CONCISE REPORTS

Serum concentrations of α tocopherol, β carotene, and retinol preceding the diagnosis of rheumatoid arthritis and systemic lupus erythematosus

G W Comstock, A E Burke, S C Hoffman, K J Helzlsouer, A Bendich, A T Masi, E P Norkus, R L Malamet, M E Gershwin

Abstract

***Objectives*—Because oxidative damage has been implicated in the pathogenesis of rheumatoid arthritis and systemic lupus erythematosus, this study was designed to see if serum concentrations of α tocopherol, β carotene, and retinol, substances believed to be involved in the prevention or repair of oxidative damage, might be lower among persons who develop rheumatoid arthritis or systemic lupus erythematosus than among those who do not.**

***Methods*—For this prospective case-control study, persons with rheumatoid arthritis and systemic lupus erythematosus that developed two to 15 years after donating blood for a serum bank in 1974 were designated as cases. For each case, four controls were selected from the serum bank donors, matched for race, sex, and age. Stored serum samples from cases and controls were assayed for α tocopherol, β carotene, and retinol.**

***Results*—Cases of both diseases had lower serum concentrations of α tocopherol, β carotene, and retinol in 1974 than their matched controls. For rheumatoid arthritis, the difference for β carotene (−29%) was statistically significant.**

***Conclusions*—These findings support those of a previous study that low antioxidant status is a risk factor for rheumatoid arthritis. They suggest a similar association for systemic lupus erythematosus.**

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Department of Epidemiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland, USA
G W Comstock
A E Burke
S C Hoffman
K J Helzlsouer

Human Nutrition Research, Hoffmann-LaRoche, Paramus, New Jersey, USA
A Bendich

Department of Medicine, The University of Illinois, College of Medicine at Peoria, Peoria, Illinois, USA
A T Masi

Biomedical Research, Our Lady of Mercy Medical Center, Bronx, New York, USA
E P Norkus

Rheumatology Practice, Hagerstown, Maryland, USA
R L Malamet

Division of Rheumatology, Allergy, and Clinical Immunology, School of Medicine, University of California-Davis, Davis, California, USA
M E Gershwin

Correspondence to:
Dr G W Comstock, Training Center for Public Health Research, Box 2067, Hagerstown, MD 21742-2067, USA.

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For more than two decades, free radical oxidation products have been identified in synovial fluid, especially in fluid drawn from inflamed joints.[1–5] Patients with rheumatoid arthritis have been reported to have lower serum concentrations of antioxidant vitamins A and E than normal controls in some but not all studies.[6 7] While such an association might be interpreted as an effect of the inflammatory joint disease on antioxidant values in the serum, vitamin E values in cases have been found to be associated with rheumatoid factor positivity but not with other evidence of inflammation or activity of the disease.[7] Furthermore, a recent study produced evidence that low serum concentrations of α tocopherol, β carotene, and selenium preceded the diagnosis of rheumatoid arthritis, and that low antioxidant values might therefore be a risk factor or marker for the disease.[8] This study adds evidence to support that conclusion, and presents similar data for a small number of cases of systemic lupus erythematosus.

Methods

A community wide campaign was conducted in Washington County, Maryland to collect basic health information and blood samples for a serum bank during September, October, and November, 1974. A few additional specimens were collected in August 1974 and July 1975. A total of 20 305 Washington County residents participated. Linking campaign records with those from a private census of the county in the summer of 1975 showed that 30% of the adult residents had participated, with somewhat higher rates among the age group 45-54 years, women, and the better educated.

Participants gave brief medical histories and their blood pressures measured. Blood was drawn into red-top Vacutainers (Becton-Dickinson, Rutherford, NJ) and allowed to stand at room temperature for three hours to facilitate clotting. It was then refrigerated at 5°C until centrifugation, almost always within a few hours. Serum was transferred to 4.5 ml Cryotubes (Nunc, Roskilde, Denmark) and stored at −70°C until aliquots were used for this and a companion study. The specimens were thawed under ice water and only exposed to dim yellow light before shipment with dry ice to the laboratory for assays of selected antioxidant concentrations.

