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Presence of Anti-Nuclear and Anti-Double-Stranded DNA Antibodies among Healthy Individuals in the Twin Cities (Rawalpindi and Islamabad) of Pakistan

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Abstract. Background: The study aimed to investigate antinuclear antibodies (ANA) and anti-double-stranded DNA antibodies among healthy individuals in Rawalpindi and Islamabad, emphasizing the need to understand their prevalence in the general population.
Objective: To determine ANA and anti-dsDNA levels, assessing their potential as disease biomarkers in the absence of clinical symptoms among healthy individuals.
Methods: A total of 659 individuals participated in the study, including 503 females (76.3%) and 156 males (23.5%). Blood samples were collected using convenient sampling technique followed by separation of serum using centrifugation. The levels of ANA and anti-dsDNA were quantified using non-competitive ELISA.
Results: The results of the study showed that only 0.91% of individuals tested positive for ANA and 0.46% for anti-dsDNA. This indicates a minimal presence of these antibodies in the healthy cohort. Furthermore, no significant association was found between ANA and anti-dsDNA.
Conclusion: This study highlights a low occurrence of ANA and anti-dsDNA among healthy subjects, suggesting their potential exclusive association with autoimmune diseases.

Keywords: anti-nuclear, anti-double-stranded, DNA, antibodies, autoimmunity

Introduction

Immunity serves as a vital defense mechanism within the human body, providing protection against various pathogens. However, autoimmunity occurs when the immune system mistakenly produces autoantibodies that target endogenous antigens, causing the body to attack its own tissues. The immune system is a highly intricate and collaborative network of cells, tissues and molecular components that work together to combat infections and cancers (Abbas *et al*., 2019). Autoimmune diseases are caused by the immune system attacking the body's own cells and tissues, triggered by self-antigens. These diseases affect approximately 1-2% of the population and are influenced by both genetic susceptibility and environmental factors. These factors can alter the body's self-tolerance and activate self-reactive lymphocytes, which contribute to the development of autoimmunity (Mir *et al*., 2022; Abbas *et al*., 2019).

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Autoimmune disorders and autoantibodies are increasingly becoming significant concerns in the field of medicine (Khan *et al*., 2018). Autoimmune disorders are most commonly observed in adults and tend to become more prevalent with age. In instances of rheumatoid arthritis, the immune system's ability to repair itself and regulate apoptosis is significantly impacted. Additionally, mutations in the p53 gene are known to play a role in the development of bowel diseases, which can result in tissue damage, inflammation and dysfunction of organs (Kumar *et al*., 2018). The immune system features several mechanisms that prevent it from mounting immune responses against self-antigens. These mechanisms are crucial in enabling the immune system to distinguish between self and nonself (typically microbial) antigens. When these mechanisms fail, the resulting immune responses are known as autoimmunity, and the diseases that arise from them are referred to as autoimmune disorders (Abbas *et al*., 2019).

The antinuclear antibody (ANA) holds significant relevance in the context of autoimmunity, as it targets

a variety of antigens. In autoimmune diseases, elevated levels of ANA are commonly detected and they are also produced in healthy individuals (Agmon-Levin *et al*., 2010; Solomon *et al*., 2002). The use of antinuclear antibodies (ANA) is a common diagnostic tool in systemic autoimmune conditions. Additionally, chronic infectious diseases can also produce ANA, which are referred to as "auto antibodies to cellular antigens." The term ANA is the most accurate and is associated with various autoimmune diseases such as systemic lupus erythematosus (SLE), idiopathic inflammatory myopathies and systemic sclerosis (Haris *et al*., 2024; Solomon *et al*., 2002). Autoantibodies are prevalent in approximately one-fifth of the general population, yet only a small fraction of individuals develop autoimmune diseases. This suggests that a substantial portion of the population harbors detectable levels of autoantibodies in addition to exhibiting clinical signs (Parks *et al*., 2014; Satoh *et al*., 2012).

