Assessment of Adenosine Deaminase Activity in Rheumatoid Arthritis Patients in Dhamar City Yemen

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Abstract

Background: Rheumatoid Arthritis is a chronic inflammatory disorder affecting joints, characterized by synovitis and systemic effects. Adenosine Deaminase is an enzyme involved in purine metabolism, with elevated levels often associated with inflammation. The aim of this study is to evaluate ADA activity levels in rheumatoid arthritis patients as well as to compare changes in Adenosine deaminase activity among patients with rheumatoid arthritis and healthy control group.

Study design: The study was conducted during October 2023 to October 2024 among rheumatoid diseases in Dhamar hospitals in Dhamar City,Yemen. The present study was conducted on (102) patients with rheumatoid arthritis and (40) apparently healthy subjects were taken as a control group; the age range is between (14–79) years. All Patients diagnosed with RA based on ACR/EULAR criteria were included whereas all patients with other autoimmune diseases, infections, or chronic inflammatory conditions were excluded. Sample Collection: Blood samples were collected to measure ADA activity and spectrophotometric was used to quantify ADA levels. In addition, data on disease duration, severity, and treatment history were collected. Results revealed that RA patients showed significantly higher levels of serum adenosine deaminase (ADA) compared to the healthy control group. In comparison with the control group, the patients with RA showed a significant increase in Adenosine deaminase ($P \leq 0.002$).

Keywords: *Adenosine deaminase (ADA), Rheumatoid arthritis (RA), antibodies against citrullinated peptides (ACPAs).*

الملخص

إلتهاب المفاصل الرثوي هو اضطراب التهابي مزمن يؤثر على المفاصل، ويتميز بالتهاب الغشاء الزليلي وتأثيرات جهازية. إنزيم ادينوسين دي إمينيز هو إنزيم يشارك في استقلاب البيورين، وغالبًا ما ترتبط المستويات المرتفعة منه بالالتهاب.

أهداف هذه الدراسة هي تقييم مستويات نشاط إنزيم ادينوسين دي امينيز في مرضى التهاب المفاصل الرثوي، بالإضافة إلى مقارنة التغيرات في نشاط إنزيم ادينوسين دي امينيز بين مرضى التهاب المفاصل الرثوي ومجموعة الأشخاص الأصحاء. تم إجراء الدراسة خلال الفتر من أكتوبر 2023 الى اكتوبر 2024 بين مرضى التهاب المفاصل الرثوي في مستشفيات ذمار -مدينة ذمار / اليمن. تمت الدراسة على (102) مريضٍ مصابِ بالتهاب المفاصل الرثوي و(40) شخصًا سليمًا ظاهريًا تم أخذهم كمجموعة ضابطة، وكانت أعمارهم تتراوح بين (14-79) عامًا.

معايير الإدراج: جميع المرضى الذين تم تشخيصهم بالتهاب المفاصل الرثوي بناءً على معايير ACR/EULAR

معايير الاستبعاد: جميع المرضى الذين يعانون من أمراض مناعية ذاتية أخرى، أو عدوى، أو حالات التهابية مزمنة.

جمع العينات: تم أخذ عينة دم لقياس نشاط إنزيم ادينوسين دي إمينيز، كما تم استخدام جهاز الطيف الضوئي لتحديد مستويات إنزيم ادينوسين دي إمينيز. بالإضافة إلى ذلك تم جمع البيانات حول مدة المرض، وشدته، وتاريخ العلاج.

النتائج: أشارت النتائج إلى أن مرضى التهاب المفاصل الرثوي لديهم مستويات عالية من إنزيم إدينوسين دي إمينيز في المصل مقارنة بالأشخاص الأصحاء، فبالمقارنة مع المجموعة الضابطة، أظهر المرضى المصابون بالتهاب المفاصل الرثوي زيادة ملحوظة في إنزيم إدينوسين دي امينيز ($P \leq 0.002$).

الكلمات المفتاحية: انزيم ادينوسين دي إمينيز، التهابات المفاصل الرثوي، الأجسام المضادة ضد الببتيد السيتروليني.

