



ASSESSMENT OF SERUM ADENOSINE DEAMINASE ACTIVITY AS A RISK MARKER FOR CARDIOVASCULAR DYSFUNCTION IN HYPOTHYROIDISM: A CASE CONTROL STUDY

Gurjinder Singh^{1*}, Dr. Ritesh Vishwakarma², Dr Shubham Girdhar³, Dr. Jasleen Kaur⁴

¹Ph. D scholar, Department of Biochemistry, Malwanchal University, Indore, MP

²Assistant Professor, Dept. of Biochemistry, Index medical college, Indore, MP

³Associate Professor, Dept. of Community Medicine, Sudha Medical College & Hospital, Kota, Rajasthan

⁴Resident Medical Officer Shri Mata Vaishno Devi Narayana Superspeciality Hospital, Katra, J&K

***Corresponding Author:** Gurjinder Singh

*Ph. D scholar, Department of Biochemistry, Malwanchal university, Indore, MP,

Email: gurjinderju@gmail.com

ABSTRACT

Background: Hypothyroidism is a clinical syndrome that results from the insufficient production of thyroid hormones leading to slow down the metabolic activities. It has been widely reported that thyroid gland is very much prone to autoimmune disorder. Adenosine deaminase (ADA), an enzyme distributed in the human tissues, is considered as nonspecific marker of cell-mediated immunity. Also it is suggested to be an important enzyme for modulating the bioactivity of thyroid hormones, but its clinical significance in thyroid disorders is not yet characterized. **Aim:** To study the correlation of TSH with ADA as possible predictor of cardiovascular dysfunction.

Materials & Methods: This was a hospital based cross sectional study. A total of 200 subjects Study population was divided into two groups based on their thyroid reports. Patients with normal TSH, values (Below 5.0 mIU/L) were considered Control (Group 1). Patients with high TSH levels were classified as being hypothyroid mentioned as Cases (Above 10.0 mIU/L) (Group 2). Among controls 21 (21%) were males and 79 (79%) females. Among cases 18 (18%) were males and 82 (82%) females reflecting the higher prevalence of hypothyroidism. This research was examined at Indore Index Medical College & Research Centre patients. After approval, the study's researchers began. Before the study, each subject gave informed consent. Healthy controls were those without history of hypothyroidism with any other medications or treatments, also patients with any medical/surgical illness like Cardio vascular disorders, Diabetes Mellitus, kidney failure, Liver disorders and other major chronic illnesses were excluded.

Results: The present investigation was designed as an age matched case-control study, including 100 hypothyroid patients (cases) and 100 healthy individuals (controls). The primary objective was to evaluate the consequences of hypothyroidism on ADA levels, and to establish correlations that highlight their role in cardiovascular risk. **Key Findings** included, ADA level was found to be

significantly higher in overt hypothyroid patients as compared to normal euthyroid subjects. Importantly ADA levels correlated positively with **TSH values**, ($r = 0.651$, $p < 0.001$, S) Reflecting that greater thyroid dysfunction is accompanied by heightened inflammatory activity. Our findings suggest that ADA may play a role in the pathophysiology of thyroid disorders; however, a large-scale study is required to predict the relationship.

Introduction:

Hypothyroidism is a clinical syndrome that results from the insufficient production of thyroid hormones leading to slow down the metabolic activities.[1] Thyroid hormone regulates almost all the metabolic process in the body and their deficiency in the tissues can develop several pathological conditions presenting with different signs and symptoms.[2,3] Hypothyroidisms is alarming as chief health problem in developed as well as in developing nation are 4-5% of the people are found to be associated with hypothyroidism in well developed nations. In contrast, Indian studies suggest a significantly higher prevalence of approximately 10.95%. [4,5] The occurrence of the diseases has been observed more in female in comparison to male and affects mostly advanced age group people.[6] Iodine deficiency is the most common cause for the hypothyroidism and endemic goiter worldwide.[7] Hypothyroidism is initiated with various symptoms like fatigue, intolerance to cold, constipation, weight gain, poor appetite, shortness of breath. In females, heavy menstrual periods, abnormal sensation, poor hearing etc and signs like Dry, coarse skin, hair loss, myxedema, swelling of limbs, slow pulse rate etc causes immune deregulation which leads to inflammation.[8,9] Several studies have reported that various type of inflammatory marker have increased during hypothyroidism inflammation. C-reactive protein (CRP) The prime source of CRP is hepatic cell which is released in the circulation in response to inflammatory cytokines like interleukine-6(IL-6), Tumour necrosis factor alpha etc.[10] The higher concentration of inflammatory cytokines in the blood prevent the conversion of thyroxine into active tri-iodothyronine which is important for the maintenance of metabolism within the cell, leading to hypothyroidism.[11]

