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Original Article

A study of immunological profile, disease characteristics and socioeconomic status of a population of rheumatoid arthritis patients in Sri Lanka

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ABSTRACT

Objective: To study the immunological profile, disease characteristics and socioeconomic status of a population of patients with rheumatoid arthritis (RA) in Sri Lanka.

Methods: A case-control study was undertaken to characterize the immunoglobulin profiles of 105 RA and, age and gender matched osteoarthritis (OA) patients ($n = 30$) from the National Hospital, Sri Lanka. Healthy, non-arthritic individuals ($n = 30$) served as controls. Sera were assayed for immunoglobulins [IgG, IgM, IgE and IgA isotypes] by establishing sandwich type ELISA. IgM, IgG and IgA rheumatoid factors (RFs) of 162 RA patients were assayed by indirect ELISA. Disease characteristics and socioeconomic factors were accrued via an interviewer-administered questionnaire.

Results: Higher IgG, IgM, IgE, IgA and lower IgG1, IgG2 levels were observed in RA sera compared with controls ($P < 0.05$). Novel correlations between disease characteristics and immunoglobulins, as well as group-specific correlation matrices of immunoglobulins and RFs ($P < 0.05$) of seropositive and seronegative patients, were found. Higher IgM-RF and IgA-RF levels in seropositives and IgG-RF in seronegatives were evident compared with controls ($P < 0.05$). Immunoglobulin and RF profiles did not reflect gender disparity of RA ($P > 0.05$). Proportions of seropositives with nodules and erosions were significantly higher than seronegatives ($P < 0.05$). While IgM-RF and erosions positively correlated in the seropositives ($P < 0.05$), the seronegatives showed an inverse correlation between IgG-RF and erosions ($P < 0.01$). Familial clustering imposed a relative risk of 4.7 for developing seropositive RA.

Conclusions: This model study provides baseline information on pathogenetic aspects of RA in Sri Lanka, which may have implications for further research on management of the disease.

Keywords: *Disease profile, ELISA, erosions, immunoglobulin profiles, nodules, rheumatoid factors, risk factors, seronegative, seropositive, socioeconomic factors.*

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune disease of widely varying expression and severity.[1,2] Although the first putative cases were described centuries ago,[3] the origin and evolution of RA remains an enigma to-date.[4] RA initiates from heterogeneous events[5] and its aetiopathogenesis continues to be a challenge.[6] Determination of serum immunoglobulin concentration is of potential importance in understanding pathogenetic processes involved in autoimmune and inflammatory disorders.[7] The rheumatoid factors (RFs) that are the specific autoantibodies directed against antigenic determinants on the Fc fragment of IgG are of four types namely, IgM-RF, IgA-RF, IgG-RF, and IgE-RF. However, their role in the pathogenesis of RA still remains obscure.[8] Furthermore, polyclonal B cell activation leading to hypergammaglobulinemia[9] and many other recognized or yet unrecognized autoantibody systems[10] are identified as immunological abnormalities that herald RA. Hence, the study of an isotype, which constitutes only a fraction of the

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immunoglobulin pool, will be of limited value. However, the contribution of all isotypes involved in the immunological process, may throw some light on aetiopathogenesis of RA.[10]

Female preponderance[11] and genetic predisposition[12] have been reported in RA. However, concordance rates in homozygous twins[13] and ethnicity[12] have supported the notion of environmental risk factors.[14] Even in egalitarian societies, hitherto unexplained environmental and lifestyle factors influence the risk of RA.[15] Hence, investigation into constitutional risk factors predicting RA remains an ongoing challenge that may offer novel approaches of disease management.[16] Data on RA in South Asia remain scanty. Thus, attempts were made in this study to investigate the associations of serum immunoglobulins and of RFs, with disease characteristics and gender disparity.