Introduction

Rheumatoid arthritis (RA) is a systemic inflammatory autoimmune disease characterized by progressive inflammation and destruction of the synovial joints, often leading to disability and a reduced quality of life. ***(Abdelhafiz et al., 2023; Atzeni et al., 2017; Radu & Bungau, 2021)***. RA involves many extra-articular complications, including cardiovascular, interstitial lung, and hematologic diseases, and reduces life expectancy by 5 to 10 years ***(Codes-Méndez et al., 2024; Salesi et al., 2012)***. The causes of rheumatoid arthritis are not fully understood, but it is believed that a combination of genetic susceptibility, exposure to environmental factors, and abnormal adaptive immune responses contribute to the development of the disease ***(C. et al., 2022; Song et al., 2024).*** B cells play a central role in the development of rheumatoid arthritis, leading to the production of pathogenic antibodies, including antibodies against citrullinated peptides (ACPAs). These antibodies can be detected years before the onset of clinical symptoms, indicating the presence of a latent immune response against citrullinated proteins in the preclinical stages of the disease ***(Talib et al., 2024)***. The synovial tissue in patients with rheumatoid arthritis contains many abnormal germinal centers, indicating that the progression of the disease is similarly affected by disturbances in the activity of T and B lymphocytes, in addition to the activation and differentiation of B cells mediated by follicular helper T cells ***(Perera et al., 2024).*** Epidemiological studies indicate that rheumatoid arthritis is the most prevalent connective tissue disease at the systemic level, affecting 1-2% of the world's population ***(Perumal et al., 2024).*** Although most of the epidemiological, pathophysiological, immunological, and genetic aspects of rheumatoid arthritis are well understood today, it remains unclear what the precise causes and factors are that support the destructive inflammatory process in this disease. The diagnosis of rheumatoid arthritis is based on identifying the underlying clinical picture and the results of imaging and laboratory tests, taking into account the modified criteria of the American College of Rheumatology (ACR) from 1987 ***(Sahin et al., 2024)***. In 2010, the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) updated the classification criteria for rheumatoid arthritis (RA). Four main areas were identified, including: joint involvement (such as the number of joints and small versus large joints), serological tests (such as antibodies associated with rheumatoid arthritis), acute phase reactants (APR) like elevated levels of C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), and the duration of symptoms (i.e., more or less than 6 weeks) ***(Clunie et al., 2018; Codes-Méndez et al., 2024)***.

Adenosine deaminase (ADA) is an important enzyme in the purine salvage pathway, as it catalyzes the irreversible deamination of adenosine and deoxyadenosine, converting them into inosine and deoxyinosine ***(Ubaid & Pandey, 2024)***. Adenosine regulates many physiological and pathological processes in the cardiovascular system, as well as in inflammation, the development and maturation of the immune system, and the maintenance of immune balance ***(Hameed et al., 2019; Vuerich et al., 2024).*** The increase in adenosine levels during periods of stress and injury is related to the regulation of the immune system and inflammation through receptor interactions. The adenosine receptor family consists of four receptors linked to G proteins: A1, A2A, A2B, and A3.***(Hussain, 2024)***. Adenosine signals are reduced by enzymes such as ecto-nucleotide triphosphate

diphosphohydrolase 1, ecto-5′-nucleotidase, and ecto-adenosine diphosphate ribosyl cyclase 1, leading to the production of signals associated with cyclic adenosine monophosphate and providing protection against excessive inflammatory responses ***(Shalini et al., 2015)***. Measuring serum ADA concentration (sADA) can be a suitable method for assessing the activity of rheumatoid arthritis (RA). The relationship between ADA and rheumatoid arthritis activity has shown conflicting results. ADA has been reported as a useful marker for the ongoing inflammatory process in rheumatoid arthritis ***(Makhe & Vagga, 2024)***. This work aimed to evaluate the performance of ADA in measuring disease activity in RA patients.

MATERIALS AND METHODS

Study Area:

A cross sectional study has been conduct during the year 2023- 2024 among rheumatoid diseases in Dhamar hospitals in Dhamar city. Dhamar governorate is the most consistently elevated governorate in Yemen, the City in general located 1,600-3200 m (5,200-10,500 Ft) above the sea level with most of the land lying at over 2,500 meters (8,200 Ft). The governorate is located approximately 100 km south to Sana`a, the capital of Yemen.

Study Population:

This study has been conduct to perform their enzymatic activity of adenosine deaminase (ADA) in Dhamar city, where the sample of RA patients, whose (14-97).

