Rheumatic Disease Among Oklahoma Tribal Populations: A Cross-sectional Study

JASMINE R. GADDY, EVAN S. VISTA, JULIE M. ROBERTSON, AMY B. DEDEKE, VIRGINIA C. ROBERTS, WENDY S. KLEIN, JEREMY H. LEVIN, FABIO H. MOTA, TINA M. COOPER, GLORIA A. GRIM, SOHAIL KHAN, and JUDITH A. JAMES

ABSTRACT. ***Objective.*** Rheumatic diseases cause significant morbidity within American Indian populations. Clinical disease presentations, as well as historically associated autoantibodies, are not always useful in making a rapid diagnosis or assessing prognosis. The purpose of our study was to identify autoantibody associations among Oklahoma tribal populations with rheumatic disease.

Methods. Oklahoma tribal members (110 patients with rheumatic disease and 110 controls) were enrolled at tribal-based clinics. Patients with rheumatic disease (suspected or confirmed diagnosis) were assessed by a rheumatologist for clinical features, disease criteria, and activity measures. Blood samples were collected and tested for common rheumatic disease autoantibodies [antinuclear antibody (ANA), anti-cyclic citrullinated peptide antibodies (anti-CCP), rheumatoid factor (RF), anti-Ro, anti-La, anti-Sm, anti-nRNP, anti-ribosomal P, anti-dsDNA, and anticardiolipins].

Results. In patients with suspected systemic rheumatic diseases, 72% satisfied American College of Rheumatology classification criteria: 40 (36%) had rheumatoid arthritis (RA), 16 (15%) systemic lupus erythematosus, 8 (7%) scleroderma, 8 (7%) osteoarthritis, 4 (4%) fibromyalgia, 2 (2%) seronegative spondyloarthropathy, 1 Sjögren's syndrome, and 1 sarcoidosis. Compared to controls, RA patient sera were more likely to contain anti-CCP (55% vs 2%; $p < 0.001$) or RF IgM antibodies (57% vs 10%; $p < 0.001$); however, the difference was greater for anti-CCP. Anti-CCP positivity conferred higher disease activity scores (DAS28 5.6 vs 4.45; $p = 0.021$) while RF positivity did not (DAS28 5.36 vs 4.64; $p = 0.15$). Anticardiolipin antibodies (25% of rheumatic disease patients vs 10% of controls; $p = 0.0022$) and ANA (63% vs 21%; $p < 0.0001$) were more common in rheumatic disease patients.

Conclusion. Anti-CCP may serve as a more specific RA biomarker in American Indian patients, while the clinical significance of increased frequency of anticardiolipin antibodies needs further evaluation. (J Rheumatol First Release Aug 15 2012; doi:10.3899/jrheum.110984)

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Rheumatic diseases among American Indian (AI) populations are highly prevalent and often atypical in clinical presentation and disease course[1,2,3,4]. Disease tends to be more aggressive and confers higher morbidity and mortality among AI populations[4,5]. Although reasons for this have not been entirely elucidated, variations in genetic expression, overlapping symptoms, and unique serological features obscure diagnosis and subsequent approaches to treatment[5,6].

The relocation of AI to present-day Oklahoma in the

From the Department of Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation, Oklahoma City; Division of Rheumatology/Allergy/Immunology, University of Oklahoma Health Sciences Center, Oklahoma City; Chickasaw National Medical Center, Ada; and the Cherokee Nation Health Services, Tahlequah, Oklahoma, USA.

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J.R. Gaddy, MD, Division of Rheumatology/Allergy/Immunology, University of Oklahoma Health Science Center and Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; E.S. Vista, MD, Divisions of Rheumatology, University of Santo Tomas Hospital; Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; J.M. Robertson, PhD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; A.B. Dedeke, MD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; V.C. Roberts, LPN, CCRP, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; W.S. Klein, BS, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; J.H. Levin, BS, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation; F.H. Mota, MD; T.M. Cooper, MD, Chickasaw Nation Medical Center; G.A. Grim, MD; S. Khan, MBBS, MPH, Cherokee Nation Health Services; J.A. James, MD, PhD, Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation and Departments of Medicine, Pathology, and Microbiology/Immunology, University of Oklahoma Health Sciences Center.