MATERIALS AND METHODS

Following approval of the Ethical Review Committee of the Faculty of Medicine, University of Colombo, Sri Lanka, the participants were recruited from the outpatient tertiary care treatment unit of the Rheumatology and Rehabilitation Unit of the National Hospital, Sri Lanka (NHSL). Informed consent was obtained. A total of 105 RA patients (1987 revised ACR criteria), 30 osteoarthritis (OA) patients from the NHSL as disease-controls, and 30 individuals asymptomatic for and with no history of musculoskeletal diseases from Colombo as healthy normals (HN) were enrolled for the assay of immunoglobulins.

In addition to those 105, a further 57 patients (seropositive $n = 30$; seronegative $n = 27$) were enrolled in the assessment of RF profiles only. However, the socioeconomic data were accrued from all 162 (105 + 57) RA patients. The collection of blood samples and the socio-economic survey was carried out from February 2006 to January, 2007. Five millilitre of venous blood was drawn under aseptic conditions and serum was stored at −20°C in aliquots.

The interviewer-administered questionnaire drawn up with the help of a medical sociologist and approved by the ethical review committee was validated using 10% of each study group. Additionally, clinical and verbal verifications were employed as data collecting instruments.

Assay of serum immunoglobulins

Levels of total IgG, IgM, IgE, IgA and IgG subclasses were determined by a 'sandwich type' enzyme linked immunosorbent assay (ELISA) developed and validated at the Immunology Laboratory of the Department of Zoology, Faculty of Science, University of Colombo, Sri Lanka. Checkerboard titrations were carried out to determine the optimum dilutions of monoclonal antibodies (mAbs), human IgG-Fc fragments, serum and conjugate.

Microtitration plates (Immulon 2HB, Dynatech Laboratories, USA) were coated with 100 µL of optimum concentration of antihuman mouse mAb; (IgM and IgG, 3 µg/mL; IgE, 0.4 µg/mL; IgA, 6 µg/mL; IgG1, 4 µg/mL; IgG2, 13 µg/mL; IgG3, 12 µg/mL; IgG4, 5 µg/mL) (Sigma Chemicals Co., St. Louis, USA) in phosphate buffer (PB; pH 7.4) and were incubated overnight at 4°C. Wells were blocked with 5% w/v non-fat milk and incubated for 1 hour. Following washing, appropriate serum dilutions (1:25–1:100) were dispensed into wells. Following 1-hour incubation, plates were washed and incubated with peroxidase conjugated polyclonal rabbit anti-human antibodies (DAKO immunoglobulins, Denmark) at optimum concentration (1:1000–1:3000). ABTS (2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) (Sigma Chemicals Co.) 0.55 mg/mL in substrate buffer with 0.04% H_2O_2 (Sigma Chemicals Co.), were dispensed and the optical density of the coloured reaction (OD) at 405 nm was determined with an automated microplate reader (BioRad, model 680, USA).

Assay of RFs

Microtiter plates were coated with 100 µL of IgG-Fc (Southern Bio-Tech; USA) at optimum concentration (IgM-RF, 0.45 µg/mL; IgG-RF, 10 µg/mL; IgA-RF, 10 µg/mL), in PB. Following overnight incubation at 4°C, the wells were treated with blocking buffer and incubated at 37°C for 1 hour. Following washing, appropriately diluted sera were added to duplicate wells (IgM-RF; 1:50, IgG-RF; 1:25, IgA-RF; 1:50) and incubated at 37°C for 1 hour. RFs were detected with HRP conjugated antibodies (IgG-RF, Sheep anti-human λ; IgM-RF, F(ab)2 fragments of polyclonal goat IgG anti-human IgM (µ-Chain specific); IgA-RF, sheep anti-human peroxidase antibodies) (Sigma Chemicals Co.) at appropriate dilution (IgM-RF, 1:1000; IgG-RF, 1:1250; IgA-RF, 1:1000). Substrate (2,2-azino-bis(3-ethylbenthiazoline-6-sulfonic acid)) (Sigma Chemicals Co.) was added and incubated at 37°C. The optical density at 405 nm was measured using a micro plate reader.