Inclusion and Exclusion Criteria:

In terms of the geographical limits, the main focus has been on rheumatoid arthritis patients in hospitals of Dhamar city were included, Patients from outside Dhamar or with other conditions were excluded.

Study Design:

A cross-sectional study has been conducted in Dhamar city, Yemen. Data were collected in a period of One Years from October 2023 to October 2024. In each hospital, were select randomly from the available hospitals list were considered in collaboration with RA patient.

Sample Size:

A total of 142 participated (102 rheumatoid arthritis patients and 40 healthy control) was select in this study. This size of sample were calculate according to study conducted by ***(Gulati et al., 2018)*** and ***(Garg et al., 2019)***. All patients met the 2010 American College of Rheumatology/European League Against Rheumatism criteria for Rheumatoid Arthritis (found as previous sources) *(Hameed et al., 2019)*.

Techniques considering 102 patients and 40 healthy people. Accordingly, a total of (142) rheumatoid arthritis patient and healthy control) has been investigating in this study.

Data Collection:

Subject before recruitment into the study. A meeting interview has been used for filling in a questionnaire that designated for matching the study need (demographic and clinic data including age, gender, duration of disease, medication history, ….etc.), dietary habits and medical history information of the participants and all interviews were conducted face-to-face by the researchers person.

About five milliliters of human blood without anticoagulant tubes were collected from different volunteers and then centrifuge to obtain serum, both males and females, diagnosed with rheumatoid arthritis is tested to ADA (found as previous sources) ***(Cennamo et al., 2024; Samuels et al., 2021)***

Estimation of serum ADA by full automatic analyzer

Catalytic activity of ADA has been determined by kit method using adenosine as a substrate, and the results has been read by an enzymatic spectrophotometer method ***(Shalini***

et al., 2015). Test analyzed on full automatic analyzer BIOSYSTEM (BA200,SPAIN) and BECKMAN COULTER (AU480,USA).

Principle:

The ADA assay relies on the process where adenosine is enzymatically deaminated to form inosine. This inosine is the n transformed into hypoxanthine by the enzyme purine nucleoside phosphorylase (PNP). Following this, hypoxanthine is converted into uric acid and hydrogen peroxide (H2O2) through the action of xanthine oxidase (XOD). The generated H2O2 then reacts with N-Ethyl-N-(2-hydroxy-3-sulphopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to produce a quinone dye, which is tracked over time. Below is the complete sch eme of the enzymatic reactions involved:

Reaction:

Adenosine + pi ADA → Inosine + NH3

Inosine + Pi PNP → Hypoxanthine + Ribose 1-phosphate

Hypoxanthine + 2H2O + 2O2 → PNP Uric acid + 2H2O2

2H2O2 + 4-AA + EHSPT → POD 4H2O + Quinone dye (max 556nm)

One unit of ADA is defined as the amount of ADA that generates one µ mole of inosine from adenosine per min at 37ºC

Reagent composition:

R1	Tris-HCl pH 8,0 4-AA PNP XOD Peroxidase	50 mM 2 mM 0,1 U/mL 0,2 U/mL 0,6 U/mL
R2	Tris-HCl pH 4,0 Adenosine EHSPT	50mM 10 mM 2 mM
ADA CAL	Ref. 1002230	

Data Analysis:

➢ Statistical analysis was carried out by SPSS version-22.

➢ The results were expressed in the mean ± SD.

➢ A 95% confidence interval was used and p-value less than 0.05 were considered statistically significant.

➢ The comparison was done using one way ANOVA and T. Test.

➢ Microsoft word and excel have been used to generate tables and graphs.

Results:

Table:-1 Describes the Gender ,Age and Education level of the sample .