Address correspondence to Dr. J.A. James, Oklahoma Medical Research Foundation, 825 NE 13th Street, Oklahoma City, OK 73104, USA. E-mail: judith-james@omrf.org

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1830s made for a heterogeneous amalgamation of indigenous people and is an ideal environment to investigate the pathology of rheumatic disease in AI populations. Tribal members comprise nearly 10% of the Oklahoma population and represent a diverse group of people with AI heritages[7]. Studies report a greater incidence of systemic lupus erythematosus (SLE) in AI compared with the general European-American (EA) population[1,3]. Oklahoma Choctaw Indians have a 40-fold increase in the incidence of systemic sclerosis (SSc) with primarily diffuse involvement and anti-topoisomerase 1 autoantibodies over non-AI populations[8,9,10,11]. Additionally, a greater overlap of rheumatoid arthritis (RA) with Sjögren's syndrome (SS) and SLE is reported in AI from Oklahoma, in which autoantibodies did not appear to correlate well with clinical outcomes[6]. These findings support the idea that rheumatic diseases manifest uniquely among Oklahoma tribal members and necessitate investigations of potential explanations for this diversity.

The aim of our study was to characterize serologic biomarkers in Oklahoma tribal patients with rheumatic diseases to help improve clinical care, as well as to develop new diagnostic and prognostic tools. Results from these studies will provide valuable strategies in the healthcare of AI in Oklahoma and may be applicable to other indigenous populations.

MATERIALS AND METHODS

Study participants and clinical evaluation. From March 2007 to January 2010, 110 AI patients in Oklahoma (patients with rheumatic disease and individuals with suspected rheumatic disease) and 110 AI controls were enrolled. Two rheumatic disease clinics were established for Oklahoma tribal patients with rheumatic disease complaints. Rheumatic disease patients were referred by primary care providers (physicians, physician assistants, or nurse practitioners) or by a tribal healthcare representative. Patients were referred to the tribal health clinic for several reasons, including presenting symptoms of systemic rheumatic disease without a clear diagnosis; abnormal blood test with rheumatic disease symptoms; systemic rheumatic disease with continued disease activity; questions regarding therapy; patient request for evaluation; or interest in being involved in a study. Healthy controls were recruited through institutional review board (IRB)-approved health fair flyers and e-mail advertisements. All patients involved in this study are members of a federally recognized AI tribe, band, or nation.

At the initial visit, history, physical examination, physician global assessment, American College of Rheumatology (ACR) disease criteria, disease activity, disease damage, and treatment histories were collected by a board-certified rheumatologist. Individuals referred to the rheumatic disease clinics were assessed for ACR criteria for classification of SLE, RA, SSc, SS, fibromyalgia (FM), and osteoarthritis (OA). Additionally, medical chart review was conducted for all participating patients referred for rheumatic evaluation according to published methods[12]. Classification of SLE required fulfillment of 4 of 11 1997 ACR criteria[13,14]. RA classification criteria required 4 of 7 for the 1987 ACR criteria[15]. FM diagnosis required 2 of 2 criteria with widespread pain present for at least 3 months[16]. SSc classification required either proximal diffuse sclerosis or 2 of the following: sclerodactyly, digital pitting scars or loss of substance of the digital finger pads, and bilateral basilar pulmonary fibrosis[17]. SS classification required 4 of 6 criteria as long as histopathology or serology was positive[18]. Patients were diagnosed with OA with 3 of the 4 criteria for OA of the hand[19]; or 2 of 3 criteria for OA of the hip[20]; or knee pain and osteophytes on a radiograph with 1 of 3 of the following: age over 50 years, stiffness lasting at least 30 minutes, or crepitus on motion[21].