Disease profile and socioeconomic survey

Personal information, clinical profile, pregnancy-related data and dietary history were recorded. Interviewer-administered

questionnaire, clinical records, clinician assisted verifications were used to obtain data on disease characteristics and history. Disease characteristics such as nodules, erosions and systemic involvement were recorded as manifestations of the disease at any time during disease progression. Hand x-rays of the RA group were analyzed to obtain data on joint destruction and erosions. Education (classified into several strata) and income were used as measures of socio-economic category. Information on monthly income was collected. A positive family history was considered as evidence for prior genetic exposure of the patient.

Statistical analysis

Statistical analyses were performed using SPSS 13.0 for windows (SPSS Inc, USA) and Epi Info 6 (version 6.04 b to c upgrade; CDC, USA & World Health Organization, Switzerland) software packages. Comparisons of normally distributed independent variables were performed using ANOVA with Bonferroni correction for multiple comparisons. Kruskal-Wallis test was used where appropriate, followed by pair-wise comparisons. Correlations between laboratory and clinical parameters, and host factors were determined using bivariate correlations. Proportions of responders for individual samples were compared using Chi-squared test. A logistic regression analysis was applied to determine the baseline risk factors of RA. Multinomial regression analysis was used to determine the optimal set of explanatory variables of the risk of RA in Sri Lanka. The level of significance was set at $P < 0.05$.

RESULTS

Mean age of disease onset and the mean disease duration of RA patients ($n = 162$) were 37 and 13 years, respectively. Serum immunoglobulins (Ig) were estimated in 105 patients with RA (73 RF positive and 32 RF negative). OA and HN groups of 30 each served as matched controls. Table 1 gives the clinical and demographic data. RFs were assayed in 162 patients with RA (103 RF positive and 59 RF negative). The test and the control groups were comparable in age and gender (ANOVA, $P > 0.05$). Table 2 gives the Ig levels (expressed as OD values) of the patients and controls.

Table 1 Demographic and clinical variables of study subjects

Group	*Age[a] (yrs)*	*Gender (F:M)*	*Duration of the disease[b] (yrs)*	*Range of disease duration (yrs)*
I. RA ($n = 103$)	51.21 ± 11.66	89:16	13.69 ± 11.00	1–52
(i) RF +ve ($n = 73$)	51.17 ± 11.64	61:12	15.23 ± 11.00	1–52
(ii) RF –ve ($n = 32$)	50.06 ± 11.79	28:04	8.5 ± 10.19	1–31
II. OA ($n = 30$)	55.1 ± 12.23	27:03	11.0 ± 10.8	1–50
III. Normal controls ($n = 30$)	49.9 ± 8.34	26:04	–	–

[a]Mean age ± standard deviation.
[b]Mean duration ± standard deviation.

Table 2 Serum immunoglobulin levels (expressed as $OD_{405\,nm}$ values)

Serum immunoglobulins[a]	*RA (n = 105)*	*Controls*			
		OA (n = 30)	*P[b]*	*Normal controls (n = 30)*	*P[c]*
IgM	1.2156 ± 0.0197	0.9286 ± 0.0360	0.000	1.0018 ± 0.0184	0.000
IgG	1.0922 ± 0.0173	1.0469 ± 0.0299	0.000	0.8724 ± 0.0256	0.000
IgE	0.7944 ± 0.0181	0.6048 ± 0.0211	0.000	0.7384 ± 0.0223	0.101
IgA	0.6266 ± 0.0236	1.450 ± 0.0293	0.000	0.8607 ± 0.0323	0.000
IgG1	0.4030 ± 0.0112	0.3717 ± 0.0175	0.212	0.4964 ± 0.0288	0.000
IgG2	0.2393 ± 0.0068	0.3107 ± 0.0011	0.000	0.3181 ± 0.0099	0.000
IgG3	0.2001 ± 0.0052	0.2106 ± 0.0066	0.288	0.2192 ± 0.0061	0.052
IgG4	0.3060 ± 0.0058	0.3249 ± 0.0044	0.090	0.3015 ± 0.0097	0.687

[a]Mean optical density at 405 nm ± SEM.
[b]Significance value of comparison of the $OD_{405\,nm}$ of RA sera with OA sera by one-way ANOVA.
[c]The significance value of comparison of the $OD_{405\,nm}$ of RA sera with healthy normal sera by one-way ANOVA.