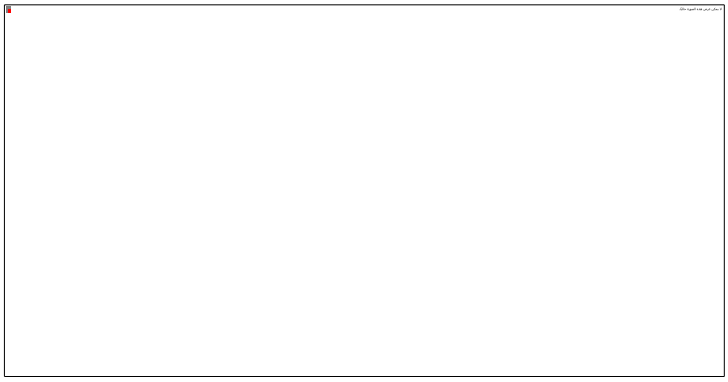
In the present study, 102 patients were recruited 19 males (18.6%) with mean(SD) of age 37.9 (14.5)) and (83 females (81.4%)) with mean(SD) of age 43.7 (15.9)) . A majority of the sample are female (81.4%).

Group	Male	Female	Total
NO. (%)	19 (18.6)	83 (81.4)	102 (100)
Age (year) mean (SD)	37.9 (14.5)	43.7 (15.9)	
Age group NO (%):			
< 30	5 (4.9)	18 (17.6)	23 (22.5)
30-39	7 (6.9)	12 (11.8)	19 (18.6)

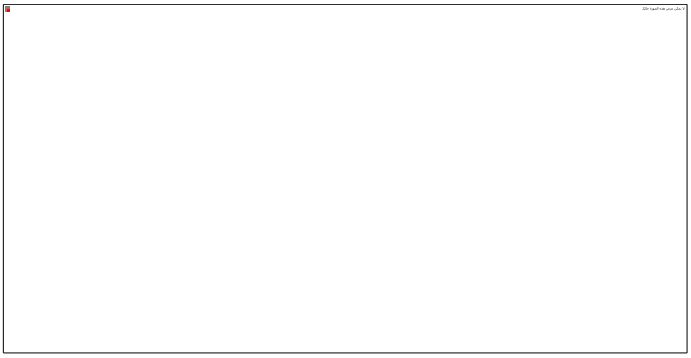
40-49	3 (2.9)	17 (16.7)	20 (19.6)
50-59	2 (2.0)	17 (16.7)	19 (18.6)
> 59	2 (2.0)	19 (18.6)	21 (20.6)
Level of Education NO (%):			
Illiterate	11(10.7)	52(50.9)	63(61.8)
Preparatory	0(0.0)	5(4.9)	5(4.9)
Secondary	2(1.96)	8(7.84)	10(9.8)
Academic	6(5.8)	18(17.6)	24(23.5)

The most common age group is lowest 30 years were recruited 24(23.5%).whereas the lowest common age group is from 30-39 were comprise 18 (17.6%), From 40-49 years comprise 20(19.6%), From 50-59 years comprise 19(19.6%) and Highest 59 years 21(20.6%) ,with a mean (20.4) .

The level of education were distributed into four groups with a mean (25.5) .the most common group is Illiterate were recruited 63 patients (61.8%) but the lowest common group is Preparatory comprise 5(4.9%) , Secondary group comprise 10(9.8%) and Academic group comprise 24(23.5%).



Graph: - 1 Gender distribution of (19 males (18.6%)) and (83 females (81.4%)) with a mean age (20.4 years).



Graph: - 2 distribution group of age The most common age group is lowest 30 years were recruited 24(23.5%).whereas the lowest common age group is from 30-39 were comprise 18 (17.6%), From 40-49 years comprise 20(19.6%), From 50-59 years comprise 19(19.6%) and Highest 59 years 21(20.6%) ,with a mean (20.4) .

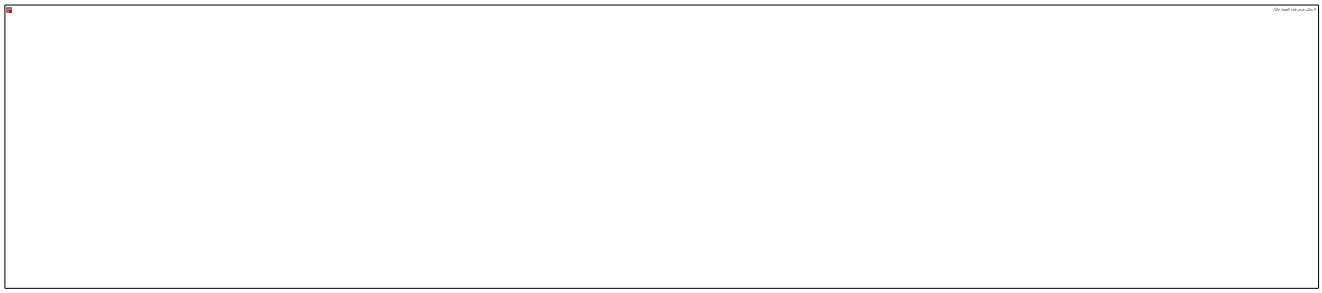
Graph: - 3 distribution of level education were distributed as the most common group is Illiterate were recruited 63 patients (61.8%) but the lowest common group is Preparatory comprise 5(4.9%) , Secondary group comprise 10(9.8%) and Academic group comprise 24(23.5%).

