Adenosine deaminase activity and ADA G22A gene polymorphism among a cohort of Egyptian patients with pulmonary and extra pulmonary tuberculosis

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Abstract

Background: Tuberculosis (TB) is infectious illness that poses a chronic threat to public health due to a variety of intricate biological and sociological factors. Examination of adenosine deaminase (ADA) allelic variations would give an idea about genetic predisposition to TB. ADA has been thoroughly investigated as a biochemical marker in pleural fluid.

Objectives: This work aimed to investigate the possible association between ADA gene polymorphism and the susceptibility to develop active TB disease and to evaluate the activity of ADA in these patients.

Patients and methods: A case-control study of 40 patients with active TB, in addition to 40 healthy,unrelated`age-and sex-matched volunteers used as controls. Clinical and radiological evaluations and routine laboratory investigations were done on all participants. The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to identify the ADA G22A gene polymorphism. Measurement of ADA activity using a colorimetric assay kit.

Results: Among the included TB patients, 85% have pulmonary TB, and 60% have abnormal chest X-ray. There was significantly higher ADA activity in TB patients (38.40 ± 2.509 U/L) compared to controls (22.30 ± 5.355 U/L) ($p < 0.001$). There was a significantly higher frequency of the GA genotype and A allele of the ADA G22A gene polymorphism among patients compared to controls, $p < 0.001$ for all. Odds ratio (95% CI) for GA genotype = 2.188 (1.470-3.254), and for A allele = 2.188 (1.322-3.620) indicating a strong genetic association between the GA genotype and A allel and increased susceptibility to TB.

Conclusion*:* The GA genotpe and A allel of ADA G22A gene polymorphism was strongly associated with increased risk of TB. ADA activity could be considerd as a biomarker for pulmonary TB.

Keywords: Adenosine deaminase (ADA) ; Tuberclosis (TB); Genetic polymorphism.

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Introduction

Mycobacterium tuberculosis (MTB) is the bacterium that causes tuberculosis (TB), an infectious illness that poses a chronic threat to public health due to a variety of intricate biological and sociological factors **(Miggiano et al., 2020).** Because it consumes financial and human resources that could be used to support the economy, tuberculosis is also thought to be a hindrance to both economic progress and the advancement of public health in those nations. Therefore, research and development of novel TB prevention strategies and therapies are urgently needed **(Rudan, 2023)**.

Adenosine deaminase (ADA), an enzyme made from lymphocytes that is involved in purine metabolism, has been thoroughly investigated as a biochemical marker in pleural fluid. The test may be carried out in most laboratories and is quick, easy, affordable, and minimally intrusive **(Wahid et al., 2023).** It is a metabolic enzyme that is expressed both intracellularly and on the cell surface complexed with CD26 **(Yegutkin, 2021).** It is a crucial part of the purine salvage pathway together with purine nucleoside phosphorylase, which is in charge of the irreversible conversion of adenosine and 2'deoxyadenosine into inosine and 2'deoxyinosine, respectively **(Flinn and Gennery, 2018).** In humans, ADA primarily contributes to immune system development and maintenance **(Gao et al., 2021).**

It has been demonstrated that the ADA enzyme's activity can be altered by a single nucleotide polymorphism (rs73598374) that results in a G–⟶…A substitution at position 22 of exon 1. Previous research has demonstrated that people with the GG genotype have 15–30% higher ADA activity than people with the GA and AA genotypes, respectively **(Verdoida et al., 2020**).

Given that gene polymorphisms may impact ADA activity, it makes sense to speculate that SNPs in the ADA gene may impact protein activity, which in turn may impact susceptibility to linked disorders. **(Farhan et al., 2017).**

So, we aim to assess the activity of ADA among patients with active TB and correlate it with routine laboratory markers and assess It's validity to discriminate between pulmonary and extra pulmonary TB. Also, to identify the genetic profile of the ADA G22A gene polymorphism among patients with active TB.

Patients and Methods

Study design

A case-control study that started from March 2023 to June 2024. The protocol approved by ethics commetee of Faculty of Medicine, South Valley University, the ethical approval code: SVU-MED-MBC004-1-23-5-643.

All participants signed a written informed consent before involving in the study.

The study included 40 Patients with confirmed tuberculosis based on clinical, radiological, and microbiological criteria, and 40 healthy volunteers used as controls. They were recroded from Luxor Fever Hospital, Luxor governrate, Egypt, and Chest Department, Qena University Hospital, Egypt .

Exclusion criteria: patients with history of immunosuppressive conditions or associated co-morbidity (liver – renal – cardiac– neurological disorders), pregnancy, or lactating females were also execluded.

Sample Size Calculation: Sample size calculated according to:

$$n\ (each\ group) = \frac{(p0q0 + p1q1)(z\ 1-\alpha/2 + z\ 1-\beta)2}{(p1 - p0)2}$$

p1= risk of exposure in cases
p0= risk if exposure in controls
q1= 1-p1
q0= 1-q0

$Z_{1-\alpha 2}$ = 1.96 for confidence interval 95%
$Z_{1-\beta}$ = 0.84 as desired power for our study 80%

so at least 38 patients should be present in the sample and we have included 40 patients with active TB in addition to 40 healthy unrelated age- and sex-machted voluteers used as acotrols. We have modified the sample size in order to get 80% statistical power and a 5% level of significance (type 1 error).

Laboratory workup

A-Routine laboratory investigations: In the form of compelet blood count (CBC), liver function test (ALT and AST)and renal function test (urea and creatinine) were recorded from patients medical files.