Disease activity and outcome measurements were performed as appropriate: the Safety of Estrogens in Lupus Erythematosus National Assessment – SLE Disease Activity Index (SELENA-SLEDAI)[22,23], the physician global assessment (PGA) and Systemic Lupus International Collaborating Clinics Damage Index (SLICC)[24] for SLE patients, Disease Activity Score (DAS28) for RA patients[25], and the Rodnan skin score for SSc disease assessment measurement[26,27]. Controls provided serum for comparison.

All participants provided written informed consent and the study was approved by the IRB of each participating organization: University of Oklahoma Health Sciences Center, Oklahoma Medical Research Foundation, the Chickasaw Nation, and the Cherokee Nation.

Historical non-AI control cohort. A historical non-AI control cohort was included for comparison of autoantibody positivity with AI control subjects. This cohort comprised healthy unaffected EA (n = 62) and African American (AA; n = 38) individuals. IRB approval and written informed consent was given at time of enrollment. Serum samples were obtained from the control subjects and tested for autoantibodies.

Autoantibody testing. Samples were tested in a College of American Pathologist/Clinical Laboratory Improvement Amendments-approved laboratory for the presence of antinuclear antibodies (ANA) by immunofluorescence for titer and pattern, anti-dsDNA, and anti-extractable nuclear antigen. ANA was detected using Hep-2 cells (Inova Diagnostics, San Diego, CA, USA), with reactivity detectable at a 1:120 dilution considered positive. In patients with rheumatic disease, testing for dsDNA antibodies was performed by Crithidia assay (Inova Diagnostics). Additionally, precipitating antibodies to Sm, nRNP, Ro, La, PM-Scl, Mi-2, and Jo-1 were examined by double immunodiffusion according to published protocols[28]. Testing for anticardiolipins (aCL; IgG, IgM, IgA) used ELISA techniques with the following ranges considered positive: low titer 11–19 units, moderate titer 20–89 units, and high titer > 90 units (Sigma, St. Louis, MO, USA).

In addition, all samples were evaluated by ELISA for extractable nuclear antigen antibodies to Sm, nuclear RNP (nRNP), Ro, La (Immunovision, Springdale, AR, USA), and ribosomal P (Ribo P; Molecular Biology Proteomics, Oklahoma City, OK, USA)[29]. ELISA detection was performed for RF IgM and IgG (Jackson Immunoresearch Laboratories, West Grove, PA, USA) with positive result as an optical density > 0.35. Patients were considered RF-positive if a positive ELISA result was obtained for RF IgM and/or IgG. These methods were as described[30]. Serum antibodies directed to cyclic citrullinated protein IgG (anti-CCP) were assessed by a commercial ELISA kit according to the manufacturer's recommendations (QuantaLite™ CCP2 IgG ELISA; Inova). Samples were considered positive when the index value was > 20.

Statistical analysis. Statistical analysis focused on chi-square methods. Contingency data tables with cells representing fewer than 5 subjects were identified and Fisher's exact test was used. Pearson correlation coefficients were used to test for an association between 2 independently measured continuous variables. ANOVA methods were used for comparisons on continuous data among subsets of patients. Levene's test was used to test for homogeneity of variance of a given variable and the Shapiro-Wilk test to investigate the assumption of normality. When either the homogeneity of variance or normality assumption required under ANOVA was not satisfied, nonparametric methods were used. P values < 0.05 were considered significant. All analyses were performed using SAS version 9.1 or GraphPad Prism 5 for Windows. Logistic regression model-building techniques used the purposeful selection method of identifying covariates of interest[31]. Models were compared using the likelihood ratio test.