In patients with systemic autoimmune diseases, such as systemic lupus erythematosus (SLE), autoantibodies are often present for several years before the onset of clinical symptoms. Previously, the progression to clinical SLE was thought to be associated with profiles of autoantibodies combined with female sex but other risk factors, including age, antinuclear antibody (ANA) titer, number of autoantibody specificities and type I interferon (IFN) signature, do not clearly distinguish ANA-positive healthy individuals who eventually develop autoimmune disease (Slight-Webb *et al*., 2016; Li *et al*., 2011).

In a study researchers focuses on individuals who test positive for antinuclear antibodies (ANA) but appear to be in good health, with the aim of demonstrating that although ANA positivity is often harmless, a subset of these individuals may be at risk for developing autoimmune diseases. The study found that higher levels of ANA were present in female participants across lupus, rheumatoid arthritis and healthy controls and were unrelated to age or levels of immunoglobulin G (IgG). Additionally, elevated levels of skin-specific autoantibodies in ANA-positive healthy controls suggest that there may be an early disruption in tolerance. The researchers also conducted gene expression analysis, which identified upregulated genes involved in autoimmune disease development, including a celiac disease autoantigen and Type I interferon components. This study provides valuable insights into the potential pathways that lead to autoimmune diseases (Agmon-Levin *et al*., 2013; Li *et al*., 2011).

Anti-ds DNA antibodies are among the most thoroughly investigated of the auto antibodies associated with lupus. It is widely accepted that these antibodies play a particularly significant role in systemic lupus erythematosus. Despite this, they are not the most abundant auto antibodies in the diagnosis of SLE and their clinical relevance is limited. Furthermore, they have been found to have minimal predictive value for disease flares (Fu *et al*., 2015). The production of an anti-dsDNA antibody in vitro within a natural setting would likely involve both the dicer and indigenous (*i.e*. manipulated) DNA as targets. Consequently, examining anti-dsDNA antibodies with purified dsDNA as the target antigen represents a synthetic, analytical approach by definition (Agmon-Levin *et al*., 2014). The utilization of Anti-dsDNA autoantibodies in the diagnostic process for Systemic Lupus Erythematosus (SLE) remains a significant and essential component. These autoantibodies serve as a classification criterion for SLE (Bentow *et al*., 2016; Petri *et al*., 2012). The standardization of anti-ds DNS antibodies is still limited and various methods have different results (Enocsson *et al*., 2015; Venner *et al*., 2013; Antico *et al*., 2010).

Previous research study aimed to examine the clinical and pathogenic roles of antinuclear antibodies (ANA) in systemic lupus erythematosus (SLE) patients, focusing on 495 individuals. Using a comparative cross-sectional approach based on the EULAR/ACR 2019 criteria, the researchers analyzed the relationship between ANA patterns and titers, complement levels and immune markers. It was revealed that the most prevalent ANA patterns were speckled (52.1%) and homogeneous (35.2%). Interestingly, the peripheral pattern was found to have the most pathogenic immune profile, which was associated with elevated levels of anti-dsDNA, reduced C4 levels and increased levels of anticardiolipin antibodies and beta-2 Glycoprotein 1 antibodies. These findings suggest that specific ANA patterns may have potential prognostic value in predicting the clinical implications of SLE (Al-Mughales, 2022).

In a recent epidemiological survey, scientists investigated the prevalence of antinuclear antibodies (ANAs) in a large, population-based cohort as part of a health checkup. The study aimed to explore potential health implications and the results showed an ANA positive rate of 7.09%. Interestingly, females demonstrated a higher positive rate (10.2%) than males (4.6%). Moreover, the ANA-positive population displayed a greater prevalence of metabolic abnormalities compared

to the control group. Additional analysis revealed a relationship between high ANA levels and inflammatory and immune dysfunction. These findings emphasize the significance of routine ANA testing in healthy individuals and suggest further examination of those with clinical symptoms to determine the specific ANA subtype in their serum and avoid potential misdiagnosis (Ge *et al*., 2022).