Adenosine deaminase (ADA) is a polymorphic enzyme which expressed in all human tissues. In addition to regulating adenosine concentration by catalyzing the irreversible deamination of adenosine to inosine [12], ADA is also essential for the differentiation and maturation of T lymphocytes, and is a general marker of cellular immunity [13]. In some autoimmune diseases, such as psoriasis [14], autoimmune hepatitis [15], inflammatory bowel disease [16], and rheumatic diseases, high serum concentrations of ADA were observed. Meanwhile, increased ADA activity was also observed in peripheral blood leukocytes of patients with GD [17] and monocytes of patients with Hashimoto disease [18]. Altered ADA activity has been reported in patients with hypothyroidism, reflecting changes in immune and inflammatory pathways associated with thyroid hormone deficiency. Reduced ADA activity has been observed in overt hypothyroidism, which may be linked to impaired cellular immunity and slower metabolic turnover [20,21]. Conversely, some studies suggest increased ADA activity in subclinical hypothyroidism, possibly due to compensatory immune activation [22] Therefore this preliminary study was undertaken to determine plasma ADA activity and sought out its association with hypothyroidism.

2. Aims & Objectives

1. To determine the TSH, and Serum ADA Levels in the study subjects
2. To compare the above parameters in hypothyroid patients (cases) and normal individuals (controls)
3. To study the correlation of TSH with ADA as possible predictor of cardiovascular dysfunction

3. Materials and Methods

3.1 Study design

This was a hospital based cross sectional study. A total of 200 subjects were enrolled from the patients visiting immunoassay lab of the teaching hospital for their thyroid function test after taking their informed consent. Five milliliter venous blood sample was collected aseptically in a plain vial and serum was separated after running it in centrifuge (REMI) at 3000 rpm for 10 minutes. Sample were stored at -20°C till the analysis of thyroid hormones, TSH and ADA. Exclusion Criteria: Patients with liver disorders, renal disorders, congestive cardiac failure; pregnant women; patients on oral contraceptive pills, statins, and other medications that alter thyroid functions and lipid levels were excluded from the study.

3.2 Study setting:

Department of Biochemistry, Index Medical College & Hospital, Indore

3.3 Division of the subjects

Study population was divided into two groups based on their thyroid reports. Patients with normal TSH, values (Below 5.0 mIU/L) were considered Control (Group 1). Patients with high TSH levels were classified as being hypothyroid mentioned as Cases (Above 10.0 mIU/L) (Group 2). Among controls 21 (21%) were males and 79 (79%) females. Among cases 18 (18%) were males and 82 (82%) females reflecting the higher prevalence of hypothyroidism.

3.4 Biochemical Assay

Thyroid hormone TSH was assessed The estimation of thyroid stimulating hormone (TSH) in serum is performed using a sandwich-type solid phase ELISA. The method employs two highly specific antibodies directed against different epitopes of the TSH molecule: Capture antibody: Mouse monoclonal anti-TSH immobilized on microplate wells Detection antibody: Goat anti-TSH conjugated with horseradish peroxidase (HRP): The normal reference values as per our laboratory were as follows: TSH (0.25–5.0 mIU/L), Serum ADA activity was measured by simple, sensitive ADA Assay (Kinetic Spectrophotometric Method) Estimations of Serum Adenosine Deaminase (ADA), Spectrophotometric Adenosine is transformed into a detectable colored byproduct by a four-step enzymatic cascade in the Diazyme ADA Assay. In the first stage, the patient sample's Adenosine Deaminase (ADA) deaminates adenosine to create inosine, which is further processed by purine nucleoside phosphorylase (PNP) to produce hypoxanthine. Xanthine oxidase (XOD) further oxidizes this hypoxanthine to produce hydrogen peroxide and uric acid. Ultimately, a quinone dye is created when hydrogen peroxide combines with EHSPT and 4-aminoantipyrine in a peroxidase-coupled Trinder reaction. The rate at which this dye develops—measured spectrophotometrically—is exactly proportional to the concentration of ADA activity. Reference ranges Serum (healthy individuals) : 0–15 U/L