B-Spescific biochemical and gentic assay

1. **Blood samples collection and processing:** 5 ml of venous blood was withdrawn from all participants divided into 2 parts, the first part (2 ml) was evacuated into EDTA cantaining tubes, and was stored at – 80 °C for later gentic analysis. The second part (3 ml) was evacuated into serum gel separator tubes ~~and~~ allawed to be clotted at 37°C for 30 min, then centrifuged at 3500 rpm for 10 min, the separated sera used for measurement of adenosine deaminase activity.
2. **Measurement of ADA activity:** The assay was done using a colorimetric assay using ELISA microplate reader(EMR 500 USA) (Cat No: E-ADA, Thermos Fisher Scientific, USA), following the manufacturer's instructions
3. **Genetic analysis of ADA gene polymorphism:**
   - **Gene extraction:** In order to extract genomic DNA, peripheral blood samples were gathered and placed in EDTA tubes. GeneJET Whole Blood Genomic DNA Purification Mini Kit (CatbNo# FD0674, Fermentas Life Sciences,Thermo Fisher Scientific Inc., USA), was used to isolate genomic DNA in accordance with the manufacturer's instructions **(Farhan et al, 2017)**
   - **Genotyping of ADA G22A Polymorphism:** The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to identify the ADA G22A polymorphism.The PCR primers are forward primer 5′-GCCCGGCCCGTTAAGAAGAGC-3′ and reverse primer 5′-GGTCAAGTCAGGGGCAGAAGC AGA-3′. **(Farhan et al, 2017).** Each sample was subjected to a PCR assay in a final reaction volume of 25 µl, utilizing 5 µl DNA, 12.5 µl universal master mix, 1 µl each of the forward and reverse primers for PTPN22 1858C>T, and 5.5 µl of distilled water (DW). The following were the conditions for the PCR , First denaturation for 15 minutes at 94°C. 36 cycles of 40 seconds of denaturation at 94°C, 80 seconds of annealing at 66°C, and 80 seconds of extension at 72°C. Final extension for eight minutes at 72°C. A thermal cycler from Applied Biosystems (Perkin-Elmer 9600, USA) was used for each reaction.Following amplification, the PCR products were digested using the Fast Digest TaqI enzyme (Thermos Fisher Scientific, Cat. No. ER0671),1 µl of the enzyme ,2 µl of enzyme buffer,7 µl of nucleas free water and10 µl of PCR products incubated for 1 hour at 65°C. The digested products were seen under a UV transilluminator after being resolved on a 2% agarose gel electrophoresis with ethidium bromide. The PCR products were loaded and 100 bp DNA ladder

(Catalog No. 24073, iNtRON Biotechnolog, Korea)was also loaded to help identification of the band size of the PCR products. The existence of two bands (245 and 152) for the ADA1/ADA1 (GG) genotype defined the ADA G22A genotype. Three bands (397, 245, and 152) were necessary to define the ADA1/ADA2 (GA) genotype. (**Fig. 1,2**) displays the representative gel with the typical band patterns.

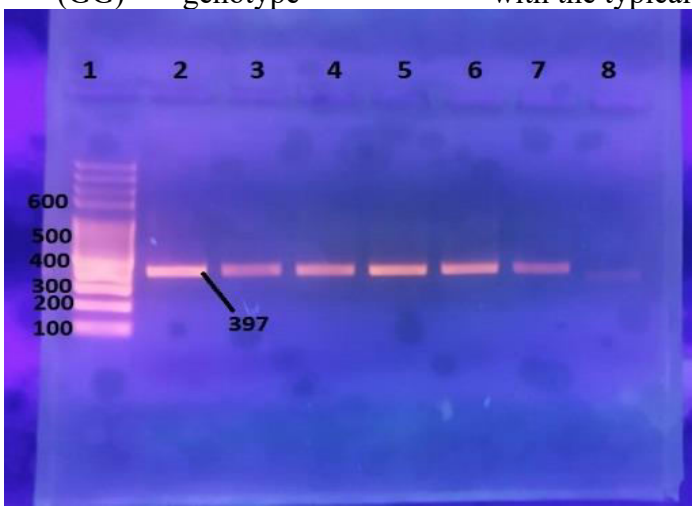

Fig. 1. Gel electrophoresis of the PCR amplification products of ADA G22A SNP. Numbers refer to lanes. Lane 1 shows a 100 bp DNA ladder. Lanes 2–5 showed amplified DNA segments of length 397 bp.

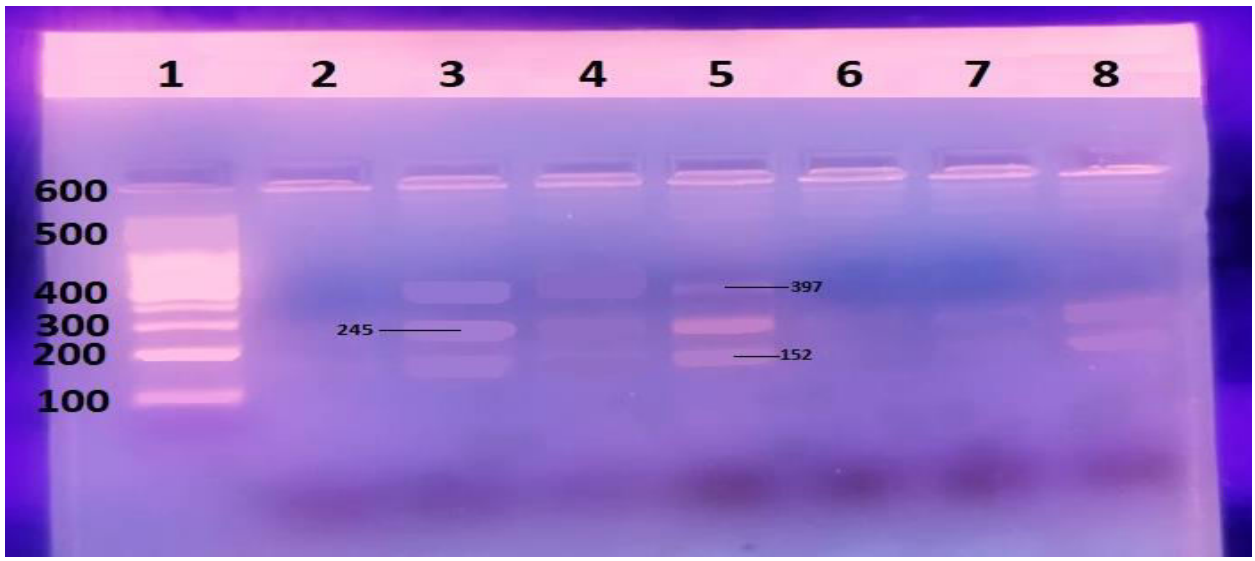

Fig. 2. Agarose gel electrophoresis (2%) showing PCR digestion products of ADA G22A SNP. Lanes are denoted with numbers. Lane 1 shows a 100 bp DNA ladder; Lanes 3, 4, 5 are heterozygous genotype (GA) with 152, 245, 397, bp band. Lane 7, 8 are homozygous genotype (GG) with 152, 245 bp.

