, non parametric method was applied for the calculation of RIs. For male and female subgroups, there was a significant difference in median ADA level so it was decided to present gender wise RIs of CSF-ADA. Because of low sample size in male and female subgroups ( $n < 120$ ), robust method was used for gender wise RIs calculation. In total population CSF-ADA upper limit of normal (ULN) was 10.7 U/L. Though there was a significant difference in the median CSF-ADA level between male and female, but there was no difference in the

ULN of CSF-ADA between two genders. The female was 12.5 U/L. ULN of CSF-ADA in male was 12.3 U/L and in

**Table1.** Demographic and biochemical characteristics of study subjects

Variables	Median (IQR)			Z value (P)
	Total (N=121)	Male (N=62)	Female (N=59)	
AGE (years)	40.0 (23.0-60.0)	39.0 (25.0-57.0)	42.0 (19.3-62.0)	0.26(0.79) <sup>NS</sup>
CSF-ADA (U/L)	7.2 (6.5-8.7)	7.2 (6.8-7.6)	8.2 (7.5-8.5)	2.37 (0.01)*
CSF- Glucose (mg/dl)	75.5 (65.5-90.5)	76.0 (65.6-86.5)	74 (63.9-96.0)	0.48 (0.62) <sup>NS</sup>
CSF-Protein (mg/dl)	30.1 (21.3-40.6)	31.0 (22.2-43.0)	29.3 (19.3-37.0)	1.23 (0.21) <sup>NS</sup>
CSF-Cell count (cells/cumm)	10.0 (10.20)	10.0 (10.0-10.0)	10.0 (5.0-20.0)	1.46 (0.14) <sup>NS</sup>

\*P value for comparison between female and male CSF-ADA level

NS= Not Significant

**Table 2.** Reference interval of CSF-ADA

Groups	Number	Method	Median	CSF-ADA (U/L)	Percentile
				2.5 <sup>th</sup> (90% CI) Lower limit	97.5 <sup>th</sup> (90% CI) Upper limit
Total population	121	Non parametric	7.6	1.3	10.7
Male	62	Robust	7.2	2.5 (1.2 to 3.4) <sup>a</sup>	12.3 (11.3 to 12.9) <sup>a</sup>
Female	59	Robust	8.2	3.9 (2.9 to 4.9) <sup>a</sup>	12.5 (11.4 to 13.3) <sup>a</sup>

<sup>a</sup> Bootstrap confidence interval (10000 iterations; random number seed: 978)

Figure 3 shows the age wise 2.5<sup>th</sup>, 95<sup>th</sup> and 97.5<sup>th</sup> percentiles of CSF-ADA in total population. The result showed that with an increase in age, the ULN (97.5<sup>th</sup> percentiles) gradually increases and at the age of 50 years the ADA ULN reaches its peak value then with further increase in age, the ULN shows decreasing trend till the age of 90 years. At the age of 10 years, the ULN was- 10.3 U/L; at

age of 20 ULN-10.8 U/L; at 30 years- ULN- 11.4 U/L; at 40 years- ULN-12.0 U/L; and at 50 years- ULN-12.4 U/L. After reaching the peak upon 50 years, ULN started to decrease. At 60 years of age, the ULN- 12.3 U/L; at 70 years ULN- 11.6 U/L; at 80 years- ULN-10.0 and at 90 years- it was 7.3 U/L.