3.5 Statistical Analysis Summary

All calculations were done using R programming language, Packages used: readxl (data import), ggplot2 (visualisation), gridExtra and grid (plot arrangement), openxlsx (Excel export), Test Used, Welch's Unpaired t-test was used to compare Controls vs Cases, p-value was Calculated, using mean and SD of each parameter entered for both groups. Significance: S (significant) if $p < 0.05$, NS (non-significant) if $p \geq 0.05$

4. RESULTS:

There were 100 hypothyroid patients (cases) and 100 healthy people (controls) in this age-matched case-control research, shown in Table 1. The main goal was to assess the effects of hypothyroidism on n Adenosine Deaminase (ADA) Activity and to find correlations that demonstrate their

significance in cardiovascular risk. Among controls 21 (21%) were males and 79 (79%) females. Among cases 18 (18%) were males and 82 (82%) females reflecting the higher prevalence of hypothyroidism.

Table 1: Sample size distribution of Controls and Cases

Table	Sample Size
CONTROLS	100
CASES	100

The study comprised 100 Controls and 100 Cases, totaling 200 subjects

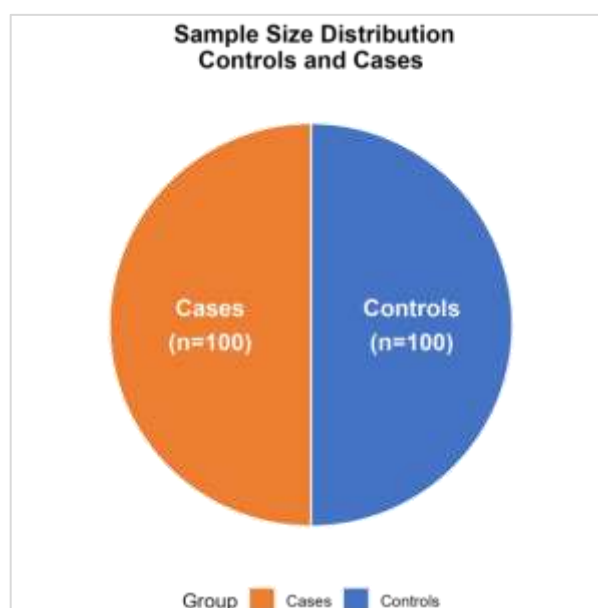


Figure 1: Sample Size Distribution of Controls and Cases

Table 2: Gender Wise Distribution of Hypothyroid Cases and Controls

S.No.	Gender	Control (N)	Cases (N)
1	Males	21	18
2	Females	79	82
Total		100	100

Among controls 21 (21%) were males and 79 (79%) females. Among cases 18 (18%) were males and 82 (82%) females reflecting the higher prevalence of hypothyroidism in females

Our results showed that the mean TSH levels in our study showed a striking elevation in hypothyroid cases compared to controls. Overall, controls had a mean TSH of 2.11 ± 0.52 μ IU/mL, which falls within the normal physiological range, whereas cases demonstrated a markedly elevated mean of 16.75 ± 3.69 μ IU/mL, confirming the diagnostic significance of TSH in hypothyroidism. With a P Value of <0.00001 in Table3

Table 3.: Comparison of TSH in Cases and Controls [Combined Males & Females] Units: mIU/L

Subjects	N	Mean	Std. Deviation	95% CI Lower	95% CI Upper	P value
Controls	100	2.11	0.5191	2.007	2.213	<0.00001
Cases	100	16.75	3.6952	16.017	17.483	

Combined TSH analysis showed mean $\pm$ SD of 2.11 ± 0.52 mIU/L in controls versus 16.75 ± 3.70 mIU/L in cases ($p < 0.00001$, highly significant).