RESULTS

Rheumatic disease presentations to tribal-based referral clinics. Of 110 patients referred for systemic rheumatic dis-

ease evaluation or therapeutic revision, 72% met ACR criteria for classification: 40 (36%) had RA (including those patients with secondary SS), 16 (15%) SLE, and 8 (7%) SSc. Additional rheumatic diagnoses comprised 8 OA (7%), 4 FM (4%), 2 seronegative spondyloarthropathy (SpA; 2%: 1 ankylosing spondylitis and 1 reactive arthritis), 1 SS, and 1 sarcoidosis. A large number of patients had had a previous rheumatic disease diagnosis (including 23 of 25 patients with RA and 13 of 16 with SLE). The distribution of cumulative ACR criteria for SLE is shown in Figure 1. Briefly, malar rash, photosensitivity, arthritis, and ANA were the most prevalent SLE ACR criteria met by these AI patients with SLE. The average number of SLE ACR classification criteria was 5.3 ± 1.2 (range 4–7). On average the patients with SLE had an SLE diagnosis for 7.8 ± 7.5 years (median 6 yrs, range 1–28). The AI patients with RA exhibited morning stiffness, arthritis of ≥ 3 joints, arthritis of the hand, and erosions on radiographs, and had detectable serum RF. On average the patients with RA had a disease diagnosis for 11.3 ± 8.8 years (median 10 yrs, range 0–37).

A group of patients referred for rheumatic disease evaluation or treatment revision did not meet ACR classification criteria for disease; they accounted for 28% (33/110) of the patient population in our study. These included 11 patients with polyarthralgia, 11 with polyarthritis, 4 with undifferentiated connective tissue disease (UCTD), 3 with anterior uveitis, and 1 with sclerodactyly. Patients with suspected systemic rheumatic disease were slightly older than controls (27% vs 13% ≥ 60 yrs of age, respectively) and 76% of all participants were female (Table 1).

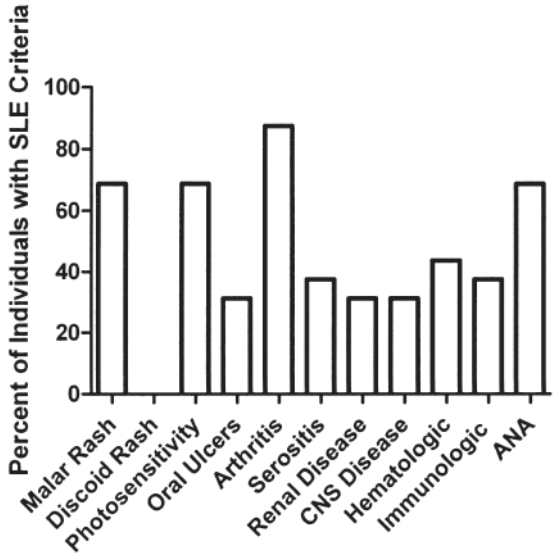

Figure 1. Distribution of cumulative SLE classification criteria in American Indian (AI) study participants. At enrollment, patients were examined for rheumatic diseases by a rheumatologist and assessed for ACR classification criteria. Medical chart review for the presence of ACR classification criteria was also performed for all AI participants referred for rheumatic evaluation. Cumulative ACR classification criteria for SLE are shown as percentage of patients positive. SLE: systemic lupus erythematosus; ACR: American College of Rheumatology; CNS: central nervous system; ANA: antinuclear antibody.

Patient sera contained autoantibodies frequently detected in other disease states. Atypical disease associations were found in patients exhibiting disease-specific antibodies within this study. Table 2 illustrates autoantibody patterns in Oklahoma tribal patients referred to the rheumatic disease clinic. Only 11 of the 16 patients (69%) with SLE had detectable ANA at the time of enrollment. Of these 5 ANA-negative patients with SLE, 4 had exhibited ANA positivity historically at some time before study enrollment. No significant differences were observed in age, ACR criteria, length from disease diagnosis, or medication use between the patients who lost ANA positivity and those who remained ANA-positive.

ANA measurements showed that Oklahoma tribal patients referred for rheumatic disease evaluation were more likely to be ANA-positive compared to controls (63% vs 18.2%, respectively; $p < 0.0001$; Tables 2 and 3). Alternatively, 13/22 patients with polyarthralgia and polyarthritis (59%), 25/40 with RA (63%), and 5/8 with OA (63%) exhibited ANA positivity. Eight patients had > 1 ANA pattern (3 RA, 2 SSc, 1 SLE, 1 OA, and 1 polyarthralgia). The ANA positivity of the individuals referred for rheumatic disease evaluation was significantly higher ($p < 0.0001$) compared to ANA positivity in controls. Anti-dsDNA positivity was more likely to be found among AI patients referred for rheumatic evaluation than in controls, although this did not reach statistical significance ($p = 0.06$).