Among the isotypes, IgM was elevated in RA and HN groups, followed by IgG. In RA, IgE ranked third followed by IgA, while in HN it was *vice-versa*. In contrast, the level of IgA was high in OA, in whom IgG was a close second followed by IgM and IgE, respectively. Cytophilic IgG1 was the most represented IgG subclass in all the study groups. The level of IgG4 ranked second in both RA and OA groups, in contrast with the HN in which IgG2 ranked second.

IgM and IgG levels of the RA patients were markedly elevated than those of healthy control sera (ANOVA; $P < 0.05$). Further, the level of IgG was higher than that of OA sera. However, the level of IgA in the RA group was higher than both the control groups (ANOVA; $P < 0.05$). IgE of the RA group was significantly higher than that of the OA group only (ANOVA; $P < 0.05$).

IgG1 was significantly reduced in RA sera than in the healthy controls, while IgG2 levels of RA patients were significantly lower than those of both control groups (ANOVA; $P < 0.05$). The differences in levels of serum IgG3 and IgG4 between RA and control groups were not significant (ANOVA; $P > 0.05$).

Immunoglobulin profile of seropositive and seronegative RA

Both seropositive and seronegative groups had elevated IgM levels followed by IgG, IgE and IgA, respectively. Among the IgG isotypes, IgG1 response was markedly high in both groups, trailed by IgG4. In both groups IgG2 and IgG3 responses were low. The IgM, IgG, IgE and IgA levels of seropositive sera were significantly higher than those of the seronegative, group (ANOVA; $P < 0.05$). In IgG isotype analysis, an apparent elevation of serum IgG1 and IgG3 was evident in seropositive sera than their negative counterparts (ANOVA; $P < 0.05$). Seropositive group exhibited a significant decline in IgG2 than their negative counterparts (ANOVA; $P < 0.05$). For IgG4, there was no significant difference in levels among both RA groups (ANOVA; $P > 0.05$).

Correlations between serum immunoglobulins

Correlations of the Ig levels were examined within each study group. Significant correlations were demonstrated in RA sera between Igs; (i) IgM with IgG ($r = 0.346$), IgG1 ($r = 0.338$) and IgG3 ($r = 0.340$), respectively; (ii) IgG with IgG1 ($r = 0.419$); (iii) IgA with IgM ($r = 0.546$), IgG2 ($r = 0.211$) and IgG3 ($r = 0.246$), respectively; (iv) IgG2 and IgG1 ($r = -0.333$) levels were significantly but inversely correlated. It is noteworthy that IgG4 of RA was not involved in correlations. All the associations were significant at $P < 0.001$ and with similar level of strength. Both seropositive and seronegative groups shared associations of IgG with IgG1 and IgG3 (Table 3).

Table 3 Significant correlations in immunoglobulin levels of seropositive and seronegative sera (by Pearson's correlation coefficient)

Associations	*Seropositive*		*Seronegative*	
	P value	*Strength (r)*	*P value*	*Strength (r)*
IgG-IgG1	< 0.01	0.304	< 0.05	0.372
IgG-IgG3	< 0.01	0.403	< 0.01	0.425
IgG-IgE	< 0.01	0.433	–	–
IgG-IgM	–	–	< 0.05	0.425
IgM-IgG3	–	–	< 0.05	0.360
IgE-IgG3	< 0.01	0.313	–	–
IgA-IgG3	–	–	< 0.01	0.533

Associations of IgM with both IgG and with IgG3, as well as IgA with IgG3 were restricted to the seronegative group. The strong concordance of IgE with IgG and IgG3 was apparent only in the seropositive group. Neither IgG2 nor IgG4 were associated with any of the significant correlations (Table 3).