Cases for this study were identified from the records of the only rheumatologist in the county (RLM). He began his practice here in 1985. All cases met the criteria for rheumatoid arthritis or systemic lupus erythematosus set by the American Rheumatism Association.[9] Because of limited resources, only 30 cases of rheumatoid arthritis could be included in the original study that involved serum hormones. These cases were selected from a list of the 50 cases of rheumatoid arthritis who had donated

Table 1 Prediagnostic serum concentrations of α tocopherol, β carotene and retinol, among selected cases of rheumatoid arthritis and systemic lupus erythematosus and their race-sex-age matched controls, Washington County, Maryland

	Cases	*Controls*	*Difference**	*95% confidence limits*	*% difference †*
Rheumatoid arthritis:					
Number	21	84			
	Mean (SD)	Mean (SD)			
α tocopherol (mg/dl)	1.07 (0.23)	1.13 (0.28)	−0.05	−0.20, 0.10	−4.7
β carotene (μg/dl)	16.6 (9.2)	23.4 (9.6)	−6.9	−13.1, −0.6	−29.3
Retinol (μg/dl)	56.2 (14.1)	60.5 (11.3)	−4.3	−10.0, 1.4	−7.1
Systemic lupus erythematosus:					
Number	6	24			
	Mean (SD)	Mean (SD)			
α tocopherol (mg/dl)	0.64 (0.09)	0.80 (0.21)	−0.17	−0.41, 0.08	−20.6
β carotene (μg/dl)	17.2 (9.6)	19.8 (5.5)	−2.6	−10.2, 5.04	−13.1
Retinol (μg/dl)	43.7 (12.6)	51.2 (3.8)	−7.5	−19.1, 4.1	−14.6

* Calculations based on values carried to three decimal places.

† $\frac{\text{Case Mean} - \text{Control Mean}}{\text{Control Mean}} \times 100$

blood in 1974 and whose onset of disease was between 1976 and 1989, and who had not developed cancer other than non-melanomatous skin cancer. First priority was given to cases under 50 years of age at onset and then to cases with the earliest date of onset. The 21 cases for this study are those from the original 30 for whom serum samples from them and their matching controls were still available for assays of micronutrients. Serum samples were also available for six of the seven cases of systemic lupus erythematosus diagnosed in this practice between 1976 and 1989 who had participated in the 1974 campaign.

Controls were selected from the list of resident donors to the serum bank. Exclusions were the same as for the cases, except for the additional exclusion of persons who were known to have died before the year of onset of symptoms of the case to which they were to be matched. Four controls were matched to each case on the basis of race, sex, and age. Two of the four controls were older than the case and nearest to the case in age. The two others were younger eligible persons who were nearest to the case in age. In most instances, cases and controls differed in age by only a few days.

Serum samples were arranged in sets, each with one case and its four matched controls. Seven of the sets also included serum from a single external quality control pool. Laboratory personnel were not told which specimens came from cases, controls, or the external quality control pool. Samples within each set were thawed and assayed on the same day within the same assay run. Assays were performed in duplicate by high performance liquid chromatography.[10]

Mean concentrations of the analyses for cases were compared with the mean values for each group of four matched controls, and the difference expressed as a percentage of the mean control concentrations. Matching was taken into account in assessing statistical significance of the case-control differences.[11]

For the seven external quality control specimens from a large serum pool, the coefficients of variation were 3.4% for α tocopherol, 7.8% for β carotene, and 2.2% for retinol.

Results

RHEUMATOID ARTHRITIS

Three of 21 rheumatoid arthritis cases had their reported onsets of disease in the period 1976-80, 13 in 1980-84, and five in 1985-89. Six cases were males and 15 were females; their median age in 1974 was 41 years and 52 years at onset of disease. There were no significant differences between cases and controls with respect to years of school completed, month of blood drawing, and hours since last meal.

Table 1 shows the mean case and control concentrations for α tocopherol, β carotene and retinol. Although cases of rheumatoid arthritis had lower values than their controls, only the difference between cases and their matched controls of −29.3% for β carotene achieved statistical significance.

