Rheumatoid arthritis in Kenya.

II. Serological observations

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SUMMARY Sera from 48 Kenyan Africans with rheumatoid arthritis, 43 patients with other diseases, and 98 blood donors were tested for the presence of rheumatoid factor by latex fixation tests using human European, human African, and rabbit immunoglobulin, and a sheep cell haemagglutination test. In the patients with rheumatoid arthritis the frequency of rheumatoid factor was comparable to that reported in series from Europe and the USA, thus differing from the findings in West Africa. In the control patients and blood donors a high frequency of positive tests for rheumatoid factor was found; a similar result has been obtained from population studies in other African countries. Broadly similar results were obtained with each of the latex tests, and these were found to be less specific for rheumatoid arthritis than the sheep cell haemagglutination test.

Epidemiological studies in Africa and other tropical countries have shown a higher prevalence of rheumatoid factor (RF) in the sera of healthy subjects than has been found in comparable populations in the temperate zone (Malawista, *et al.*, 1959; Houba and Allison, 1966; Shaper *et al.*, 1968; Greenwood *et al.*, 1971). Indeed, it has been reported from West Africa that the prevalence of RF positivity in the sera of control populations does not differ significantly from that found in indigenous patients with rheumatoid arthritis (RA). Hence the statement in one Nigerian study that the detection of RF 'has limited value for diagnosis of RA in some tropical countries' (Greenwood, 1969). In East Africa, however, a higher prevalence of positive tests for RF has been found in patients with RA, the figures being comparable to those reported from Europe and the USA. Unfortunately only small numbers of patients have been investigated in the past, and in some of them only latex slide tests were carried out (Hall, 1963, 1966; Kanyerezi, 1969; Bagg *et al.*, 1976).

In order to verify the limited data from East Africa we have examined the sera of Kenyan patients with RA for the presence of RF by standard quantitative tests. This paper presents the findings of these tests, and for comparison with other studies we have also included the results of tests carried out on the sera of

patients with disease other than RA and the sera of presumptively healthy blood donors.

Material and methods

SERA

Sera from 48 patients with RA (15 classical, 29 definite, and 4 probable) were tested. The sera were collected from patients attending the outpatient department or in the wards of the Kenyatta National Hospital, Nairobi. All the patients were Kenyan Africans from Nairobi or the surrounding area. There were 41 females and 7 males, aged between 15 and 75 years. A detailed clinical description of those patients with classical or definite RA has been given elsewhere (Bagg *et al.*, 1979). Classification into categories was in accordance with the criteria of the American Rheumatism Association.

Sera from 43 African patients with disease other than RA were examined. In this group there were 34 females and 9 males, aged between 11 and 63 years. In addition sera from 98 African blood donors were examined.

All blood samples were left for about 2 hours at room temperature, then centrifuged. Separated sera were frozen and kept at -20°C until examined.

LATEX FIXATION TESTS

The method employed has been described previously (Houba and Allison, 1966). Three different immunoglobulins were used for coating the latex particles:

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(a) human IgG of European origin (supplied with the testing kit); (b) IgG from the pooled sera of 2 African patients with IgG myeloma, separated by DEAE chromatography (Winblad, 1964); (c) IgG from the pooled sera of several rabbits, isolated as described above. The tests employing these immunoglobulins were designated LFT-E, LFT-A, and LFT-R respectively. All 3 globulins were made up to identical protein concentrations for coating the latex particles. Sera were doubly diluted with glycine-saline buffer (0.1M, pH 8.2 with 1% NaCl) from 1:20. Sera giving definite agglutination of particles in a final dilution of 1:80 and above were regarded as positive.

SHEEP CELL AGGLUTINATION TEST (SCAT)

A haemagglutination technique, using inactivated and absorbed sera and sheep red blood cells sensitised with rabbit amboceptor, was carried out as previously described (Houba and Allison, 1966). Sera were doubly diluted from 1:4, and positive haemagglutination at 1:32 or above was regarded as positive.

International Standard rheumatoid arthritis serum was used as the standard positive control in all estimations (Anderson *et al.*, 1970). The lyophilised preparation was reconstituted with distilled water and diluted with saline to 1:20. Further dilutions were carried out as described above. The titres obtained with this positive control varied between 1:2560 and 1:10 240 in the latex fixation tests and 1:1280 and 1:5120 in the SCAT. Control negative sera and diluents alone gave negative results in all the tests.