Statistical analysis

Data was collected, coded, revised, and entered into the Statistical Package for Social Science (IBM SPSS) version 27. The data were presented as numbers and percentages for the categorical variables,

and mean, and standard deviations, for the numerical variables. Data was tested for normality using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The chi-square test compares cases and controls regarding qualitative variables. When the chi-square assumptions were unmet, the Fisher exact test was used. The independent t-test compares 2 groups regarding numerical variables with parametric distribution.The Mann-Whitney test compares 2 groups regarding numerical variables with non-parametric distribution. One-way ANOVA (Analysis of variance) compares 3 groups regarding numerical variables with parametric distribution followed by a post hoc test using Tukey correction for pairwise comparison. Pearson correlation was used to assess the association association between ADA activity and routine laboratory investigations. Receiver operating characteristics (ROC) curve analysis was used to assess the accuracy of ADA activity in discriminating pulmonary from extrapulmonary tuberculosis and healthy controls from T.B. cases. The allowable margin of error was set at 5%, while the confidence interval was set at 95%. A p-value < 0.05 was considered statistically significant. The studied SNP follows the Hardy-Weinberg equation.

Results

Demographic data of study participants (n=80)

The study shows no statistically significant difference between cases and controls regarding age, gender, and occupation ((p >0.05) **(Table 1).**

Table 1. Demographic data of study participants

Variables		Case (n=40)	Control (n=40)	P-value
		Number (%)	**Number (%)**	
Gender	**Male**	21 (52.50%)	19 (47.50%)	0.655(1)
	Female	19 (47.50%)	21 (52.50%)	
Job	**Housewife**	18 (45%)	18 (45%)	1.00(1)
	Worker	22 (55%)	22 (55%)	
		Median (IQR)	**Median (IQR)**	
Age (years)		38 (23-51.25)	29 (25-31)	0.051(2)

(1)Chi-square test (2) Mann-Whitney test

Clinical data of the studied patients (n=40)

The shows that 12 cases out of 40 (30%) had a positive family history of tuberculosis, 34 cases (85%) had pulmonary T.B, and 24 cases had abnormal chest X-rays in the form of pulmonary infiltration, pleural effusion, and fibro cavitary lesion. Among the extrapulmonary T.B cases, 2 (2.5%) had spinal T.B, 2 (2.5%) had pleural T.B, one (1.25%) had T.B in the cervical lymph nodes, and one (1.25%) had urinary bladder T.B (**Table. 2)**.

Routine laboratory investigation of the studied groups

The mean serum creatinine was significantly higher among TB than controls (0.8475 ± 0.31257 mg/dl vs. 0.5550 ± 0.18940 mg/dl) (p<0.001);. No statistically significant difference exists between cases and controls regarding other laboratory investigations such as hemoglobin level, white blood cells, platelets, AST, ALT, and urea (p>0.05) **(Table. 3; Fig. 3).**

Table 2. Clinical data of the studied patients (n=40)

Clinical Parameters		Number	Percentage (%)
Family history	Positive	12	30%
	Negative	28	70%

Type of T. B	Pulmonary	34	85%
	Extrapulmonary	6	15%
Extrapulmonary T.B site (6)	Spine	2	2.5%
	Pleura	2	2.5%
	Lymph nodes	1	1.25%
	Urinary bladder	1	1.25%
Chest X-ray	Normal	16	40%
	Abnormal	24	60%

Table 3. Routine laboratory investigation of the studied groups

Parameters	**Cases (n=40)**	**Controls (n=40)**	**P-value**
	Mean ± SD	**Mean ± SD**	
Hemoglobin (g/dl)*	13.278 ± 1.9750	13.375 ± 1.7641	0.816
WBCs ($\times10^3/mm^3$)*	7.595 ± 1.8779	7.270 ± 2.0742	0.465
Platelets ($\times10^3/mm^3$)*	304.83 ± 83.787	292.02 ± 46.371	0.401
AST (u/l)*	24.28 ± 14.173	25.58 ± 6.324	0.598
ALT (u/l)*	25.13 ± 12.940	25.78 ± 7.280	0.783
Urea (mg/dl)*	26.43 ± 13.397	23.95 ± 7.776	0.316
Creatinine (mg/dl)*	0.8475 ± 0.31257	0.5550 ± 0.18940	**<0.001***

*Student's t-test; **Bold**: significant difference; WBCs: White blood cells; AST: aspartate aminotransferase; ALT: alanine aminotransferase