RF profiles

RF profiles of the study groups are shown in Table 4. IgM-RF levels of the seropositive RA group were markedly elevated than those of the other three groups (ANOVA; $P < 0.05$). Although the level of IgG-RF of the seropositives did not significantly differ from that of the seronegatives (ANOVA; $P > 0.05$), it was significantly higher than that of the controls (ANOVA; $P < 0.05$). The level of IgA-RF of seropositive patients was significantly higher than that of seronegatives and healthy normals (ANOVA; $P < 0.05$).

Correlations between RFs and serum immunoglobulins

Correlations between RF and other serum immunoglobulin levels were examined within each study group (Table 5). Significant correlations were demonstrated in RA sera between, (i) IgM-RF of seropositive RA group with IgE ($r = 0.464$, $P < 0.01$) and IgG3 ($r = 0.288$, $P < 0.05$), respectively, and (ii) IgG-RF with IgG ($r = 0.607$, $P < 0.01$), IgG1 ($r = 0.382$, $P < 0.05$), and with IgG3 ($r = 0.488$, $P < 0.001$)

Table 4 Comparison of RFs, IgM-RF, IgG-RF and IgA-RF among the four study groups

RF	*Seropositive RA (OD_{405nm})*	*Seronegative RA (OD_{405nm})*	*OA (OD_{405nm})*	*Healthy control (OD_{405nm})*
IgM-RF[a]	0.4171 (±0.2361)	0.1172[b*] (±0.1151)	0.0186[b**] (±0.0830)	0.0027[b**] (±0.0610)
IgG-RF[a]	0.3669 (±0.2736)	0.3159 (±0.1653)	0.1536[b*] (±0.1378)	0.2055[b*] (±0.0809)
IgA-RF	0.7614 (±0.2421)	0.5352[b*] (±0.2754)	0.6871 (±0.2387)	0.3762[b*] (±0.2819)

[a]The mean optical density at 405 nm ± SEM.
[b]Comparison with the seropositive group by one-way ANOVA.
*Significant at the 0.05 level.
**Significant at the 0.01 level.

Table 5 Correlations between rheumatoid factors and other serum parameters

Association between serum parameters	*Strength of Pearson's correlation coefficient (r)*			
	Seropositive RA group	*Seronegative RA group*	*OA*	*Healthy control*
IgM-RF/IgE	0.464**			
IgM-RF/IgG3	0.288*			
IgG-RF/IgG		0.607**		
IgG-RF/IgG1		0.382*		
IgG-RF/IgG3		0.488**		
IgG-RF/IgE			0.452*	
IgG-RF/IgM-RF				0.551*
IgG-RF/IgG4				0.550**
IgG-RF/IgA		0.307*		
IgM-RF/IgA	0.405*			
IgG-RF/IgA		0.367*		
IgA-RF/IgM			0.405*	
IgG-RF/IgA				0.370*

*Significant at the 0.05 level.
**Significant at the 0.01 level.

respectively. A correlation was evident between IgG-RF and IgE of the OA group ($r = 0.452$, $P < 0.05$). Further, exclusive correlations were detected in the HN group between IgM-RF with IgG-RF ($r = 0.551$, $P < 0.05$), IgM-RF with IgG4 ($r = 0.550$, $P < 0.01$) and IgG-RF with IgG4 ($r = 0.495$, $P < 0.05$) (Table 5).

Disease characterization

The clinical disease of the RA patients was categorized according to presence of nodules, radiographic erosions and systemic involvement. Among the RF-positive patients, the prevalence of erosions, nodules and systemic involvements were 38%, 16% and 7%, while in the seronegatives these values were 21.05%, 3.5% and 12.28%, respectively. The percentage of erosions and nodules of seropositives was significantly higher compared with seronegative patients (Chi-squared test; $P < 0.05$). However, the percentage of systemic involvements did not differ between seropositive and seronegative groups (Chi-squared test; $P > 0.05$). A single seropositive patient was diagnosed with erosions coupled with systemic involvement. Percentage of seronegative patients who did not suffer from any of these three disease characteristics was significantly higher than their seropositive counterparts (Chi-squared test; $P < 0.05$).