Table (2) Statistical analysis to Comparison between RA Patients with Healthy control .

	Groups	N	Mean ± SD	p.v
ADA	RA Patients	102	29.20±16.85	
	Control Healthy	40	13.44±3.52	0.002

* Significant at P ≤ 0.05, **P-value:** Probability value, N: Number, SD: Stander division, ADA: adenosine deaminase

From this table show that there is a significant difference (0.002) in the measured parameter between RA patients and healthy controls, with RA patients showing higher values. Also The mean value and The standard deviation for the RA patients (29.20±16.85) which is relatively high compared to the control group's (13.44±3.52). The data suggest that there is a significant difference in the measured parameter between RA patients and healthy controls**.**



Graph: - 4 The graph is described as a comparison between Rheumatoid Arthritis Patients and Healthy control.

Table: (3) **Effect of Age on ADA level** in rheumatoid arthritis.

	Age	N	Mean ± SD	P.V
ADA	Lowest 30	24	26.09±11.04	0.774
	From 30 To 39	18	27.60±11.67	0.774
	From 40 To 49	20	29.04±11.89	0.562
	From 50 To 59	19	37.05±31.08	0.036
	Highest 59	21	27.18±9.90	0.829

* Significant at P ≤ 0.05, **P-value:** Probability value, N: Number, SD: Stander division, ADA: adenosine deaminase

From this table show The analysis suggests that age impacts the measured parameter, particularly noticeable in the 50 to 59 age group, which shows both the highest mean and a significant p-value. The variability indicated by the high standard deviation in this group also highlights the diverse responses among individuals. The lack of significance in the other age groups suggests that the effect of age

may become more pronounced as individuals reach middle age. Further investigation could explore the reasons behind this trend and the implications for treatment or intervention strategies

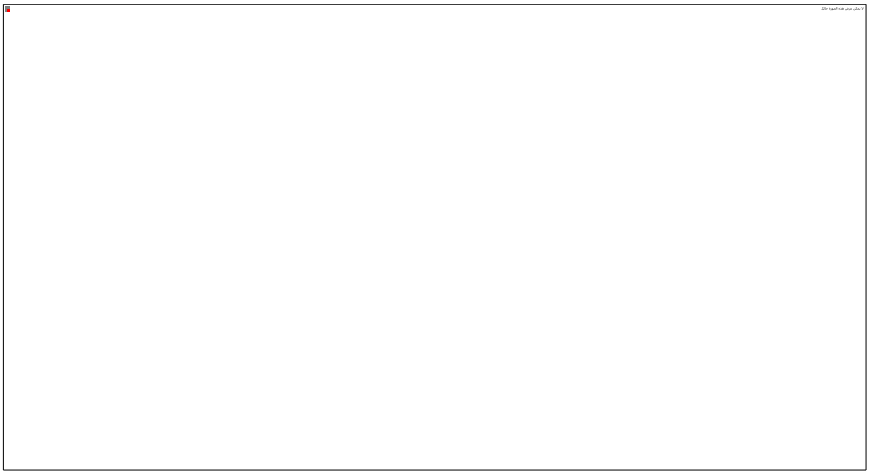
.

Graph: - 5 effect of age on ADA level in RA

Table: (4) **Effect of gender on ADA level** in rheumatoid arthritis.

	Sex	N	Mean ± SD	P.V
ADA	Male	19	29.00±13.18	0.880
	Female	83	29.07±17.73	

From this table shows- There were no significant differences in ADA activity between males and females (P = 0.880).