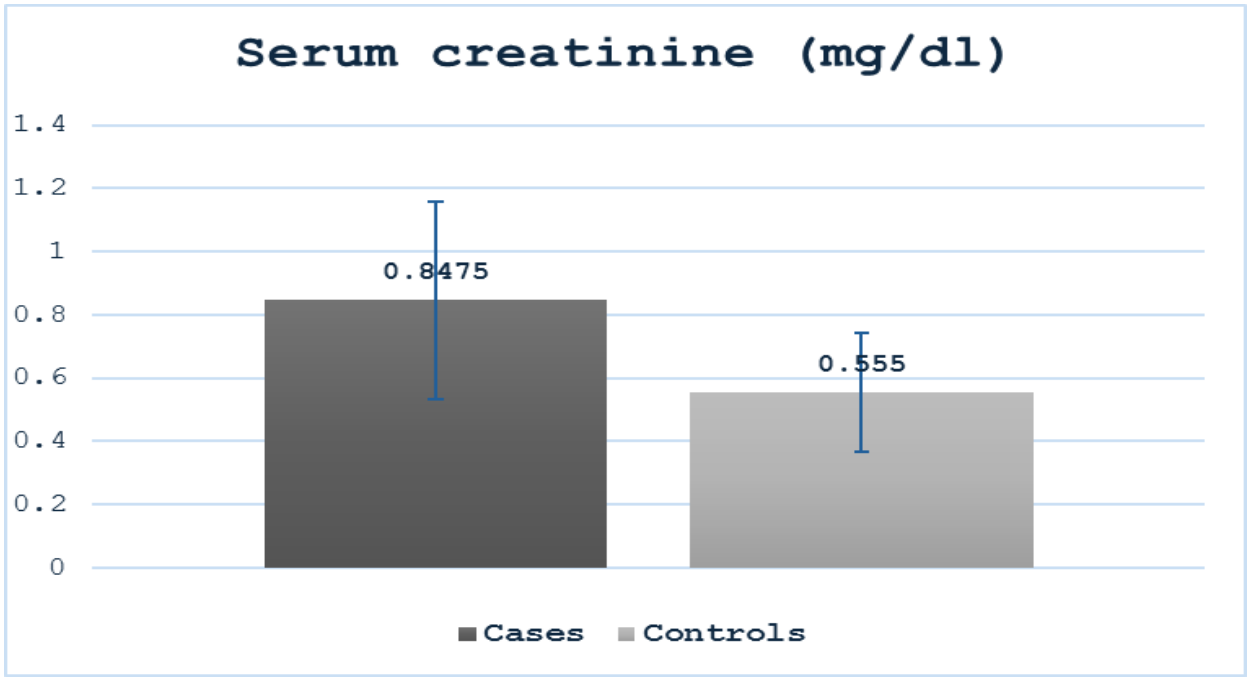

Fig.3. Serum creatinine among cases and controls

Serum ADA activity among case and controls

The mean ADA activity was significantly higher among TB cases than controls (38.40 ± 2.509 U/L vs. 22.30 ± 5.355 U/L) ($p<0.001$), **(Table.4) .**

Table 4. Adenosine Deaminase activity among cases and controls

Variables	Cases (n=40)	Controls (n=40)	P-value
	Mean ± SD	Mean ± SD	
Adenosine deaminase (ADA) Activity (U/L)*	**38.40 ± 2.509**	**22.30 ± 5.355**	**<0.001**

*Student's t-test, **Bold**: significant difference

ADA activity among cases regarding family history

There are no statistically significant differences between cases with a positive family history and cases with a negative family history regarding ADA activity (p>0.05), **(Table.5).**

Table 5. ADA activity among cases regarding family history

Variables	Family history		P-value
	Positive (n=12)	Negative (n=28)	
	Mean ± SD	Mean ± SD	
Adenosine deaminase (ADA) Activity (U/L)*	38.42 ± 2.937	38.39 ± 2.362	0.978

*Student's t-test

ADA activity among cases concerning TB types.

The study shows statistically higher ADA activity in cases with pulmonary TB compared to cases with extrapulmonary TB (p=0.027) (**Table.6).**

Table 6. ADA activity among cases concerning T.B. types

Variables	Type of TB		P-value
	Pulmonary (n=34)	Extrapulmonary (n=6)	
	Mean ± SD	Mean ± SD	
Adenosine deaminase (ADA) Activity (U/L)*	38.76 ± 2.413	36.33 ± 2.160	**0.027**

*Student's t-test, **Bold:** significant difference

ADA activity among cases concerning chest X-rays

The study shows no statistically significant differences between TB cases with normal chest X-rays and cases with abnormal chest X-rays regarding ADA activity (p>0.05**), (Table.7).**

Table 7. ADA activity among cases concerning chest X-rays

Variables	Chest X-rays		P-value
	Normal (n=16)	Abnormal (n=24)	
	Mean ± SD	Mean ± SD	
Adenosine deaminase (ADA) Activity (U/L)*	38.50 ± 2.898	38.33 ± 2.278	0.840

* Student's t-test

Correlation of ADA activity with routine laboratory investigations

There was statistically significant positive correlation between ADA activity and serum creatinine ($p< 0.001$, $r = 0.472$), **(Table.8, Fig.5).**

Table 8. Correlation of ADA activity with routine laboratory investigations among all participants

Routine laboratory parameters	ADA activity	
	r*	P-value
Creatinine (mg/dl)	0.472	**<0.001**
Urea (mg/dl)	0.122	0.283
Hemoglobin (gm/dl)	-0.090	0.427
Platelets (×103/mm3)	0.065	0.566
WBCs (×10^3/mm^3)	0.047	0.678
ALT (u/l)	0.037	0.741
AST (u/l)	-0.014	0.905