Of patients whose sera tested positive for anti-dsDNA, 1 had polyarticular inflammatory arthritis, while only 2/16 patients with SLE (13%) had anti-dsDNA antibodies (Table 2). Among RNA-binding protein antibodies, 1/16 SLE patient sera had detectable anti-nRNP, while none had anti-Sm. Anti-Ro antibodies were detected in 5 of the 110 referred patients, including 2 with RA and 1 each with SSc, SS, and SLE.

Of the 33 patients who did not meet ACR classification criteria, 24 had detectable ANA (5 polyarthralgia, 8 polyarthritis, 2 FM, 8 OA, 4 other disorders; Table 2). Additionally, sera from 2 patients with polyarthralgia and 1 patient with polyarthritis were positive for RF IgM; and 1 patient with polyarthralgia and 2 patients with polyarthritis were positive for anti-RF IgG (Table 2), although these indi-

Table 1. Baseline characteristics of Oklahoma American Indian tribal members referred for rheumatic disease evaluation and healthy controls.

Characteristic	Clinic Patients, n = 110	Controls, n = 110	p
Mean age, yrs (SD)	49.2 (± 13)	40.7 (± 14.4)	< 0.001
Range	17–83	16–75	
Women, n (%)	88 (80)	80 (73)	

Table 2. Autoantibody specificities detected in sera from Oklahoma tribal patients referred to the rheumatic disease clinic.

Antibody	RA, n = 40	SLE, n = 16	SSc, n = 8	SS, n = 1	Polyarthritis, n = 11	Polyarthralgia, n = 11	FM, n = 4	OA, n = 8	Other, n = 11
ANA	25*	11	7	1	8	5	2	5**	5***
Anti-dsDNA	1	2	1	0	0	1	0	0	0
aCL IgG	10	2	3	0	5	1	2	1	3†
aCL IgM	3	0	0	0	0	0	0	0	1††
Anti-Ro	2*	1	1	1	0	0	0	0	0
Anti-La	1	0	0	0	0	0	0	0	1
Anti-Sm	0	0	0	0	1	0	0	0	1††
Anti-nRNP	0	1	0	0	1	0	0	0	1#
Anti-Ribo P	0	0	0	0	0	0	0	0	0
Anti-Jo 1	0	0	1	0	0	0	0	0	0
Unidentified	2	2	2	0	0	0	0	0	0
Anti-CCP	22	0	0	0	0	1	0	0	1††
RF IgM	23	1	1	1	1	2	0	1	0
RF IgG	12	0	0	0	2	1	0	0	0

FM: fibromyalgia; OA: osteoarthritis; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; SSc: systemic sclerosis; SS: Sjögren's syndrome; ANA: antinuclear antibody; aCL: anticardiolipin antibodies; Ig: immunoglobulin; CCP: cyclic citrullinated peptide; RF: rheumatoid factor. Other: undifferentiated connective tissue disease (UCTD) (4), anterior uveitis (3), sarcoidosis (1), sclerodactyly (1), seronegative spondyloarthropathy (2). Unidentified: antibody binding lines detected during immunodiffusion that do not match known rheumatic disease antigens. * Patient with both RA and Sjögren's syndrome (1); ** patient with both FM and OA (2); *** UCTD (3) and anterior uveitis (1); † UCTD (2) and sclerodactyly (1); †† UCTD (1); # inflammatory eye disease.

Table 3. ELISA autoantibody profiles of American Indian (AI), European-American (EA), and African American (AA) control subjects. Data are percentages.