Graph: - 6 Effect of gender on ADA level in RA

Discussion

This study aimed to evaluate the adenosine deaminase (ADA) levels in rheumatoid arthritis (RA) patients and the demographic characteristics and compare them with healthy controls. A total of 102 patients were included in the study, with a predominance of females (81.4%). The mean age of male participants was 37.9 years (SD = 14.5), while the mean age of female participants was 43.7 years (SD = 15.9), suggesting a slightly older age group among females. The sample was stratified into different age groups, with the most common age group being patients aged over 59 years (20.6% of the total sample), while the least common group consisted of patients aged 30–39 years (17.6%).

Participants in the study exhibited a wide range of educational backgrounds, with a significant portion being illiterate (61.8%). This finding suggests a possible connection between education levels and the prevalence of rheumatoid arthritis (RA) within the sample. The percentage of illiterate individuals is

considerably higher than those with other educational qualifications: preparatory (4.9%), secondary (9.8%), and academic (23.5%). These results indicate that socio-economic factors, particularly education, may influence the health outcomes seen in this population. Nonetheless, additional research is necessary to fully explore these relationships.

In our study, we observed significant differences in ADA levels between patients with rheumatoid arthritis (RA) and healthy controls. RA patients had notably higher ADA levels (mean ± SD: 29.20 ± 16.85) compared to the control group (mean ± SD: 13.44 ± 3.52), with a p-value of 0.002. These findings align with previous research indicating that ADA activity could serve as a valuable biomarker for RA, reflecting the inflammatory processes linked to the condition. The elevated ADA levels in RA patients may suggest increased immune system activity and could be useful for diagnostic or prognostic applications.

The impact of age on ADA levels in patients with rheumatoid arthritis (RA) was examined. While most age groups—specifically those aged 30–39, 40–49, and over 59—showed no significant differences, a notable increase in ADA levels was observed in patients aged 50–59 years ($p = 0.036$). This finding suggests that ADA levels may rise with age in RA patients, possibly indicating that older individuals experience more intense or prolonged inflammatory responses. However, the data for patients under 30 and those over 59 did not show significant results, underscoring the complexity of the relationship between age and ADA levels in RA, which may warrant further investigation.

One limitation of this study is the absence of detailed information about the duration of the disease, medication use, and severity of rheumatoid arthritis (RA) in the patients, all of which could have affected ADA levels. Additionally, the relatively small sample size, especially the number of healthy controls (40), may restrict the generalizability of the findings. Future research with larger sample sizes and more thorough data on disease characteristics would be beneficial for validating these results.

Adenosine deaminase (ADA) is recognized as a valuable marker for diagnosing, predicting outcomes, and monitoring treatment in rheumatoid arthritis(Al-Rubaye et al., 2016). While the precise reason for the rise in ADA levels remains unclear, it may be attributed to the release of the enzyme from damaged cells and heightened cellular proliferation in rheumatoid arthritis (RA)(Atta et al., 2024). ADA facilitates the irreversible breakdown of adenosine into inosine. Research indicates that adenosine serves as a powerful natural anti-inflammatory agent(Kutryb-Zajac et al., 2020). and therefore is expected to influence the inflammatory processes. Consequently, measuring serum adenosine levels is a suitable method for evaluating disease activity in rheumatoid arthritis (RA). (Chimenti et al., 2015) the enzyme ADA serves as a regulatory checkpoint for extracellular adenosine levels(Zhulai et al., 2022). ADA appears to serve as a predictive indicator of the inflammatory process in rheumatoid arthritis(RA) (Wijbrandts & Tak, 2017)**.** ADA has been proposed as a useful biochemical marker for the inflammatory process in patients with rheumatoid arthritis (RA)(Makhe et al., 2024).

Conclusion

This study demonstrates that ADA levels are significantly higher in patients with rheumatoid arthritis (RA) than in healthy individuals, indicating that ADA may be a potential biomarker for RA. Notably, the effect of age on ADA levels is particularly pronounced in the 50–59 age group, highlighting the need for further research to understand how age

influences inflammatory biomarkers in RA. Additionally, the socio-demographic characteristics of the sample, such as education and gender, offer valuable insights into the RA-affected population. Further investigation is needed to assess the impact of education, socio-economic factors, and other biomarkers on the diagnosis and management of RA.

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