r*: Pearson correlation coefficient, **Bold**: significant difference

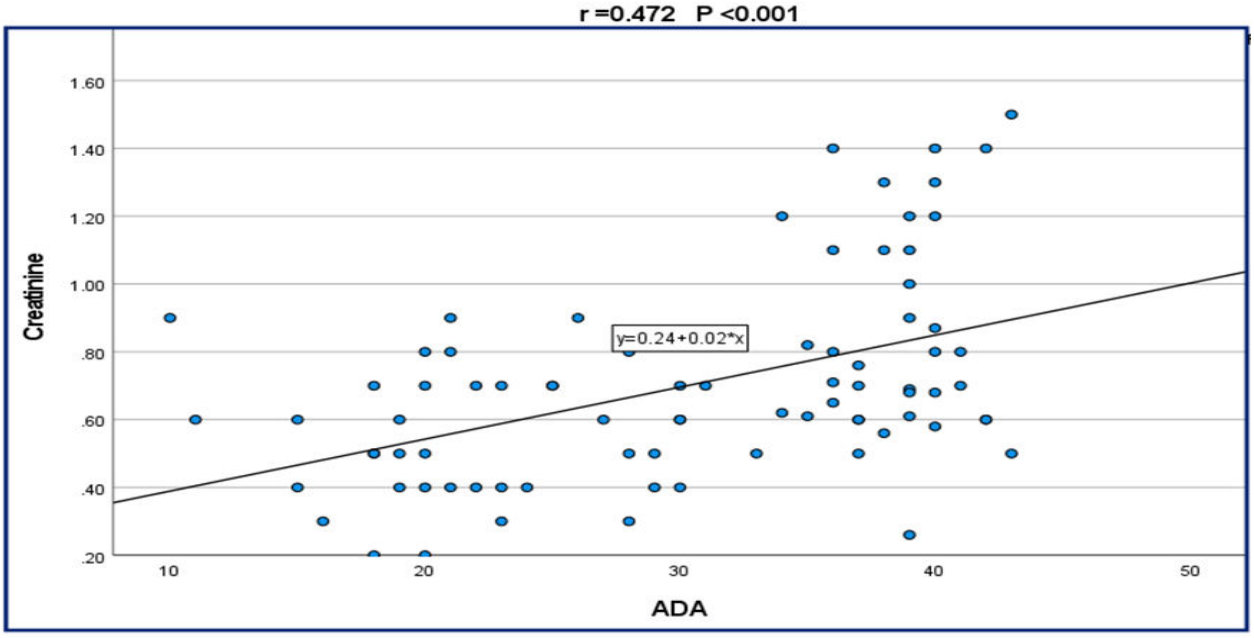

Fig.5. Pearson correlation of ADA activity with serum creatinine

ADA activity among cases based on TB types in comparison to controls

The study shows a statistically significant difference between cases with pulmonary and extrapulmonary tuberculosis and controls regarding ADA activity (P-value <0.001), where the mean ADA activity was lower among controls than cases with pulmonary and extrapulmonary T.B (22.3 ± 5.355 vs. 38.76 ± 2.413 and 36.33 ± 2.160, respectively). So, post hoc testing with Tukey adjustment was done to determine the nature of the difference between the three groups and revealed that the mean ADA activity was significantly lower among controls than cases with pulmonary and extrapulmonary T. B. (p-value <0.001).**(Table.9).**

Table 9. ADA activity among cases based on TB types and controls

Variables	Pulmonary TB (n=34)	Extrapulmonary TB (n=6)	Control (n=40)	P-value
	Mean ± SD	Mean ± SD	Mean ± SD	
ADA activity	38.76 ± 2.413	36.33 ± 2.160	22.3 ± 5.355	**<0.001***
Pairwise comparison (P-value)	Pulmonary TB vs. extrapulmonary TB = 0.389**	Pulmonary TB vs. control **<0.001****	Extrapulmonary TB vs. control **<0.001****	

*One-way ANOVA, **Post hoc test with Tukey correction, **Bold**: significant difference

ROC curve analysis of ADA activity to distinguish pulmonary TB from extrapulmonary TB

The shows that ADA can predict pulmonary tuberculosis at the cutoff point of ≥37.50 with the area under the curve (AUC) = 0.772, sensitivity = 76.6%, specificity = 83.3%, positive predictive value =97.99%, NPV =33.33%, and the overall accuracy =72.50% (p-value =0.036). **(Table.10, Fig.6).**

Table 10. ROC curve analysis of ADA activity to distinguish pulmonary TB from extrapulmonary TB

Parameters	AUC	Cut off value	Sensitivity (%)	Specificity (%)	P-value	PPV	NPV	Accuracy	95% CI	
									Lower	Upper
ADA activity	0.772	≥37.50 (UL)	70.6%	83.3%	0.036	95.99%	33.33%	72.5%	0.566	0.978

AUC: Area under the curve, PPV: Positive predictive value, Negative predictive value

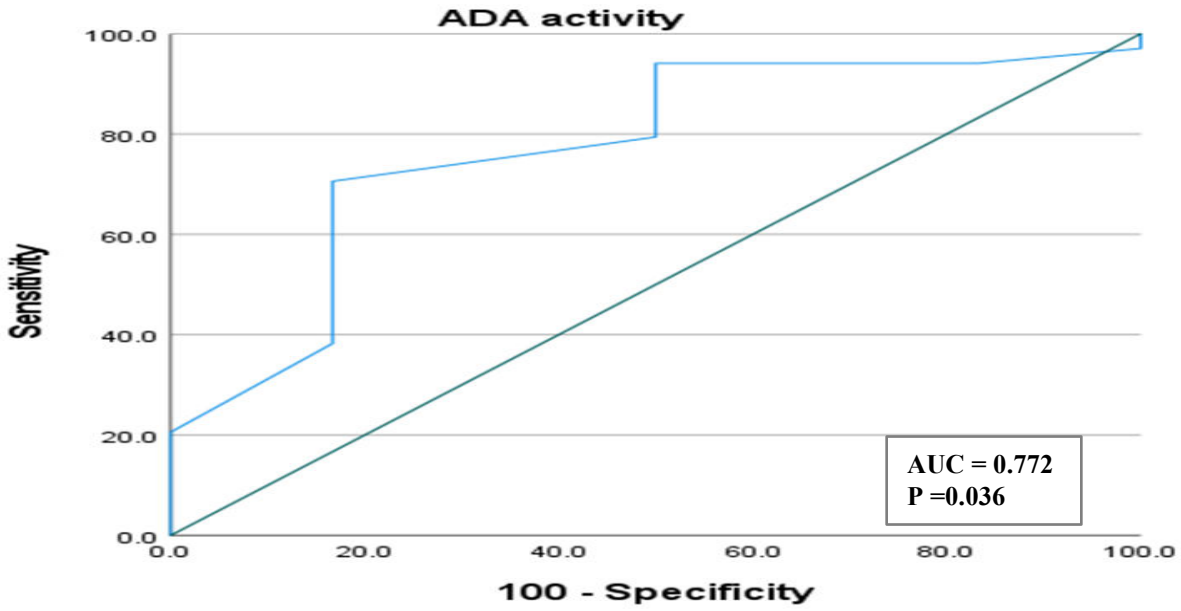

Fig.6. ROC curve analysis of ADA activity to distinguish pulmonary TB from extrapulmonary TB