Results

Table 1 shows the numbers of positive tests in each of the groups tested. Sera from patients with classical RA were all positive by each of the LFTs and all but 2 by SCAT in addition. Patients with definite RA

Table 1 Number of positive results by each test in each group tested

Source of sera	No. tested	No. of positive tests			
		LFT-E	LFT-A	LFT-R	SCAT
Rheumatoid arthritis					
Classical	15	15	15	15	13
Definite	29	21	16	20	19
Probable	4	1	1	1	0
Classical and definite	44	36	31	35	32
Total	48	37	32	36	32
Control patients	43	6	6	4	1
Blood transfusion sera	98	7	10	7	1

had a lower percentage of positive results, ranging from 55%, with the LFT-A to 72% with the LFT-E. One seronegative patient in this group had subcutaneous nodules; he was on steroid therapy at the time of testing. One out of the 4 patients with probable RA was seropositive by each of the LFTs.

Table 2 shows the titres obtained in the 44 cases of classical and definite RA. There was a significant positive correlation between the titres obtained with each of the tests in these cases, with the latex tests showing a higher degree of correlation with each other than with the SCAT (Table 4). All 4 tests were in agreement (positive or negative) in 36 (75%) cases and 1 test alone was positive in only 2 (4%) cases.

Out of the 141 blood donors and control patients 24 were seropositive by at least 1 test, and the remainder were negative by all tests. In 2 cases high

Table 2 Titres obtained by LFT and SCAT in 15 cases of classical and 29 cases of definite RA

Case no.	LFT-E	LFT-A	LFT-R	SCAT
Classical RA				
1	>320	>320	>320	>1024
2	>320	>320	>320	512
3	>320	>320	>320	128
4	>320	>320	>320	64
5	>320	>320	>320	<16
6	>320	320	160	>1024
7	>320	320	160	1024
8	>320	320	80	512
9	>320	320	80	32
10	>320	160	>320	32
11	>320	160	160	>1024
12	320	160	160	64
13	160	>320	160	>1024
14	160	>320	160	256
15	160	160	160	16
Definite RA				
16	>320	>320	>320	512
17	>320	>320	>320	512
18	>320	>320	>320	512
19	>320	>320	>320	128
20	>320	>320	>320	16
21	>320	>320	320	>1024
22	>320	>320	320	>1024
23	>320	>320	320	512
24	>320	>320	320	512
25	>320	>320	320	256
26	>320	>320	320	64
27	>320	>320	320	64
28	>320	320	160	>1024
29	>320	320	80	>1024
30	>320	40	160	>1024
31	>320	<40	320	<16
32	320	320	160	>1024
33	320	40	160	64
34	160	320	40	<16
35	160	40	80	512
36	80	<40	40	64
37	40	40	40	<16
38	40	<40	80	16
39	40	<40	40	<16
40	40	<40	<40	<16
41	<40	40	40	128
42	<40	<40	40	<16
43	<40	<40	<40	<16
44	<40	<40	<40	<16

Table 3 Titres obtained by LFT and SCAT in 13 out of 43 control cases and 11 out of 98 blood donor sera seropositive by at least one test

Case no.	LFT-E	LFT-A	LFT-R	SCAT
Control patients				
1	320	<40	320	256
2	160	160	40	<16
3	160	80	40	16
4	80	<40	40	<16
5	80	<40	40	<16
6	80	<40	<40	<16
7	40	160	40	<16
8	40	80	40	<16
9	40	<40	80	<16
10	<40	160	<40	<16
11	<40	80	<40	<16
12	<40	<40	80	<16
13	<40	40	80	<16
Blood donors				
14	320	160	160	128
15	160	80	80	<16
16	160	80	80	<16
17	160	<40	80	<16
18	80	160	80	<16
19	80	80	80	<16
20	80	80	80	<16
21	<40	160	<40	<16
22	<40	160	<40	<16
23	<40	80	<40	<16
24	<40	80	<40	<16

Table 4 Correlation between LFTs and LFTs with SCAT in 44 cases of classical and definite RA

Test	r*	P
LFT-A	0.7828	<0.001
LFT-R		
LFT-E		
LFT-R		
LFT-E	0.7798	<0.001
LFT-A		
LFT-E		
LFT-A		
SCAT	0.6862	<0.001
LFT-A		
LFT-E		
LFT-R		
SCAT	0.5133	<0.001
LFT-A		
LFT-E		
LFT-R		
SCAT	0.4566	<0.001
LFT-A		
LFT-E		
LFT-R		
SCAT	0.3067	0.022
LFT-A		
LFT-E		
LFT-R		

*r=Spearman's correlation coefficient with patients being ranked by titre.

titres of rheumatoid factor were found, but in the others the titres tended to be lower than in the seropositive cases of RA (Table 3). In addition there was less agreement between the tests, all 4 tests being positive in only 1 case and 1 test alone being positive in 14 cases.