This study addresses a significant research gap by investigating the levels of antinuclear antibodies (ANA) and anti-double-stranded DNA antibodies in healthy individuals from Rawalpindi and Islamabad, Pakistan. The scarcity of data on these antibodies in the Pakistani population hampers our understanding of regional autoimmune patterns. By shedding light on the prevalence of ANA and anti-dsDNA in this specific geographic context, the research contributes valuable insights to the global understanding of autoimmune phenomena.

Materials and Methods

Study design. A formal descriptive study was undertaken at University of Lahore and excel labs Islamabad over a period of 10 months, from January 2019 to October 2019, after obtaining approval from the institutional ethical review committee. The research utilized a convenient sampling technique to obtain blood samples from participants who were healthy and willing to participate. A total of 659 individuals from Rawalpindi and Islamabad were included in the study to evaluate the levels of ANA and Anti dsDNA in healthy individuals. Prior to testing, written informed consent was obtained from each participant and those with any autoimmune diseases were excluded from the study. The levels of anti-nuclear antibodies (ANA) and anti-double-stranded DNA antibodies were determined using the enzyme-linked immunosorbent assay (Cal Biotech, USA).

Determination of serum autoantibodies levels. The determination of serum autoantibodies levels was carried out using a convenient sampling technique. Blood samples were collected from healthy individuals to assess the prevalence of ANA and anti-ds DNA. The samples were collected in gel-clotted vials and serum was separated from the blood samples by centrifugation at 7000 rpm for 5 min. The individual serum was then added to antigens coated wells and if present an Ag-bound ANA IgG or Anti-ds DNA specific antibody would bind to the antigen. The wells were washed to remove any other materials and the enzyme conjugate was applied to bind the antibody-antigen complex. The extra enzyme conjugate was then washed off and substrate was added. The ELISA plate was then incubated to allow the enzyme to hydrolyze the substrate and the concentration of the colour produced was measured by a spectrophotometer at a wavelength of 490nm, which was proportionate to the quantity of IgG-specific antibody in the sample. Maglumi ANA and anti-dsDNA kits manufactured by Snibe are employed for enzyme-linked immunosorbent assay (ELISA) procedures.

Statistical analysis. SPSS version 25.0 was utilized (IBM-SPSS, Inc, Armonk, New York) to analyze the data obtained from patients who were recruited for the study. The data was subjected to descriptive and inferential statistical analysis. The chi-square test, a common test for categorical variables, was employed to determine the p-value. A p-value less than 0.05 was considered to be statistically significant.

Results and Discussion

The study comprised 659 individuals, of whom 503 were female and 156 were male. Participants were grouped based on their age into four categories ranging from 1 to 80 years old, with 76.3% females and 23.7% males. The autoantibody level was higher in females than males. The participants were divided into groups 1 through 4, with group 1 consisting of individuals aged between 1 and 20 years, group 2 including those aged between 21 and 40 years, group 3 comprising individuals aged between 41 and 60 years and group 4 consisting of those aged between 61 and 80 years. There were 97 individuals in group 1, 318 in group 2, 194 in group 3 and 50 in group 4 (Fig. 1). The study found that only 6 (0.91%) individuals were positive for ANA out of the 659 participants, with 1 (0.15%) being male and the rest being female (Fig. 2). Additionally, only 3 (0.46%) individuals tested positive for anti-ds DNA, with 2 (0.30%) being female and 1 (0.15%) being male (Fig. 3).

Identification of ANA positive and negative results according to gender and age groups. The distribution of ANA results is presented in Table 1, categorized by age group and gender. In the negative category, the majority of participants across all age groups and genders tested ANA-negative. Notably, in the 1-20 age group,

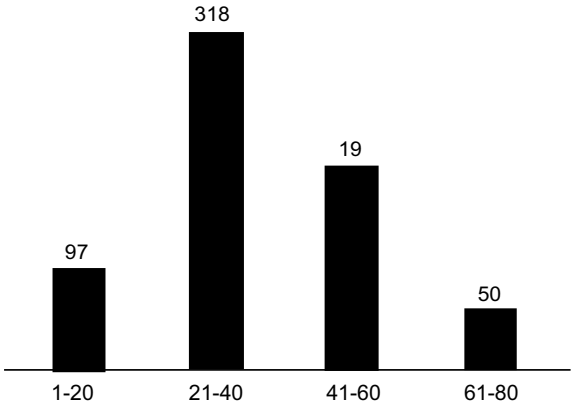

Fig. 1. The participation of total number of individuals on their age groups.