ROC curve analysis of ADA activity to discriminate healthy controls from T.B cases

The study shows that ADA can predict tuberculosis at the cutoff point of 32 with the area under the curve (AUC) = 1.00, sensitivity = 100%, specificity = 100%, positive predictive value =100%, NPV =100%, and the overall accuracy =100% (p-value <0.001). **(Table.11 ,Fig.7).**

Table 11. ROC curve analysis of ADA activity to discriminate healthy controls from T.B cases

Parameter	AUC	Cut off value	Sensitivity (%)	Specificity (%)	P value	PPV	NPV	Accuracy	95% CI	
									Lower	Upper
ADA activity	1.00	32	100%	100%	<0.001	100%	100%	100%	1.00	1.00

AUC: Area under the curve, PPV: Positive predictive value, Negative predictive value

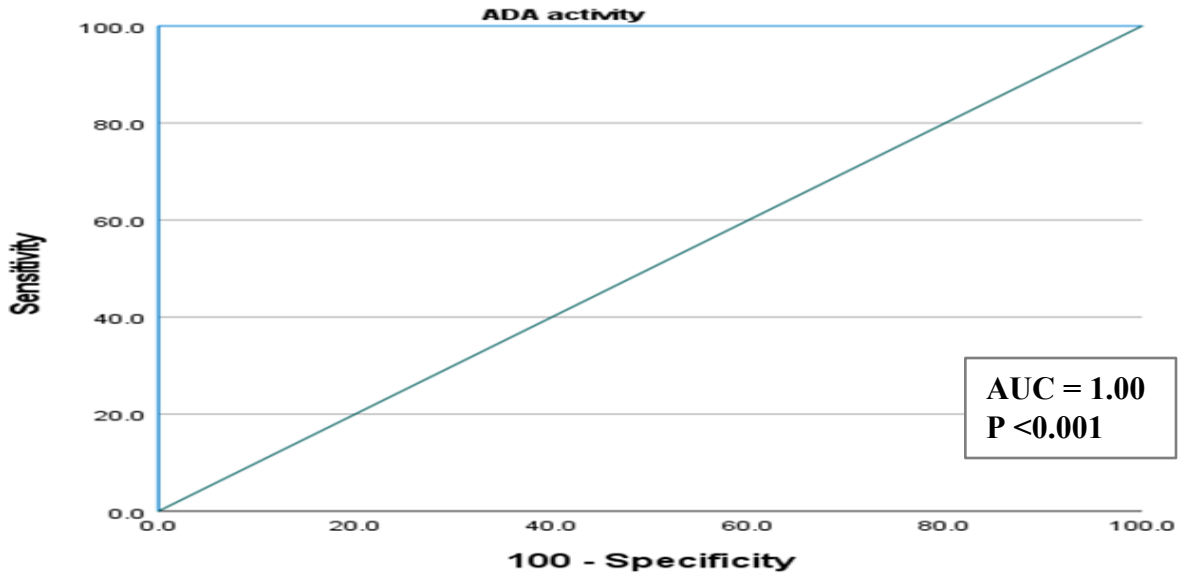

Fig.7. ROC curve for the ADA diagnostic accuracy in T.B

Genotypic and allelic frequencies of ADAG22A (G/A) among study groups

A statistically significant difference exist between TB cases and controls concerning ADAG22A ($p < 0.001$); 87.50% of cases had genotype GA polymorphism compared to 40% of cases. A-allele represents 43.75% of cases compared to 20% of controls, with a statistically significant difference at p=0.001. Odds ratio (95% CI) for GA genotype = 2.188 (1.470-3.254), and for A allele = 2.188 (1.322-3.620) **(Table.12, Fig.8)**

Table 12. Genotypic and allelic frequencies of ADAG22A (G/A) among study groups

Variables		TB cases (n=40) Number (%)	Controls (n=40) Number (%)	P-value	Odds ratio (95% CI)
Genotypes*	**GG**	5 (12.50%)	24 (60%)	**<0.001**	1 (reference)
	GA	35 (87.50%)	16 (40%)		2.188 (1.470-3.254)

Alleles*	**G-allele**	45 (56.25%)	64 (89%)	**0.001**	1 (reference)
	A-allele	35 (43.75%)	16 (20%)		2.188 (1.322-3.620)