Antibody	AI, n = 110	EA, n = 62	AA, n = 38
ANA	18.2	33.9	47.4
Anti-dsDNA	0	4.8	7.9
aCL IgG	11.8	6.5	5.3
Anti-Ro	0.9	1.6	5.3
Anti-La	0	1.6	2.6
Anti-Sm	0.9	4.8	5.3
Anti-nRNP	1.8	1.6	0
Anti-Ribo P	0	6.5	2.6

ANA: antinuclear antibody; aCL: anticardiolipin antibodies; Ig: immunoglobulin.

viduals did not meet the ACR classification criteria for RA.

Anticardolipin IgG antibodies were enriched in individuals referred for rheumatic disease evaluation. In patients with rheumatic disease symptoms or diagnoses, 27/110 (25%) had detectable IgG aCL, with 3/27 (11%) also exhibiting IgM aCL (Table 2). Of these rheumatic disease patients with detectable aCL, 18/27 (67%) met ACR criteria for disease as follows: 10 RA, 3 SSc, 2 SLE, 1 OA, and 2 FM. Of those patients not meeting criteria for disease-associated conditions (9/27, 33%): 6 had polyarthralgia, 2 UCTD, and 1 sclerodactyly. Compared to controls, patients with rheumatic disease were more likely to be positive for IgG aCL (27 vs 13; p = 0.022). Of these patients, 16/27 (59%) were low-titer (11–19 units) and 11/27 (41%) moderate-titer (20–89 units) for aCL. In the 3 rheumatic patients with detectable IgM aCL, 1 patient with RA exhibited low-titer IgM aCL and also had detectable low-titer IgG aCL; 1 patient with RA and 1 with UCTD exhibited moderate-titer for both IgM and IgG aCL. In contrast, 13/110 controls had detectable aCL with 8/11 positive for low-titer IgG aCL, and the remaining 5 had moderate-titer aCL. No control tested positive for IgM aCL autoantibodies. None of the aCL-positive individuals had a history of clots, pulmonary embolism, or deep vein thrombosis as determined through medical chart review.

Combination of anti-CCP and RF antibodies is a sensitive and specific biomarker of RA in AI patients with rheumatic disease. A comparison was made between RF and anti-CCP among all patients with rheumatic disease to determine the strongest association with RA. Anti-CCP antibodies appeared to be found almost exclusively in patients with RA, while several patients without RA had detectable RF IgM and/or IgG (Table 2). The diagnostic accuracy of anti-CCP in our AI study population had 55% sensitivity, 98.6% specificity, a positive predictive value (PPV) of 0.96 (95% CI 0.78–0.99), and a negative predictive value (NPV) of 0.79 (95% CI 0.69–0.87). The diagnostic accuracy of RF (IgM and IgG) in our AI study population had 57.5% sensitivity, 87.1% specificity, PPV of 0.72 (95% CI 0.53–0.86), and NPV of 0.78 (95% CI 0.67–0.86). In patients with RA, 18/40 patients were anti-CCP antibody-positive and RF-positive, 13/40 were anti-CCP-negative and RF-negative, 5/40 were anti-CCP-negative and RF-positive, and 4/40 were anti-CCP-positive and RF-negative. Used together, anti-RF and anti-CCP antibodies are better biomarkers of

RA than either measure alone (p = 0.007). Anti-CCP antibody positivity (DAS28 5.6 vs 4.45; p = 0.021) or having both anti-CCP and RF IgM and/or IgG antibodies (DAS28 5.7 vs 4.64; p = 0.039) was associated with higher disease activity scores. Among patients with RA, those ANA-positive (62%, 25/40) had higher DAS28 scores (5.46 vs 4.42; p = 0.031), but only anti-CCP antibodies were independently associated with higher disease activity when evaluating both anti-CCP antibody and ANA positivity in these patients (p = 0.025 vs p = 0.067).

Of the 40 patients with RA, 35 (88%) were receiving disease-modifying antirheumatic drug (DMARD) therapy, 17 (43%) were maintained on prednisone (average dose 8.8 mg), and 11 (28%) were taking or had been taking biological therapy. No relationship was observed between current medications and DAS28 scores.

