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The Impact of Lifestyle Factors and Biomarkers on Patients with Autoimmune Disorders

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Abstract: Rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are chronic autoimmune diseases shaped by genetic predisposition and modifiable lifestyle factors. Anti-cyclic citrullinated peptide (anti-CCP) antibodies are central to RA diagnosis, but their relevance in AS remains unclear. This study investigates the impact of BMI, stress, and other lifestyle variables on anti-CCP levels in a Georgian cohort, highlighting potential mechanisms of stress-induced citrullination in AS. A cross-sectional study was conducted involving 219 Georgian participants diagnosed with RA or AS. Anti-CCP levels were analyzed against BMI, smoking status, physical activity, perceived stress, age, and sex. Statistical significance was assessed using correlation and regression analyses to evaluate lifestyle modulators of systemic inflammation and autoantibody expression. Overweight or obesity (BMI ≥ 25 kg/m²) was prevalent in 71.5% of RA patients ($p = 0.0001$) and 66.2% of AS patients ($p = 0.0497$). Most participants were non-smokers (85%, $p = 0.0001$). Physical activity showed no correlation with anti-CCP levels ($p = 0.6415$). RA patients were predominantly female (96.7%), with anti-CCP levels increasing with age ($p = 0.0001$). In AS, elevated anti-CCP levels were rare and primarily observed in younger females aged 35–44 reporting high stress ($p = 0.0075$). Perceived stress was significantly associated with increased anti-CCP levels in both RA ($p = 0.0001$) and AS ($p = 0.0034$). Anti-CCP antibodies remain diagnostically specific for RA but may be elevated in AS under stress. These findings underscore the interplay between metabolic and psychosocial factors in autoimmune disease and support lifestyle assessment in personalized care.

Keywords: Autoimmune diseases, Rheumatoid arthritis, Ankylosing spondylitis, Lifestyle

Introduction

Autoimmune diseases affect an estimated 7–9% of the global population and represent a growing public health concern due to their chronic nature, diagnostic complexity, and long-term impact on morbidity and disability (Miller, 2023). Among these, rheumatoid arthritis (RA) and ankylosing spondylitis (AS) are prototypical autoimmune disorders where genetic predisposition interacts with lifestyle and environmental factors to shape disease onset and progression. The rising prevalence of these conditions, coupled with diagnostic delays and treatment challenges, underscores the urgent need for improved biomarkers and personalized management strategies (Rosenbaum et al., 2019; Tański et al., 2022). RA affects approximately 0.5–1% of the global population, with increasing prevalence projected through 2050 (Black et al., 2023), while AS affects an estimated 0.1–1.4%, depending on genetic background and HLA-B27 distribution (Dean et al., 2014). Early and accurate

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diagnosis remains particularly challenging in AS due to limited serological markers, necessitating investigation of potential contributors such as stress-mediated autoantibody production.

The pathogenesis of RA and AS involves a complex interplay of immunological mechanisms and environmental factors. Central and peripheral tolerance mechanisms, including thymic deletion and regulatory T cell function, normally eliminate autoreactive lymphocytes. However, genetic risk alleles, environmental triggers, and modifiable lifestyle factors, including infections or smoking-induced activation of peptidyl arginine deiminase (PAD) enzymes, can disrupt these safeguards, allowing autoreactive clones to persist and initiate inflammation (Curran et al., 2020; Chauhan, et al., 2023). Molecular mimicry further contributes to disease onset, as microbial epitopes resembling joint or enthesal proteins can cross-activate autoreactive lymphocytes, amplifying immune responses through bystander activation and epitope spreading (He et al., 2024; Klareskog et al., 2013).

Under inflammatory conditions, Peptidyl arginine deiminase type 2 (PAD2) and Peptidyl arginine deiminase type 4 (PAD4), which are most strongly implicated in RA at both genetic and cellular levels (Curran et al., 2020), catalyze citrullination of proteins such as vimentin and fibrinogen, generating neoepitopes that provoke B cell production of autoantibodies including rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) (del Val del Amo et al., 2006; Erdő-Bonyár et al., 2024). These autoantibodies contribute directly to joint pathology via immune complex formation, complement activation, and synovial tissue inflammation. Emerging evidence suggests that stress-induced PAD activation may transiently elevate anti-CCP even in AS, indicating a broader role for citrullination beyond classical RA (Autoantibodies in Autoimmune Disorders, n.d.).