SYSTEMIC LUPUS ERYTHEMATOSUS

For the six cases of systemic lupus erythematosus, the onset of disease occurred three to 13 years after donating blood in 1974 and at ages ranging from 39 to 52 years. All cases were white females, and like the situation for the rheumatoid arthritis cases, the ages of the cases and controls differed by only a few days on average. There were only slight differences between cases and controls with respect to years of school completed and hours between blood drawing and the last meal.

As shown in table 1, prediagnostic values for serum α tocopherol, β carotene, and retinol were all lower among systemic lupus erythematosus cases than controls. With the small number of cases, none of the differences achieved statistical significance.

Discussion

The findings of this study show that serum concentrations of several important antioxidants were lower among people who subsequently developed rheumatoid arthritis than among the general population. The importance of this report is its agreement with the findings of another small study conducted somewhat differently and in an entirely different population.[8] This concordance reduces the likelihood that this is a chance finding and suggests that low concentrations of these antioxidants may in some way be related

to the development of rheumatoid arthritis, either directly or as associates of an aetiological factor.

Cases of systemic lupus erythematosus were included in this study because findings in animals and in patients suggested that free radical damage might play a part in this disease.[12–14] Although the number of cases is too small to allow definitive statements about the association of serum antioxidants with systemic lupus erythematosus, it is hoped that this report will stimulate others to see if our results can be replicated.

Selection of cases from a single practice specialising in rheumatic diseases has advantages and disadvantages. Such cases are probably true cases both because of the presumptive greater severity of their disease and greater accuracy of diagnosis by a specialist. On the other hand, it is possible that patients seen by a specialist may come from a higher socioeconomic level than patients seen by general practitioners. However, the cases in this study did not differ from their age-race-sex matched controls with respect to years of school completed nor with respect to other factors related to dietary intake and absorption such as month blood was drawn, and hours between last meal and blood drawing.

It is also possible that diagnosable but unrecognised disease was present at the time of blood drawing despite the fact that cases with onsets within two years of blood donation were excluded from the study. A clue to whether or not unrecognised disease was present in 1974 when blood was drawn may be gained from the medication histories taken at that time. Five cases were taking drugs that might have been for rheumatic symptoms: one was taking 'arthritis pills', another a non-steroidal anti-inflammatory drug, two were taking aspirin, and a fifth, acetaminophen. However, the serum concentrations of the three antioxidant micronutrients among these five cases were not appreciably or significantly different from the concentrations among cases not taking these drugs. Furthermore, there were no demonstrable differences in serum concentrations among cases with early and late onsets. Hence it seems unlikely that the presence of disease at the time of blood drawing in 1974 accounts for the association of low serum antioxidants and subsequent arthritis.

Controls were selected in a way that should make them representative of the general population, even though they happened to contain no cases as defined for this study. Among the 83 controls with histories of drugs taken during the 48 hours preceding blood donation, one was taking a non-steroidal anti-inflammatory drug and 17 were taking some form of aspirin. Again the mean serum concentrations among those taking drugs were almost identical with those among persons not taking aspirin or non-steroidal anti-inflammatory drugs. With an estimated prevalence of definite rheumatoid arthritis in four American communities ranging from 0.5 to 1.0%,[15] it seems highly unlikely that enough unrecognised cases could have been present in our control population to have had an important effect on the results.

Findings of a lowered antioxidant status prior to the recognition of rheumatoid arthritis (and possibly systemic lupus erythematosus as well) show that oxidative damage may precede the onset of definite symptoms and the medical diagnosis. The pathogenic mechanisms whereby low serum concentrations of antioxidants are related to rheumatoid arthritis and systemic lupus erythematosus are unlikely to be ascertained by epidemiological studies such as the present one. It is possible that in the prediagnostic stage, serum antioxidants are low because they have been used in reducing inflammatory products. Or perhaps low antioxidant status, whether because of decreased intake, absorption, or transport, increases the potential for oxidative damage. The order of events that lead to low serum concentrations of α tocopherol, β carotene, and retinol in people who later develop rheumatoid arthritis or systemic lupus erythematosus will have to be elucidated by a variety of studies designed to answer this question.

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