Autoantibody prevalence in AI control subjects was not enriched. To fully define the differences between AI autoantibody profiles, we compared the ELISA serology of our AI controls (n = 110) to a historic cohort of healthy unaffected European-American and African American controls (n = 100). The EA controls were age 44.74 ± 14.81 years and consisted of 89% women; whereas the AA controls were age 37.45 ± 10.60 years and comprised 87% women. The age of the non-AI controls was not significantly different from that of the AI controls (p > 0.05 for both AA and EA). Differences in autoantibody positivity were observed between AI controls and the EA and AA controls (Table 3). ANA positivity (39% vs 18.2%, respectively; p = 0.0012), antibody positivity to dsDNA (6% vs 0%; p = 0.011) and Ribo P (5% vs 0%; p = 0.023) were more likely to be found in EA and AA controls than in AI. No statistically significant differences in aCL, anti-Ro, La, Sm, or nRNP positivity were observed.

DISCUSSION

Several studies involving specific rheumatic diseases in Native Americans have indicated a higher prevalence of RA (122 cases in 100,000 vs 48 cases in 100,000 in the non-Native American population)[32] and SLE (42.3 cases in 100,000 vs 20.6 cases in 100,000 in the non-Native American population)[4]. Our study was not designed to serve as a population-based investigation to evaluate the incidence and prevalence of each systemic autoimmune rheumatic disease, but focuses on the clinical and serologic presentations of these patients that impair diagnosis. At the start of our study, Oklahoma was home to 395,500 American Indians. However, only a subset of these individuals is cared for in the Chickasaw and Cherokee health systems (and therefore would be available for referral to our clinics). To date, over 150 patients have been provided care at the Chickasaw and Cherokee tribal health clinics. With the prevalence rates outlined above[4,32], we should expect 27 cases of RA and 9 cases of SLE. Over the course of this study (2007-2010), 110 patients with rheumatic diseases were recruited. Of these, 40/110 were diagnosed with RA and 16/110 with SLE. While these numbers are slightly higher than the 2007 population estimate, we believe this population is representative of American Indian patients with rheumatic disease.

Most of the initial data regarding correlation of specific autoantibodies and rheumatic diseases have been generated from cohorts that were predominantly patients with European or African heritage[33,34,35,36]. In our study, the majority of the Oklahoma tribal patients evaluated for rheumatic disease tested positive for ANA, with varying titers and patterns, while only 69% of patients with SLE had a positive ANA at the time of evaluation. No differences in age, length of time from disease diagnosis, ACR criteria, or treatment were observed between patients with SLE who remained ANA-positive compared to those whose sera lost ANA positivity. A numerical difference in SLEDAI scores was observed (5.45 ± 4.80 for ANA-positive SLE patients vs 2.67 ± 1.15 for ANA-negative). While this difference was not statistically significant (p = 0.46; Mann-Whitney test), a finding of ANA-positive patients with SLE demonstrating higher disease activity is consistent with previous work. Additionally, other studies have identified a subset of patients with SLE who are ANA-negative[37,38,39].

SLE-specific antibodies were detected in 3% of the AI patients with rheumatic disease; however, the majority of these patients lacked clinical features of SLE. Further, anti-Ro antibodies are historically found in sera from almost 50% of patients with SLE and can also be detected in the vast majority of patients with SS[40]. In our cohort, anti-Ro antibodies were present in patients with SLE and SS, as well as in patients with RA, SSc, and anterior uveitis. Interestingly, the myositis-specific antibody, anti-Jo-1, was detected in a patient with SSc without clinical features of inflammatory myositis. In this study, 5% of patients referred for rheumatic disease evaluation had antibodies detected by immunodiffusion that were unidentifiable. These findings support evidence of overlapping antibodies and rheumatic diseases and highlight the lack of prognostic knowledge of autoantibody specificities in this AI population.

The aCL encompass a heterogeneous group and are associated with SLE as well as risk of clinical complications such as arterial and venous thrombosis[41]. They are associated with vascular impairment in certain connective tissue diseases[42]. However, aCL have also been observed in rheumatic diseases without the presence of antiphospholipid syndrome[43,44]. Detectable aCL were found in a number of patients with systemic rheumatic diseases (RA, SSc), along with alternative diagnoses (FM, OA, polyarthralgia, and polyarthritis). The clinical significance of the role of these aCL in AI patients and the pathologic risk for thrombosis remains to be determined.