*Chi-square test, **Bold**: significant difference

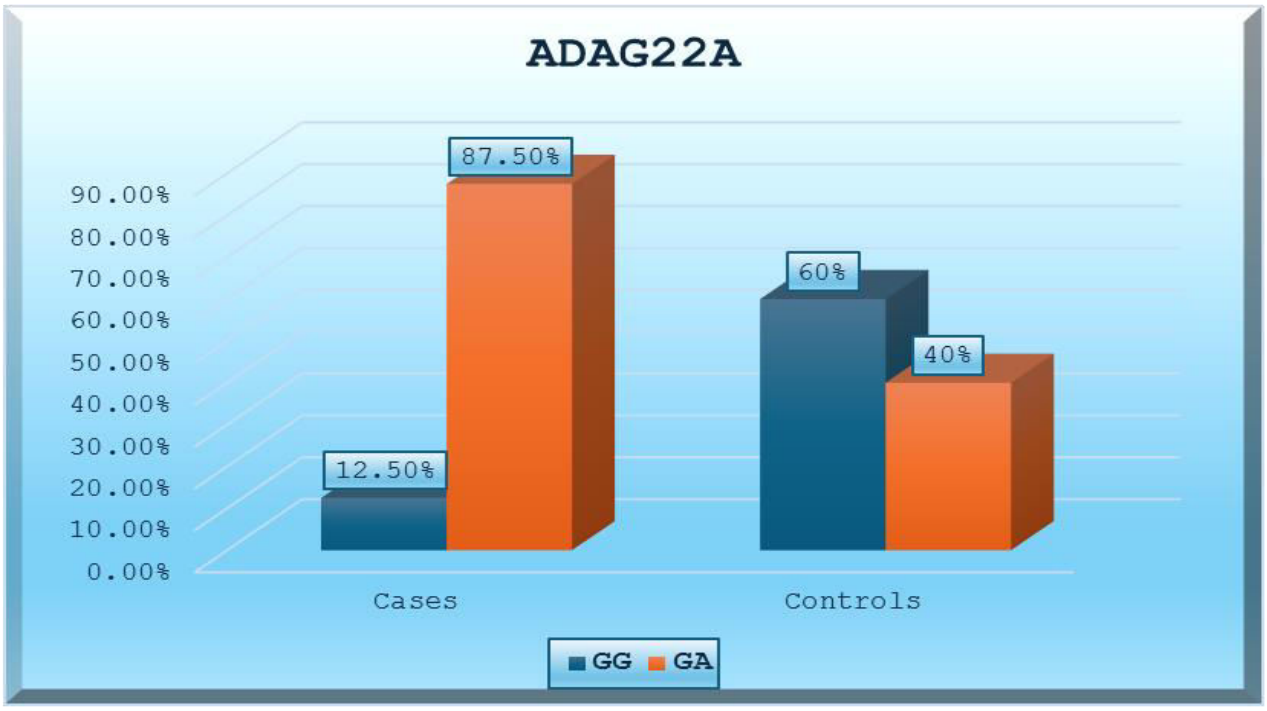

Fig.8. ADAG22A genotypes among cases and controls

Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning family history

There is no statistically significant differences between cases with a positive family history and cases with a negative family history regarding ADAG22A (p=0.118); 100% of cases with a positive family history had Genotype GA polymorphism compared to 82.1% of cases with negative family history. A-allele represents 50% of cases with a positive family history compared to 41.1% of cases with a negative family history, with an insignificant difference at p- =0.461. **(Table.13).**

Table 13. Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning family history

Variables		**Family history**		**P-value**	**Odds ratio (95% CI)**
		Positive (n=12)	**Negative (n=28)**		
		Number (%)	**Number (%)**		
Genotypes*	**GG**	0 (0%)	5 (17.9%)	0.118	1 (reference)
	GA	12 (100%)	23 (82.1%)		1.217 (1.024-1.447)
Alleles*	**G-allele**	12 (50%)	33 (58.9%)	0.461	1 (reference)
	A-allele	12 (50%)	23 (41.1%)		1.217 (0.732-2.024)

*Chi-square test

Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning types of T.B

The study shows no statistically significant differences between cases with pulmonary and cases with extrapulmonary TB regarding ADAG22A (p=0.738); 88.235% of cases with pulmonary TB had Genotype GA polymorphism compared to 83.33% of cases with extrapulmonary TB. A-allele represents 44.117% of cases with pulmonary TB compared to 41.667% of cases with an extrapulmonary TB, with an insignificant difference at p = 0.875. **(Table .14).**

Table 14. Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning types of TB

Variables		Pulmonary TB (n=34)	Extrapulmonary TB (n=6)	P-value	Odds ratio (95% CI)
		Number (%)	Number (%)		
Genotypes*	GG	4 (11.765%)	1 (16.667%)	0.738	1 (reference)
	GA	30 (88.235%)	5 (83.333%)		1.059 (0.725-1.546)
Alleles*	G-allele	38 (55.88%)	7 (58.333%)	0.875	1 (reference)
	A-allele	30 (44.117%)	5 (41.667%)		1.059 (0.515-2.177)

*Chi-square test

Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning chest X-rays

The study shows no statistically significant differences between cases with normal chest X-rays and cases with abnormal chest X-rays regarding ADAG22A (p=0.329); 89.75% of cases with normal chest X-rays had Genotype GA polymorphism compared to 83.33% of cases with abnormal chest X-rays. A-allele represents 46.875% of cases with normal chest X-rays compared to 41.667% of cases with abnormal chest X-rays, with an insignificant difference at p = 0.465**. (Table.15)**

Table 15. Genotypic and allelic frequencies of ADAG22A (G/A) among cases concerning chest X-rays

Variables		Chest X-rays		P-value	Odds ratio (95% CI)
		Normal (n=16)	Abnormal (n=24)		
		Number (%)	Number (%)		
Genotypes*	GG	1 (6.25%)	4 (16.667%)	0.329	1 (reference)
	GA	15 (93.75%)	20 (83.333%)		1.125 (0.904-1.401)
Alleles*	G-allele	17 (53.125%)	28 (58.333%)	0.645	1 (reference)
	A-allele	15 (46.875%)	20 (41.667%)		1.125 (0.684-1.851)

*Chi-square test

ADA activity among T.B cases concerning genotypes

The study shows that ADA activity was higher among the GA genotype than the GG genotype, but the difference was insignificant (p =0.056). **(Table. 16).**

Table 16. ADA activity among TB cases concerning genotype

Variables	Genotype		P value
	GG (n=5)	GA (n=35)	
	Mean ± SD	Mean ± SD	
Adenosine deaminase (ADA) Activity (U/L)*	36.40 ± 1.140	38.69 ± 2.529	0.056

*Student's t-test

Discussion

The Mycobacterium TB complex, a collection of closely related bacterial species, is the primary cause of TB **(Sarkar et al., 2023).** TB is a complicated, multifaceted illness, and mounting data indicates that a person's risk of contracting active tuberculosis may be influenced by genetic variations in immune response-regulating genes. Several genetic variants have been linked to active tuberculosis in numerous studies, yet the results frequently varied between the investigations (**Rudko et al., 2016).**