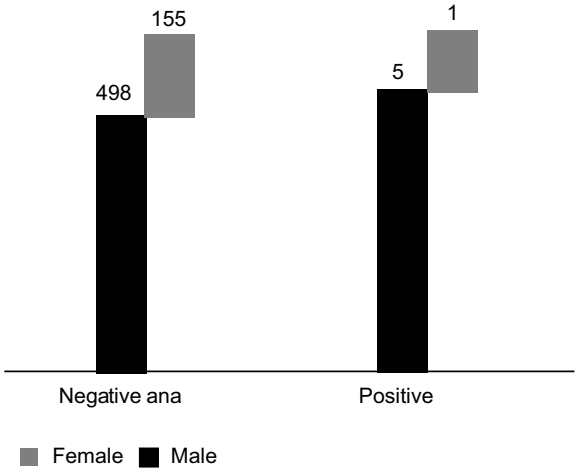

Fig. 2. ANA positive and negative result according gender wise in the all participants.

72 females and 25 males were ANA-negative, contributing to a total of 97. Similar patterns were observed in the 21-40, 41-60 and 61-80 age groups. A chi-square test was performed to evaluate the statistical significance of the findings, resulting in a p-value of 0.786902, suggesting that there was no statistical significance present.

On the other hand, the positive category includes individuals who tested ANA-positive. Across all age groups, there were a total of 6 ANA-positive cases. In the 21-40 age group, 3 females and 1 male tested positive, while in the 41-60 and 61-80 age groups, 1 female each tested positive. The total ANA-positive cases were 5 females and 1 male, contributing to a total of 6. A p-value of 0.062882 was also obtained for

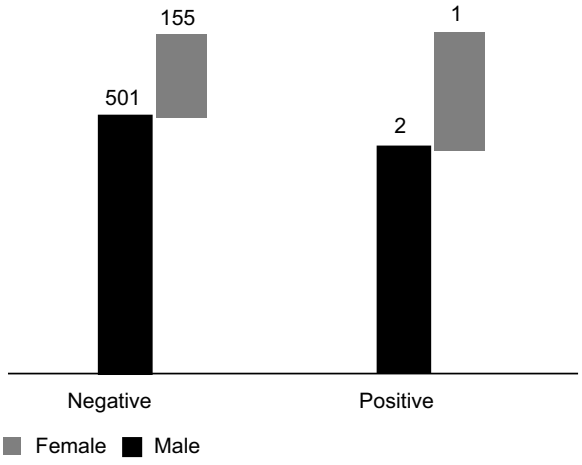

Fig. 3. Anti ds DNA positive and negative result according gender wise in all participants.

positive results, indicating non-significance. These findings shed light on the prevalence of ANA positivity across different age groups and genders.

Identification of anti Ds DNA positive and negative results according to gender and age. Table 2 presents the distribution of anti-Ds DNA results across various age groups and genders, offering valuable insights into the prevalence of this autoantibody. In the negative category, the majority of participants in each age group and gender tested anti-Ds DNA-negative. For example, in the 1-20 age group, 72 females and 25 males had negative results, totaling 97 individuals (P-Value: 0.761003). Similar trends were observed in the 21-40, 41-60 and 61-80 age groups, with varying numbers of negative cases. A chi-square (χ^2) test revealed p-values of 0.761003 for negative results, indicating no statistical significance.