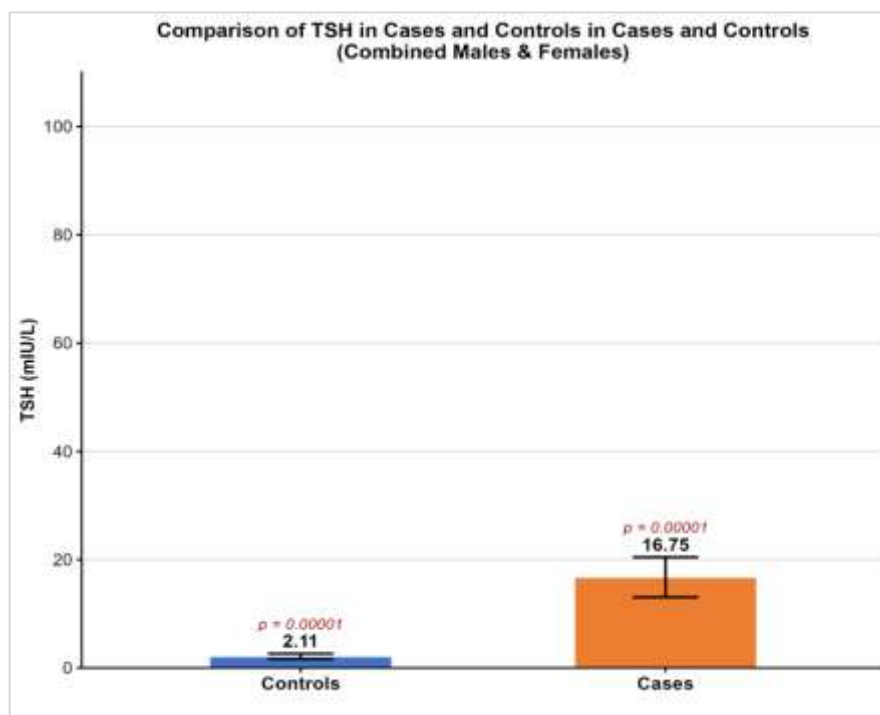


Figure 2: Comparison of TSH Levels in Cases and Controls in Males and Females (Combined)

Our study demonstrated a significant elevation of adenosine deaminase (ADA) activity in hypothyroid cases compared to controls, with mean ADA levels of 36.25 ± 5.12 U/L in cases versus 9.45 ± 1.36 U/L in controls. This sharp increase reflects enhanced lymphocyte function and immune activation, consistent with the autoimmune and inflammatory nature of thyroid disease. Elevated ADA has been linked to increased T-cell activity and cytokine release, which may contribute to vascular inflammation, dyslipidemia, and endothelial dysfunction with a P Value of <0.00001 shows significant difference among cases compared to controls.

When stratified by gender, the same pattern was evident. In males, hypothyroid cases had a mean ADA values of 34.56 ± 5.23 , compared to 8.56 ± 1.2 U/L in controls. In females, cases showed even higher mean values 36.63 ± 5.12 U/L compared to controls 9.69 ± 1.23 U/L. These findings highlight both the magnitude of ADA elevation in hypothyroidism and the female predominance, consistent with epidemiological evidence that Immunological changes in hypothyroidism occur more frequently in women as shown in Table 5.

Table 4: Comparison of Adenosine Deaminase (ADA) Levels in Cases and Controls [Combined] Units: U/L

Subjects	N	Mean	Std. Deviation	95% CI Lower	95% CI Upper	P value
Controls ADA	100	9.45	1.36	9.180	9.720	<0.00001
Cases ADA	100	36.25	5.12	35.234	37.266	