Discussion

Our observations have shown a high frequency of positive tests for RF in the sera of Kenyan patients with RA that is 77% positive by LFT-E and 67% by SCAT. In cases with classical or definite RA these percentages rise to 83% and 73% respectively. These figures are similar to those reported from Europe and the USA (Singer and Plotz, 1956;

Cecchi and Ferraris, 1959; Harter, 1959; Houba and Tesarek, 1961; Wager *et al.*, 1961; Waller *et al.*, 1961; Ball *et al.*, 1962; Winblad, 1964) and to those previously reported from East Africa. In Kenya, using a slide latex test only, Hall (1966) reported 5 out of 6 cases as being seropositive, and Bagge *et al.* (1976) found 46 out of 60 cases of classical or definite RA to be seropositive. In Uganda Karayerezi (1969) reported 20 out of 25 sera from patients with classical, definite, or probable RA to be seropositive by LFT and/or SCAT. The high prevalence of seropositivity in Kenyan patients with rheumatoid nodules accords with the findings in European and American studies (Ziff, 1957; Block and Buning, 1959; Hill and Greenberg, 1965; Sievers, 1965; Houba, 1970).

The frequency of RF positivity in patients with RA in West Africa is reported to be much lower (Greenwood (1969) and Greenwood *et al.* (1971) found 13% of cases positive by LFT and 7% by HEAT (human erythrocyte agglutination test) in 53 Nigerian patients with definite or probable RA. The tests and techniques used were comparable to those employed in this study. One possible explanation for these differences is that in the Nigerian study many more cases with probable RA were included than in our series, but this cannot be ascertained from the data given.

We have found a positive LFT-E in 14% of control patients and 7% of blood donors. A similarly high prevalence rate for the presence of rheumatoid factor has been found in population studies in several African countries including Liberia (Malawista *et al.*, 1959; Hijmans *et al.*, 1964) Nigeria (Valkenburg and Hijmans, 1961; Greenwood, 1969; Greenwood *et al.*, 1971), Uganda (Shaper *et al.*, 1968) and South Africa (Beighton *et al.*, 1975). A previous study in Kenya showed that both SCAT and LFT were positive in 6-7% of a population in the Nairobi area, but that higher figures of 15-17% were present in members of the Luo tribe living in malarial areas around Kisumu (Houba and Allison, 1966). In this study, however, a positive SCAT was found in 1 control patient and in 1 blood donor only.

Several reasons for a higher prevalence of RF positivity in the sera of Africans have been postulated. In particular it has been suggested that there is an association with the high prevalence of chronic infections occurring in the populations studied, for example, malaria (Houba and Allison, 1966; Wells, 1967; Shaper *et al.*, 1968; Greenwood, 1969; Greenwood *et al.*, 1971), trypanosomiasis (Klein and Mattern, 1965; Houba and Allison, 1966; Klein *et al.*, 1970), kala-azar (Houba and Allison, 1966), leprosy (Cathcart *et al.*, 1961; Abe *et al.*, 1967), and

pulmonary tuberculosis (Singer *et al.*, 1962). To date no direct evidence is available to confirm a causal relationship.

A differing reactivity of RF with globulins of European and African origin has been reported from Nigeria (Greenwood *et al.*, 1971), and was found in some of our cases. Indeed in the cases of classical and definite RA the correlation between LFT-E and LFT-A was lower than that between either of them and LFT-R. Genetic factors must be considered amongst the possible causes of such differences, with special reference to the allotypic and idiotypic specificities (Hollander and Rawson, 1968; Ropartz *et al.*, 1971).

It has been suggested that a latex test using rabbit gammaglobulin may be even more specific for RA than the SCAT (Lawrence, 1977). In the total series here the LFT-R differed from all other tests in only 4 sera and showed a larger number of positive results than the SCAT in both control patients and blood donors. Interestingly, in the cases of classical and definite RA the correlation between LFT-R and SCAT, being the 2 tests employing rabbit immunoglobulin, was the lowest obtained. The reason for this is not apparent.

In conclusion, our observations have confirmed a high frequency of RF in Kenyan patients with classical or definite RA. In this respect there is a marked differences between our data and that obtained from West Africa. A collaborative study between East and West African centres in a form suggested in a recent WHO memorandum (1974) with the intention of elucidating the cause for these differences would be of great interest. In particular, further study is required of the influence of genetic factors (Brewerton, 1975), the part played by anti-globulins of different immunoglobulin classes (Torrigiani and Roitt, 1967), and the possible effect of circulating and intra-articular immune complexes (Zubler *et al.*, 1976).

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