The enzyme ADA, which catalyzes the conversion of adenosine to inosine and deoxyadenosine, is mostly generated by T cells. It is indicative of cellular immunity that is active. Exudative effusions of many etiologies, including parapneumonic, tuberculous, and malignant effusions, exhibit elevated levels of ADA **(Abdelaziz et al., 2023).**

The ADA gene's enzymatic activity can be influenced by modifications to its function. Previous studies have shown the diagnostic value of ADA in TB, particularly its usefulness in evaluating effusions associated with TB. While pleural fluid ADA is not a perfect diagnostic tool, its levels are much greater in individuals with tuberculous pleural effusion (TPE), and ADA activity is enhanced in TB patients (**Passos et al., 2018).**

Our finding agreed with **Tenzin et al. (2020)**, who showed that there is no significant difference between cases and controls regarding age .

The routine laboratory data revealed that serum creatinine was significantly higher in TB patients than in controls; this increase may indicate impaired kidney function, possibly due to TB or the effects of anti-tuberculous drugs.

Our result aligns with findings from **Sakashita et al. (2018) and Du et al. (2022)** that report increased serum creatinine levels in TB patients, often associated with acute kidney injury during treatment.

The study found significantly higher ADA activity in TB patients compared to controls. Serum ADA levels that are elevated indicate serious TB and damage to the lung tissue. Serum ADA tests can be utilized as a prognostic indicator for patients with pulmonary tuberculosis and as an extra criterion for evaluating the effectiveness of TB treatment **(Sarkar et al., 2023).** Interestingly, no significant difference in ADA activity was found between patients with a positive versus negative family history of TB, suggesting that ADA levels are more influenced by active infection than by genetic predisposition.

ADA activity is mostly a consequence of cellular immunological activation in response to TB infection, rather than genetic determinants. Increased levels of ADA are regularly found in instances of activeTB, highlighting its use as a biomarker for the diagnosis of the illness and tracking the immune system's reaction to infection **(Ahmed et al., 2021).**

When comparing pulmonary to extrapulmonary TB cases, ADA activity was significantly higher in pulmonary TB (p =

0.027), reinforcing the idea that ADA is particularly elevated in more immune-active forms of the disease, such as pulmonary TB.

For instance, a study indicated that serum ADA levels were higher in pulmonary TB patients than in controls and higher in pulmonary compared to extrapulmonary TB, confirming its role as a biomarker of pulmonary TB infection.

No significant differences in ADA activity were found between patients with normal and abnormal chest X-rays, indicating that ADA activity may not correlate with the radiological extent of TB but rather with the systemic immune response.

Soedarsono et al. (2020) have established that ADA activity is more closely linked to immune system activation than to the severity of a particular disease. Elevated ADA levels are reported in active TB cases, but these levels may not necessarily match to the extent of lung involvement shown on chest X-rays.

Kim et al. (2020), have demonstrated that although ADA levels are raised in TB patients, there is no discernible relationship between the severity of radiological signs and ADA levels. This shows that rather than being a direct indicator of the disease's progression as seen by imaging, ADA activity is more indicative of the body's immunological response to the infection.

A striking finding of this study was the significant difference in the distribution of ADA G22A polymorphism between TB patients and controls. The GA genotype was more prevalent in TB patients (87.5%) compared to controls (40%), while the GG genotype was much more common in controls (60%) than in TB patients (12.5%). This suggests a strong association between the GA genotype and increased susceptibility to TB.

Moreover, the frequency of the A allele was significantly higher in TB patients (43.75%) compared to controls (20%) ($p = 0.001$), further indicating that the A allele may be a risk factor for TB. These findings align with previous studies suggesting that genetic polymorphisms in the ADA gene might influence susceptibility to infectious diseases like TB by modulating immune responses.

Wodelo et al. (2024) highlighted that numerous genetic polymorphisms, particularly those in immune-related genes, have a major effect on a person's susceptibility to tuberculosis. The analysis reaffirms the notion that genetic variables are important in disease susceptibility by highlighting the ways in which particular alleles can affect immune responses and the probability of contracting tuberculosis.

Additionally**, Shafiek et al. (2022)** have shown substantial correlations, especially across different ethnic groups, between specific genetic markers and elevated TB susceptibility. This implies that some alleles, such as the A allele in the ADA G22A polymorphism, may influence immune system performance and thereby increase risk.

Interestingly, no significant differences in ADA G22A gene polymorphism were observed between TB patients with and without a family history, between pulmonary and extrapulmonary TB cases, or even between patients with normal and abnormal chest X-rays. This indicates that while the ADA G22A gene polymorphism may predispose individuals to TB, it does not seem to influence the clinical presentation or severity of the disease.

Harishankar et al. (2018) investigated how genetic variations affected TB susceptibility. While some genetic variants are linked to a higher risk of acquiring TB, the analysis pointed out that there is an inconsistent relationship between these variants and the

disease's clinical severity or radiological findings. It specifically showed that while immune-related genetic variables may predispose people to TB, they do not always determine the severity of the disease's manifestation in its various forms.

This supports the finding that, without influencing the disease's severity or clinical results, ADA polymorphism may increase a person's susceptibility toTB (**Wodelo et al., 2024).**

Recommendation: further studies are needed to explore the mechanisms underlying the association between ADA polymorphisms and TB susceptibility.

Study limitation: Small sample size, not include negative TB, number of cases with positive family history was small (30%) compaird with cases with negative family history,number of extra pulmonary TB patients (85%) was greater than pulmonary TB (15%).

Conclusion

From the findings of our results, we conclude that ADA activity is significantly elevated levels in TB patients compared to controls. Additionally, the ADA G22A gene polymorphism, particularly the GA genotype and A allele, was strongly associated with an increased risk of TB. These findings suggest that ADA activity and genetic polymorphism could be used in conjunction with other diagnostic tools for TB screening and risk stratification

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