In addition to immunological mechanisms, modifiable lifestyle factors play a critical role in disease expression. Smoking, elevated body mass index (BMI), low physical activity, and psychological stress have all been linked to increased risk and severity of RA and AS (Conrad et al., 2023; Schäfer & Keyßer, 2022). Stress, in particular, may act through psychoneuroimmunological pathways to enhance PAD activity and autoantibody generation, representing a novel modifiable factor influencing systemic inflammation. Poor dietary quality, early-life malnutrition, and occupational strain may exacerbate inflammation and bone remodeling in AS, while smoking and socio-economic disadvantage can compound disease burden. Recent studies suggest that certain occupations, such as agricultural labor, construction work, mining, and manufacturing may increase the risk of RA and AS due to prolonged physical strain, repetitive joint stress, and exposure to environmental toxins (Work Limitations and Associated Factors in Rheumatoid Arthritis, Axial Spondyloarthritis, Osteoarthritis and Fibromyalgia - PubMed, n.d.), (Occupational Inhalable Agents Constitute Major Risk Factors for Rheumatoid Arthritis, Particularly in the Context of Genetic Predisposition and Smoking | Annals of the Rheumatic Diseases, n.d.) However, the influence of these lifestyle factors on serological markers such as anti-CCP antibodies remains unexplored, particularly in AS, where they are often treated as confounders rather than contributors.

Demographic disparities also shape disease prevalence and outcomes. RA predominantly affects older adults, with higher rates observed in women (87.8 per 100,000) compared to men (22.5 per 100,000), and regional variation ranging from 0.46% in Australasia to approximately 0.16% in East and Southeast Asia (Corrado et al., 2025; Versini et al., 2014). AS typically begins before age 45 and disproportionately affects working-age populations, with prevalence estimates ranging from 0.9–1.4% in the U.S. and 1.3–4.98 million cases across Europe and Asia (Mathkhor et al., 2022; Voigt et al., 1994).

These lifestyle and demographic factors may also influence serological markers such as anti-CCP antibodies. Anti-CCP arises from PAD-mediated citrullination and is highly specific for RA, correlating with disease severity and prognosis (Yasmeen et al., 2024). However, low-level anti-CCP positivity has been occasionally observed in AS (2–4%), particularly in women or patients reporting high psychosocial stress, suggesting potential stress-mediated PAD activation or subclinical immune overlap (Ediz et al., 2011; Mease et al., 2022; Singh Sangha et al., 2018). Citrullinated neoepitopes and anti-CCP reactivity have been detected in sacroiliac and enthesal tissues, indicating local PAD activity and the potential for neoantigen formation within AS-affected joints. Anti-CCP testing may therefore help distinguish atypical AS presentations from early or seronegative RA, refine diagnostic accuracy, and guide treatment decisions. This highlights the dual role of anti-CCP as both a diagnostic biomarker and a mechanistic readout of systemic inflammatory processes. (Basic Science Department, Dentistry College, Mustansiriyah University, Baghdad -Iraq. et al., 2025; Pisetsky, 2023). Despite these insights, the significance of anti-CCP antibodies in AS remains poorly defined, particularly within Caucasian populations. While conventionally considered RA-specific, occasional detection of anti-CCP in AS underscores the potential influence of modifiable lifestyle factors, such as BMI and psychological stress, on citrullination pathways. To date, no studies have systematically examined anti-CCP levels in RA and AS patients from the Republic of Georgia, adding to the novelty and regional relevance of this research.

The aim of this study is to evaluate the relationship between anti-CCP antibody levels and life-style factors-including BMI, smoking, physical activity, stress, age, and sex-in RA and AS patients from a Georgian cohort. By characterizing these patterns, we aim to provide mechanistic and clinically relevant insights into the interplay between modifiable factors and autoimmune activity, informing personalized management strategies.

Method

This cross-sectional observational study evaluated the relationship between anti-cyclic citrullinated peptide (anti-CCP) antibody levels and demographic and modifiable lifestyle factors-including BMI, smoking status, physical activity, stress level, age, and sex-among patients with RA and AS in the Republic of Georgia. The study design aimed to capture real-world associations between lifestyle factors and serological markers at a single time point, providing mechanistic insights into autoimmune regulation.