Historically, RF has been the serologic criterion used in

the diagnosis of RA. More recently, the combination of anti-CCP along with RF antibodies appears to be more sensitive and specific for RA diagnosis and a better predictor of joint destruction[45,46,47]. This finding was reinforced among our Oklahoma tribal patients with RA, suggesting that anti-CCP antibodies may be more strongly associated with RA and a biomarker of disease in this population. In addition, nearly 60% of our RA tribal patient sera samples contained ANA, much higher than previously reported in this geographic population[48], but in agreement with findings from other AI tribes[3,32,49]. Interestingly, our study cohort had a high percentage (32.5%) of individuals who were seronegative for anti-CCP and anti-RF antibodies compared to other AI studies[3,5,49]. Taken together this emphasizes ethnic and potentially tribal differences in autoantibody expression, specifically in AI populations, and has potential to elicit changes in current evaluation and treatment practices.

Of the Oklahoma AI patients referred for rheumatic disease evaluation, 28% were unclassifiable by ACR criteria. This percentage is significantly smaller than the 48% seen with the Nuu-Chah-Nulth tribe[50]. However, these populations of individuals with unclassifiable rheumatic disease highlight differences in disease presentation between AI and other demographic groups. Interestingly, a subset of our study patients with polyarthritis and polyarthralgia exhibited detectable antibody production. It would be of considerable interest to follow those patients who did not fulfill the ACR criteria to assess the progression of disease. This also illustrates that use of the current ACR criteria may not hold distinct applicability to the AI population.

Limitations exist in our study. Rheumatic disease patients on average were older than our healthy AI controls, potentially confounding the autoantibody comparisons. However, the percentage of antibody-positive individuals in the control population was 18% compared to 39% of controls of either EA or AA descent with a similar age at participation, suggesting this difference would not significantly change the results. Medication reporting in our study has a potential limitation. While no statistical significance was observed between medication, disease activity scores, and antibody positivity, the medication list at the time of evaluation may not fully represent what the participant had taken in the past. This limitation can be minimized in the future with the recent implementation of electronic medical records. Patients were also referred to this clinic for a variety of reasons, many of which may bias the severity or presentation of rheumatic disease clinical manifestations. Additional population-based studies are warranted to confirm and expand these observations. Historical serologies obtained by medical record review may be variable because of difference in laboratory used, types of testing, sample shipping, and other such confounding issues. These limitations may explain in part some of the difference between SLE ACR ANA criteria and ANA at time of evaluation.

Another potential limitation is that the results might not be fully representative of those seen in rheumatology clinics because the patients referred for rheumatologic evaluation may have included a subset who presented with more atypical disease features, were difficult to treat, or had increased disease severity. However, the majority of the rheumatic disease presentations did match the clinical characteristics of disease classification, with the exception of the serology. Additionally, our study subjects included a number of different AI tribes evaluated in the Chickasaw and Cherokee catchment areas and may exhibit differences in rheumatic disease presentation unique to the Oklahoma area. Thus, while our subjects might not represent the typical patient with rheumatic disease, they are representative of patients with rheumatic disease seen in Oklahoma. A future direction that would alleviate some of the study limitations is the development of a cohort of newly diagnosed AI and non-AI patients with rheumatic disease and evaluation of differences in clinical symptoms, serology, and disease activity. Additionally, the development of a longitudinal cohort that would follow AI and non-AI individuals with rheumatic disease could give valuable insights into the differences in disease progression.

Oklahoma AI tribal members with rheumatic disease failed to exhibit all the typical disease-specific autoantibody markers. ACR criteria for classification of disease do not appear to be as inclusive in this unique population as in previously studied ethnic groups. Further studies are needed to better define more reliable diagnostic biomarkers to help guide treatment and improve outcomes in AI patients with rheumatic disease.

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