Shifting to the positive category, Table 2 highlights the number of individuals testing anti-Ds DNA-positive in each subgroup. In the 21-40 age group, 1 female and 1 male tested positive, contributing to a total of 2 positive cases (P-Value: 0.058328). In the 41-60 age group, 1 female tested positive. Notably, no positive cases were found in the 1-20 and 61-80 age groups. Overall, there were 2 females and 1 male in the anti-Ds DNA-positive category, resulting in a total of 3 positive cases. A chi-square (χ^2) test revealed p-values 0.058328 for positive results, indicating no statistical significance. This breakdown provides a comprehensive view of the distribution of anti-Ds DNA results within specific age

Table 1. Identification of ANA positive and negative results according to gender and age groups

ANA			Female	Male	Total	P-Value
Negative	Age	1-20	72	25	97	0.786902
	groups	21-40	238	76	314	
		41-60	148	45	193	
		61-80	40	9	49	
	Total		498	155	653	
Positive	age	1-20	0	0	0	0.062882
	groups	21-40	3	1	4	
		41-60	1	0	1	
		61-80	1	0	1	
	Total		5	1	6	
Total	age groups	1-20	72	25	97	
		21-40	241	77	318	
		41-60	149	45	194	
		61-80	41	9	50	
	Total		503	156	659	

Table 2. Identification of Anti Ds DNA positive and negative results according to gender and age

AntidsDNA			Female	Male	Total	P-Value
Negative	Age groups	1-20	72	25	97	0.761003
		21-40	240	76	316	
		41-60	148	45	193	
		61-80	41	9	50	
	Total		501	155	656	
Positive	age groups	1-20	0	0	0	0.058328
		21-40	1	1	2	
		41-60	1	0	1	
		61-80	0	0	0	
	Total	2	1	3		
Total	age groups	1-20	72	25	97	
		21-40	241	77	318	
		41-60	149	45	194	
		61-80	41	9	50	
	Total		503	156	659	

and gender subsets, aiding in understanding the prevalence of this autoantibody in different demographics.

Autoantibodies, produced by pathogenic B cells targeting one's own tissue, are recognized markers of autoimmune disorders like lupus and rheumatoid arthritis. These diseases, marked by chronic immune activation and inflammation affecting various body tissues, are more prevalent in women, influenced by hormonal factors (Xiao *et al*., 2021). Autoantibodies are a hallmark of both autoimmune disease and cancer, but they also occur in healthy individuals (Shome *et al*., 2022). The progression of autoimmune diseases, which are characterized by the presence of autoantigen-specific lymphocytes and autoantibodies, can vary and may ultimately result in clinical disease. A key area of ongoing research is the identification of individuals who test positive for autoantibodies and the development of strategies to monitor and prevent the progression of these individuals to inflammatory autoimmune conditions (Bieber *et al*., 2023).

ANA and dsDNA are essential indicators for the purpose of screening, diagnosing and evaluating the activity of

diseases in autoimmune disorders (Yadav *et al*., 2020). The existence of anti-nuclear antibodies (ANA) and anti-double-stranded DNA (anti-ds DNA) expression in the general population suggests that there may be widespread immunological disturbances, which could increase the risk of autoimmune disorders. It is important to note that ANA expression may be an indicator of this underlying vulnerability, which is analogous to the visible portion of an iceberg (Pisetsky *et al*., 2011).

The primary objective of this study was to assess the frequency of antinuclear antibodies (ANA) and anti-double-stranded DNA antibodies (Anti dsDNA) in healthy individuals residing in Rawalpindi and Islamabad. The findings revealed that out of the 659 participants, only a small fraction displayed positive results for ANA (0.91%) and anti dsDNA (0.46%). The low prevalence among healthy individuals highlights important implications for comprehending autoimmune markers in the absence of clinical symptoms. It is essential to consider the physiological role of the immune system as a sensory and analytical instrument that contributes to the maintenance and even formation of the multicellular organism by recognizing its own antigens. This normal function of auto-recognition is associated with the existence of physiological autoimmunity (Pashnina *et al*., 2021).