Participants were assessed once between February 2023 and May 2024. A total of 219 participants were enrolled: 93 patients with rheumatoid arthritis (RA), 62 patients with ankylosing spondylitis (AS), and 64 healthy controls. Healthy controls were age- and sex-matched where feasible to minimize confounding. Participants were recruited via convenience sampling from two urban outpatient clinics-JSC Seamen's Medical Center 2010 and SoloMed LLC - selected based on patient volume and access to rheumatology services. Eligible patients were approached consecutively during routine visits. All participants were ethnically Georgian and aged 16–65+ years. RA and AS diagnoses were confirmed using the 2010 ACR/EULAR criteria for RA and ASAS criteria for spondyloarthritis. Exclusion criteria included pregnancy, current immunosuppressive therapy, recent infection (within 4 weeks), and other autoimmune or inflammatory conditions (e.g., SLE, psoriasis, psoriatic arthritis, PCOS) that could confound inflammatory markers.

All participants provided written informed consent, and the study was approved by the Institutional Ethics Committee of BAU International University Batumi (reference N-MED006). Participant confidentiality was maintained using unique study codes, and all data were stored in secure databases. Blood samples (2 mL) were collected into K₃-EDTA tubes, transported within one hour, centrifuged at 3000 rpm for 15 minutes, and stored at –20°C until analysis. Anti-CCP levels were measured using the Anti-CCP hs ELISA Kit (ORGENTEC Diagnostika GmbH, Germany) according to the manufacturer's protocol.

Demographic and lifestyle data-age, sex, BMI, smoking, physical activity, and perceived stress-were collected using standardized questionnaires adapted from validated tools. Physical activity was assessed via a four-point ordinal scale: no activity, rarely active, irregularly active, or regularly active. Stress was assessed using a four-point Likert item (high, average, low, unnoticed). While feasible for outpatient settings, these measures may be subject to recall bias.

The primary outcome variable was anti-CCP antibody level (U/mL); exposure variables included age, sex, BMI, smoking, physical activity, and stress. Descriptive statistics were expressed as means $\pm$ standard deviation (SD) or percentages. The one-way ANOVA test assessed group differences (RA, AS, controls). When significant ($p < 0.05$), Tukey's post hoc test was applied for pairwise comparisons. Normality and homogeneity were evaluated using Shapiro-Wilk and Levene's tests, respectively. All variables met assumptions for parametric testing (Shapiro-Wilk $p > 0.05$; Levene's $p > 0.05$).

Missing data (<5%) were handled via pair-wise deletion without imputation. Regression analysis was not performed due to sample size and exploratory scope. Potential biases included selection bias from convenience sampling and recall bias in self-reported lifestyle data. While findings may not generalize beyond the Georgian population, they provide insight into autoimmune disease expression in a unique regional context. Ethical considerations, standardized laboratory protocols, and structured lifestyle assessments ensured methodological rigor, supporting the validity of associations between anti-CCP levels and modifiable risk factors.

Results and Discussion

A total of 219 participants were included: 93 with rheumatoid arthritis (RA), 62 with ankylosing spondylitis (AS), and 64 healthy controls (HC). No participants were excluded or lost to follow-up. Key demographic and lifestyle characteristics are summarized in Table 1 and Figures 1–3.

Table 1. Demographic and lifestyle characteristics of study participants across diagnostic

Variable	RA (n = 93)	AS (n = 62)	Control (n = 64)	p-value*
Female (%)	96.7	48.4	61.0	<0.0001
Male (%)	3.3	51.6	39.0	
Age group (%)				
16–34	10.0	11.0	5.0	<0.0001
35–44	12.0	23.0	14.0	
45–54	24.0	31.0	25.0	
55–64	37.0	15.0	37.0	
≥65	17.0	15.0	19.0	
BMI category (%)				
Underweight	2.0	2.0	6.0	0.0003
Normal weight	29.0	25.0	42.0	
Overweight	32.0	36.0	27.0	
Obesity class I	22.0	27.0	17.0	
Obesity class II	11.0	7.0	5.0	
Obesity class III	4.0	3.0	3.0	
Smoking status (%)				
Smoker	17.0	24.0	30.0	<0.0001
Non-smoker	83.0	76.0	70.0	
Stress level (%)				
High	39	34	23	0.0025
Average as all	24	32	36	
Doesn't bother	16	20	14	
Unnoticed	21	14	27	
Physical activity (%)				
regularly	16	14	21	0.015
irregularly	59	46	34	
Rarely	19	21	37	
None	6	19	8	

Sex distribution differed significantly across groups ($p < 0.0001$), with RA patients predominantly female (96.7%), controls predominantly male (73.4%), and AS patients showing near-equal gen-der representation (Figure 1). BMI classification also varied significantly ($p = 0.0003$), with overweight and obesity more prevalent in RA and AS patients compared to controls (Figure 2). Other lifestyle variables such as smoking status ($p < 0.0001$), stress level ($p = 0.0025$), and physical activity ($p = 0.015$) showed group differences and were analyzed in relation to anti-CCP levels.