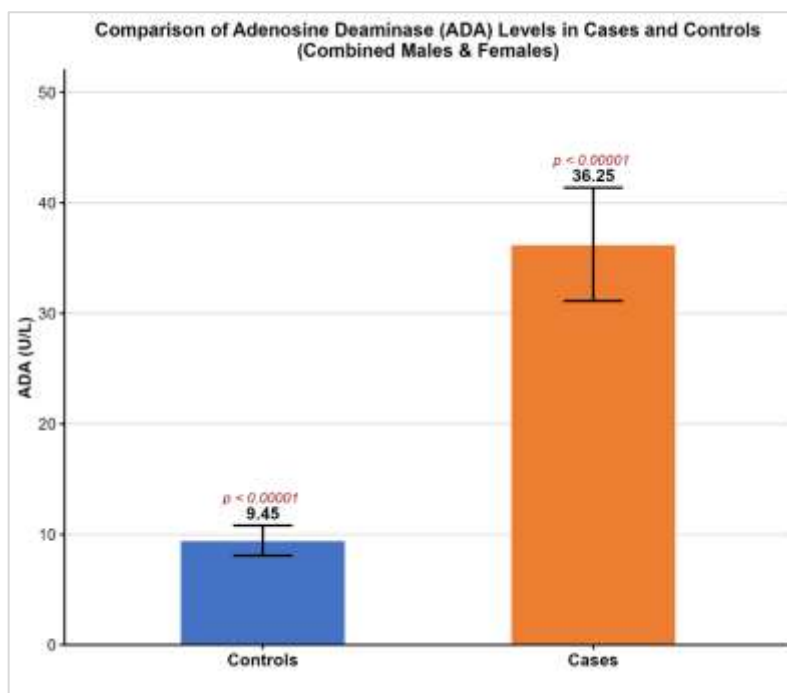


Figure 3: Comparison of Adenosine Deaminase Levels in Cases and Controls in Males and Females

Table 5: Comparison of Adenosine Deaminase (ADA) Levels in Cases and Controls [In Males & Females] Units: U/L

Gender	Subjects	N	Mean	Std. Deviation	95% CI Lower	95% CI Upper	P value
Male	Controls ADA	21	8.56	1.23	8.000	9.120	<0.00001
	Cases ADA	18	34.56	5.23	31.959	37.161	
Female	Controls ADA	79	9.69	1.45	9.365	10.015	<0.00001
	Cases ADA	82	36.63	5.12	35.505	37.755	

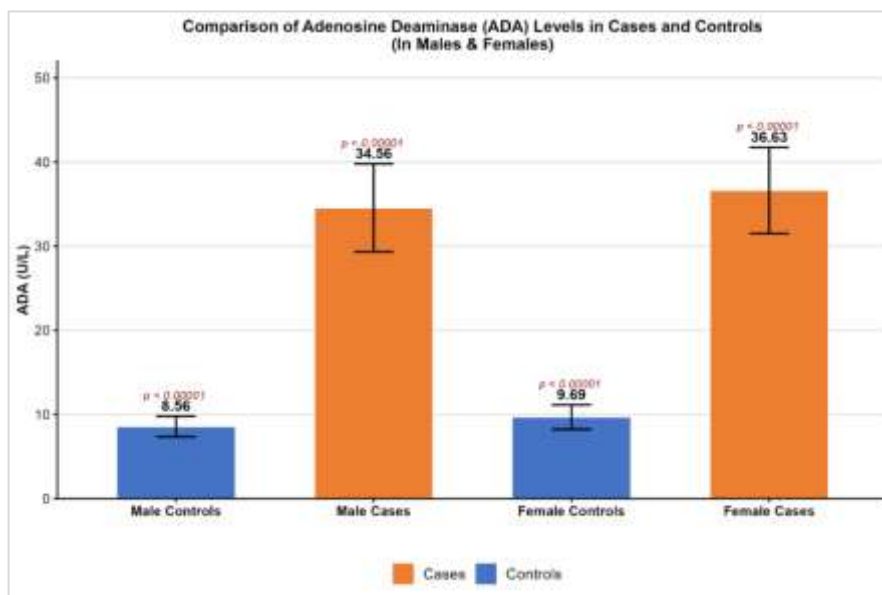


Figure 4: ADA levels were significantly elevated in hypothyroid cases compared to controls in both males (8.56 ± 1.23 vs 34.56 ± 5.23 U/L) and females (9.69 ± 1.45 vs 36.63 ± 5.12 U/L), with $p < 0.00001$ in both groups.