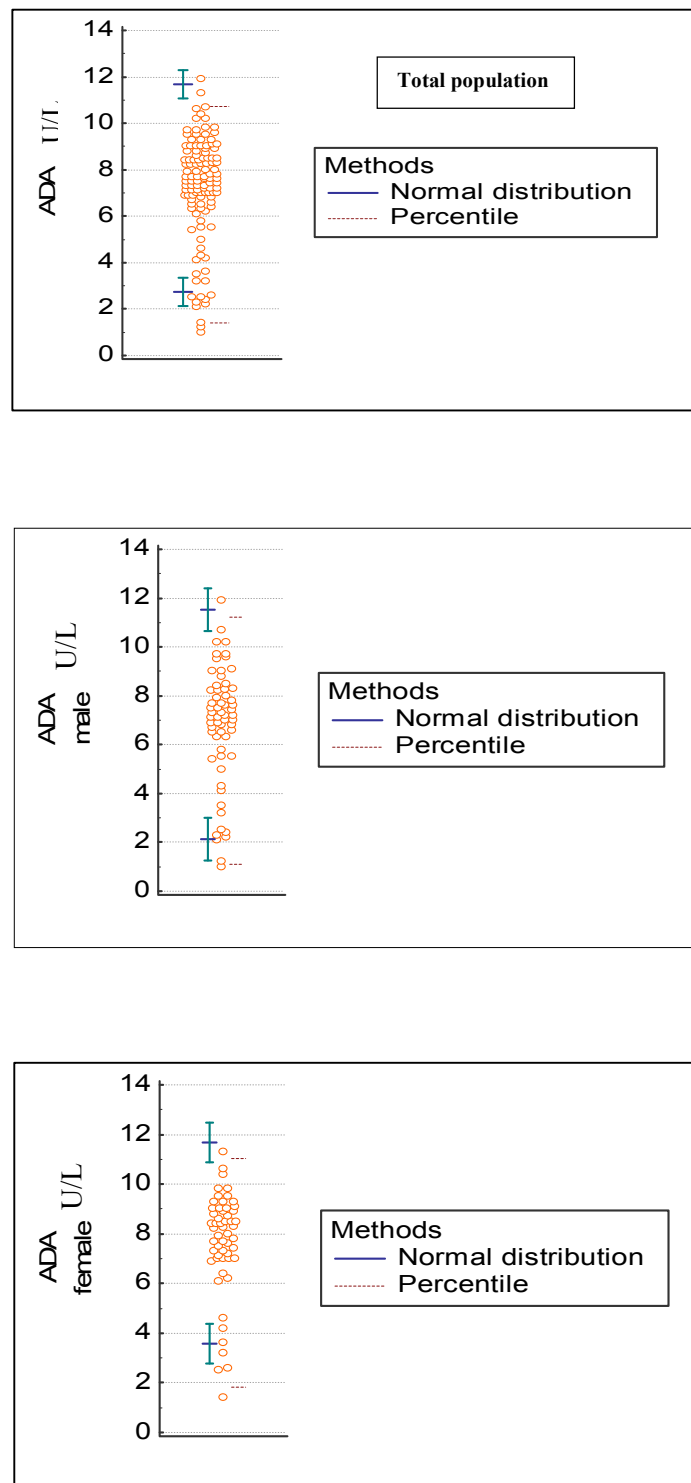


Fig. 2. Box-Whisker plot of RIs of CSF-ADA in total, male and female subjects

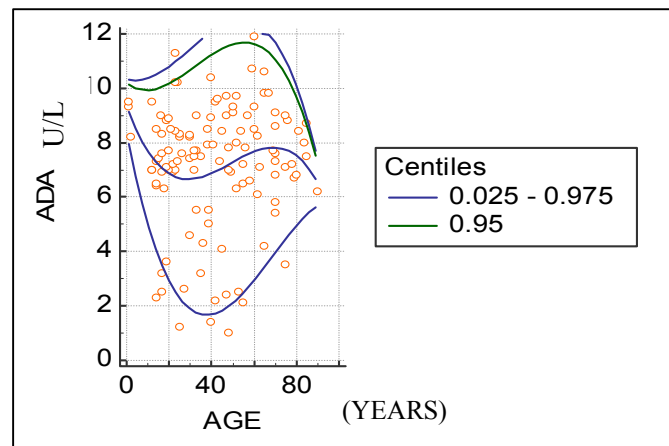


Fig. 3. Age related reference intervals for the total population

## Discussion

In the reagent kit insert, the normal range of CSF-ADA was given 0-5 U/L. The reagent manufacturer has also recommended to the laboratories to establish their own reference range to reflect the variation due to age, sex, diet and the geographical location of the population. Here our laboratory ULN for CSF-ADA was high (10.7 U/L) compared to the value given in the kit literature (5.0 U/L). These results clearly indicate that normal range of CSF-ADA was affected by age, but not by gender for our reference population. The RIs may be affected by the method and the techniques used by the individual laboratory. We could not find any similar study from internet database search to compare our results. Our study can provide useful information to our peers about ULN of CSF-ADA and can encourage them to establish their own in-house RI or to validate the RIs used in their laboratories.

Our study was based upon a retrospective database search for a healthy reference

population, which may not provide all the information about the health status of the subjects included in the study. So, our study group may not reflect the absolutely healthy reference cohort. Due to small sample size upon gender wise partition of subjects, robust method was used for RI calculation in male and female subgroups.

## Conclusion

Our study quantifies the RIs of CSF-ADA and age related changes in its ULN. The data reported in our study reflected the characteristics of the population served by our laboratory. Updated ULN of CSF-ADA would help clinicians in better interpretation of this parameter for early diagnosis and prognosis of cerebral diseases.

## Conflict of Interest

The authors declare no competing interest.

## Acknowledgment

There is no acknowledgment to declare.

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