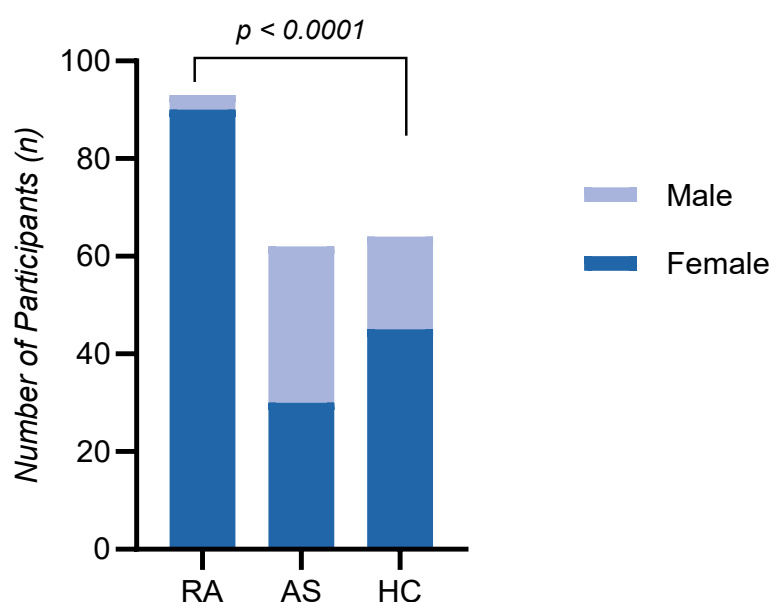


Figure 1. Distribution of RA, AS, and control groups based on sex

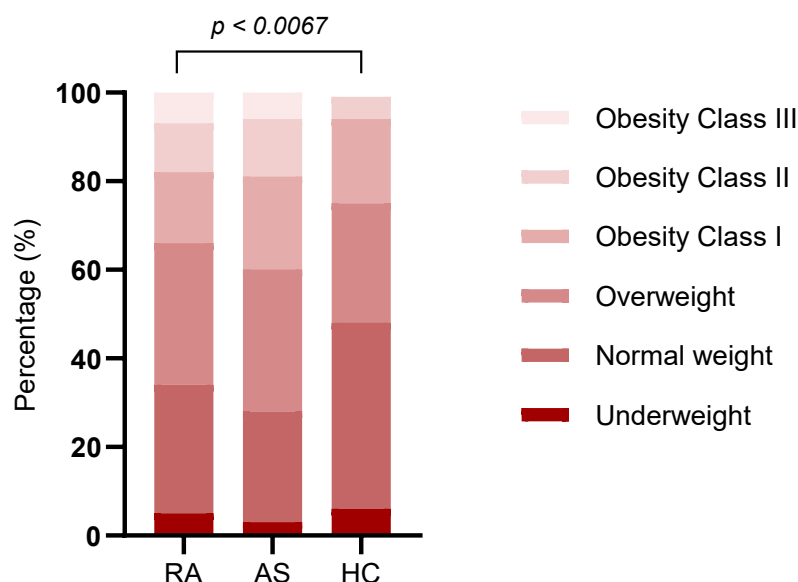


Figure 2. Distribution of RA, AS, and control groups based on BMI

Anti-CCP antibody levels were analyzed by age group and diagnosis to assess associations with demographic factors. In RA patients, anti-CCP levels increased with age, peaking in the 55–64 and ≥ 65 categories (ANOVA, $F = 9.34$, $p < 0.0001$), supporting the hypothesis that older age is associated with elevated autoantibody expression. In AS patients, elevated anti-CCP levels were observed primarily in females aged 35–44 (ANOVA, $F = 7.12$, $p = 0.0075$), suggesting a potential age- and sex-specific pattern despite anti-CCP not being a standard AS biomarker. These results are illustrated in Figure 3.