Table 6: Correlation of TSH with ADA in Hypothyroid Subjects

Parameters	r value	p value	Significance
ADA	0.651	< 0.001	S

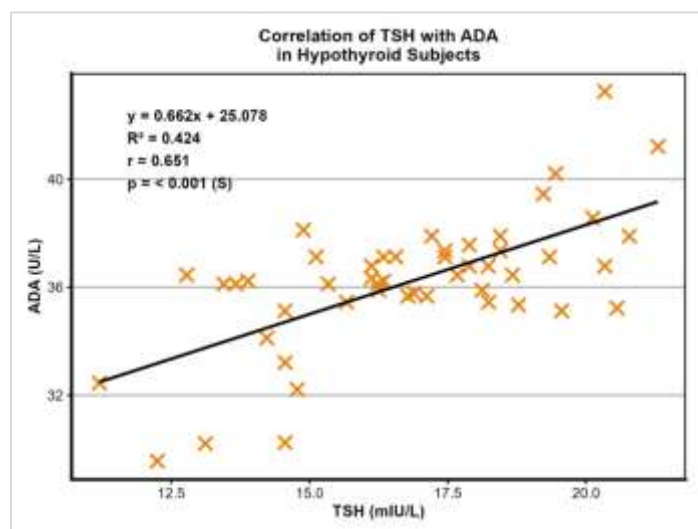


Figure 5: Correlation of TSH with ADA in Hypothyroid Subjects

A significant positive correlation ($r = 0.651$, $p < 0.001$, S) indicates that as TSH levels increase ADA activity rises significantly suggesting enhanced purine metabolism and immune activation in hypothyroid subjects.

5. Discussion

Immunological disturbances of cell-mediated origin, are believed to initiate from T-lymphocyte dysfunction. ADA plays a crucial role in lymphocyte proliferation and differentiation and shows its

highest activity in T-lymphocytes. In the present study we studied the ADA activity in 100 age matched controls with hypothyroid cases related as inflammatory marker. Since this is the first study of its kind in the central Indian population, therefore very few literatures are available. Similar study has been carried out in other disorders like diabetes mellitus (DM), autoimmune diseases, tuberculosis. Our study report that the females are much prone to thyroid disorders as compare to male, owing to their greater susceptibility to increased autoimmunity. ADA level was found to be significantly higher in overt hyperthyroid and overt hypothyroid as compared to normal euthyroid subjects. Our finding is in accordance to the study done by Abbas *et al.* Whereas the study done by Vishnu *et al.*, shows that ADA level in hyperthyroid patients were within normal limit, which contradicts to our study. This marked elevation in figure 5 indicates that hypothyroidism is associated with a strong pro-inflammatory state, ADA acting as a key mediator of enhanced purine metabolism and immune activation. Importantly ADA levels correlated positively with **TSH values**, ($r = 0.651$, $p < 0.001$, S) Reflecting that greater thyroid dysfunction is accompanied by heightened inflammatory activity.

Moreover, ADA levels have also been correlated with lipid abnormalities and inflammatory markers in hypothyroid patients. Elevated ADA activity has been associated with increased LDL cholesterol and triglycerides, suggesting that ADA may contribute to the dyslipidemic profile and cardiovascular risk seen in thyroid dysfunction (Patel et al., 2015). Our findings exhibited a positive correlation ($r = 0.430$, $p = 0.002$, S) data not shown, suggests that higher cholesterol levels are significantly associated with increased ADA activity reflecting enhanced purinergic signalling and immune cell activation in the context of hypothyroid related hypercholesterolaemia., Furthermore, ADA interacts with cytokines such as IL-6 and TNF- α , amplifying inflammatory responses and endothelial dysfunction in hypothyroidism (Turemen et al., 2011; Kebapcilar et al., 2007) . Taken together, ADA serves as a potential biomarker of immune and metabolic dysregulation in hypothyroidism. Its association with lipid abnormalities and inflammatory cytokines highlights its role in the pathogenesis of cardiovascular complications in thyroid disease.

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