The current analysis of the distribution of positive and negative cases with respect to age and gender revealed that females exhibited a higher prevalence of autoantibodies than males. This finding aligns with existing literature, which suggests that females are more susceptible to autoimmune diseases than males. The higher prevalence of autoantibodies in females is linked to differences in circulating antibodies, which are potentially evolved for offspring protection. This increased susceptibility to autoimmune diseases in women, up to four times that of men, is a well-established phenomenon (Kronzer *et al*., 2021). The categorization into age groups also revealed variations in the prevalence of ANA and Anti dsDNA, with no significant statistical differences observed.

The distribution of ANA-positive cases was diverse across various age groups, with the 21-40 age bracket exhibiting the highest prevalence. However, the overall low incidence of positive cases, at 0.91%, coupled with the non-significant p-value, was observed. Previous research disclosed that ANAs targeting cell nucleus structures are detected in 7.09% of healthy individuals, with a higher prevalence in females (10.2%) than males (4.6%). Elevated ANA levels are associated with metabolic irregularities, inflammation and immune dysfunction, highlighting their potential influence on health. To prevent misdiagnosis and ensure complete evaluation, especially for individuals with clinical symptoms, routine ANA testing in healthy populations is recommended (Ge *et al*., 2022).

The number of anti dsDNA-positive cases was small and the highest incidence was observed in the 21-40 age group. It is noteworthy that there were no positive cases in the 1-20 and 61-80 age groups, which may indicate an age-related pattern in the prevalence of anti dsDNA. A previous study conducted in a healthy elderly population revealed a significant prevalence (7.6%; $P<0.006$) of anti-double-stranded DNA antibodies. The antibodies found in the elderly population were distinct, characterized by low titers, IgA class dominance, lack of complement-fixing ability and negative results in the Farr assay, suggesting a unique profile in elderly individuals without symptoms of disease (Ruffatti *et al*., 1990). The occurrence of antibodies against double-stranded DNA (dsDNA) in individuals with systemic lupus erythematosus (SLE) has been reported to vary widely, from 60% to 80%. This variation is attributed to factors such as patient selection and assay methods. Furthermore, the prevalence and levels of these antibodies have been reported to fluctuate in accordance with disease activity (Alnaqdy *et al*., 2007).

Autoimmune diseases are caused by a combination of genetic susceptibility and environmental factors that influence the expression of immune regulatory genes through mechanisms such as epigenetics. Autoantibodies, which are commonly present in most autoimmune diseases prior to the onset of clinical symptoms, can indicate the risk and sometimes the severity of developing a corresponding autoimmune disorder. The factors affecting autoantibodies include genetic predisposition, environmental influences and epigenetic modifications. However, the precise role of autoantibodies in the progression of autoimmune conditions remains unclear for many diseases, highlighting the need to identify additional events that trigger the transition from the preclinical to the clinical disease phase (Ma *et al*., 2017).

In the broader context, these findings contribute to the understanding of autoantibody prevalence in healthy individuals and emphasize the importance of considering multiple factors in autoimmune research. Further

investigations and longitudinal studies could provide valuable insights into the dynamics of autoantibodies in various demographic subsets and their potential implications for autoimmune diseases.

Conclusion

In summary, our research focused on analyzing the seroprevalence of antinuclear antibodies (ANA) and anti-double-stranded DNA antibodies (Anti-Ds DNA) in 659 healthy individuals from Rawalpindi and Islamabad. The results revealed that the prevalence of ANA and Anti-Ds DNA was minimal, at 0.91% and 0.46%, respectively. Although females showed a higher prevalence than males, no significant associations with age were detected. These findings highlight the importance of investigating autoimmune markers in asymptomatic individuals and contribute valuable insights into the baseline autoantibody levels in the general population.

However, it's essential to acknowledge the potential limitation of the study, such as the relatively small sample size and the need for further research to explore the implications of these markers for autoimmune diseases.

Conflict of Interest. The authors declare they that have no conflict of interest.

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