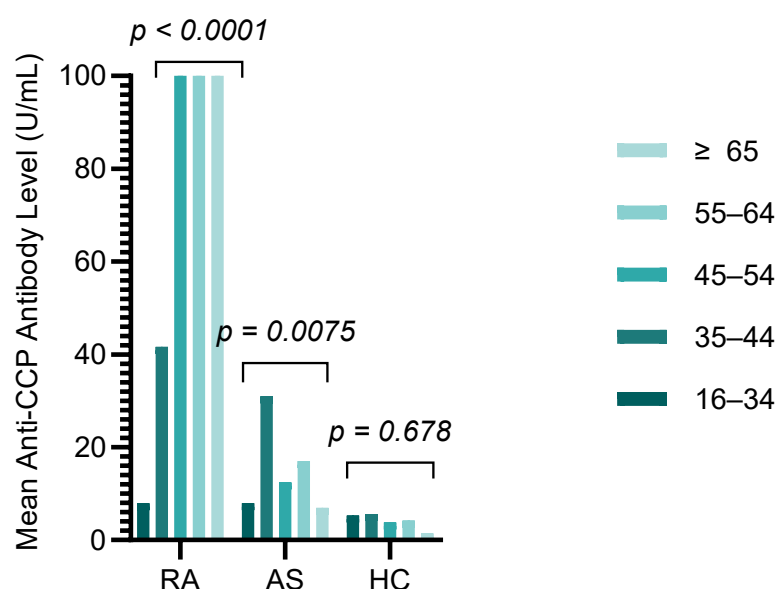


Figure 3. Anti-CCP antibody levels (U/mL) by age group across rheumatoid arthritis (RA), ankylosing spondylitis (AS), and healthy control (HC) cohorts.

Perceived stress was examined in relation to anti-CCP elevation. Among RA patients with elevated anti-CCP levels, 41% reported high stress, despite 81.7% of all RA patients having low CCP levels ($\chi^2 = 19.67$, $p < 0.0001$). In AS patients, 12% had elevated CCP levels, of whom 62.5% re-reported high stress ($\chi^2 = 8.52$, $p = 0.0034$). These findings indicate that psychological stress may act as a modifiable enhancer of citrullination in both RA and AS. Other lifestyle factors showed limited associations. Physical activity did not correlate with anti-CCP levels in either group ($p = 0.6415$), and smoking prevalence was low across cohorts as 85% were reported non-smokers. Overall, the results identify key demographic and modifiable factors-particularly age, sex, BMI, and perceived stress-that influence anti-CCP antibody levels. These associations provide a foundation for exploring behavioral and immunological interactions in autoimmune disease expression.

Conclusion

This study demonstrates that anti-cyclic citrullinated peptide (anti-CCP) antibodies remain a robust serological marker for rheumatoid arthritis (RA), with levels increasing particularly in older women and in patients with elevated BMI and high perceived stress. These findings highlight the significant interplay between metabolic and psychosocial factors in shaping systemic inflammation and autoantibody production. In ankylosing spondylitis (AS), anti-CCP elevation was rare and primarily observed in younger females reporting high stress, suggesting that psychological strain may act as a nonspecific enhancer of citrullination even outside classical RA pathology. This observation challenges the traditional view of anti-CCP as an RA-specific marker and underscores the potential role of stress-induced activation of peptidylarginine deiminase (PAD) enzymes in transient antibody production. Although smoking prevalence was low and physical activity did not directly correlate with anti-CCP levels in this cohort, these lifestyle factors remain clinically relevant as modulators of systemic inflammation and overall disease activity. The results emphasize the importance of assessing and addressing modifiable lifestyle determinants, particularly obesity and psychological stress, in personalized rheumatologic care. Future longitudinal and mechanistic studies are warranted to elucidate PAD regulation under stress, characterize tissue-specific citrullinated proteomes in AS, and evaluate interventions targeting obesity, psychosocial strain, and lifestyle factors to mitigate autoimmune disease progression. Collectively, these findings support integrating metabolic and psychosocial assessment into clinical management, enhancing both diagnostic precision and individualized therapeutic strategies for RA and AS.

Scientific Ethics Declaration

* The authors declare that the scientific ethical and legal responsibility of this article published in EPHELS journal belongs to the authors.

*All participants provided written informed consent, and the study was approved by the Institutional Ethics Committee of BAU International University Batumi (reference N-MED006). The authors declare that all procedures performed in this study were in accordance with the ethical standards.

Conflict of Interest

* The authors declare that they have no conflicts of interest

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