Serum immunoglobulins and RF as a function of disease characterization

Levels of immunoglobulins did not significantly differ either between the nodular and the non-nodular disease groups or erosive and non-erosive disease groups (Kruskal-Wallis; $P > 0.05$). Serum immunoglobulins were exclusively and significantly related with nodular disease ($P < 0.05$). In contrast, the non-nodular disease sera demonstrated the following correlations at $P < 0.05$; IgE with IgG, IgG1 and IgG3 ($r = 0.435$, 0.265, 0.281 respectively) and IgG with IgG1 and IgG3 ($r = 0.258$, 0.410).

Erosions were weakly correlated with IgE level ($r = 0.277$, $P < 0.05$). There was a strong correlation between IgG and IgM ($r = 0.524$, $P < 0.01$) of erosive patients. A significant inverse correlation was demonstrated between IgG3 and IgG4 in the group positive for both nodules and erosions ($r = -0.733$, $P < 0.05$). The isotype associations present in non-nodular-disease sera were apparent in the non-nodular non-erosive form as well. IgA profile was not associated with either disease characteristic. A significant correlation was evident between IgM-RF and erosions of the seropositive RA group ($r = 0.199$, $P < 0.05$), while a significant inverse correlation was demonstrated between IgG-RF and erosions of seronegative RA group ($r = -0.458$, $P < 0.01$).

Gender disparity of immunoglobulins and RFs in RA

Though female preponderance was pronounced among local incident cases, the gender disparity of Igs and RFs among test groups was insignificant (Kruskal-Wallis test; $P > 0.05$).

Socio-economic status and the risk of RA

RA group recorded a high degree of positive family history than the controls (Chi-squared test; $P < 0.05$). Seropositive patients exhibited a statistically significant level of familial clustering than the seronegatives, with proportions of 31% and 14.1%, respectively (Chi-squared test; $P < 0.05$).

Manual occupation was more common among the seropositives than the HN group (Chi-squared test; $P < 0.05$). 10.1% of the seropositives and only one seronegative individual were reported with permanent work cessation. The postpartum period seemed to be of high risk as 15.6% seronegative and 19.8% seropositive females complained of disease onset within a year of delivery. During the first 5 years of delivery, 35.5% of seropositive and 31.25% seronegative females have developed the active disease.

The proportion of individuals with tertiary education was higher in the HN controls than in both RA groups (Chi-squared test; $P < 0.05$). Income level of both RA and OA groups were lower than that of the HN group (ANOVA; $P < 0.05$). The dietary habits of the study groups were comparable (Chi-squared test; $P > 0.05$).

Relative risk of RA owing to the sociodemographic variables were determined via multinomial and logistic regression analyses, where familial clustering imposed a relative risk of 3.95 (CI: 1.179–13.24). Further, genetic predisposition was apparent on seropositives over seronegatives with a relative risk of 4.844.

DISCUSSION

Immunoglobulin and RF profiles of Sri Lankan RA patients were characterized using in-house established ELISAs. Among Ig abnormalities, polyclonal B cell activation[9] leading to augmentation of IgM, IgG and IgA have been recorded in RA.[17–19] Further, IgE augmentation[20] exclusively in seropositive RA was recorded.[21] The current study confirmed elevated levels of IgG. The actuarial reason for IgG elevation was unknown, but may be due to augmentation of galactose-free IgG,[22] antiphospholipid and antioxidized low-density lipoprotein antibodies in RA.[23] Confirming previous findings,[17–19,24] RA sera reported augmentation of IgM in this study. In contrast, IgA demonstrated an extraordinary decline.

Mechanisms underlying the IgG augmentation of sera also influence the subclass distribution.[25] Among IgG subclasses, IgG1 was the most prevalent followed by IgG4[25] as was evident in the present study. IgG1 preponderance reflected the augmentation of IgG1-AhFibA (anti-human fibrinogen autoantibodies) in RA sera.[26] Despite prevalence data, the effector mechanisms of IgG4 are evasive. However, the IL-4 stimulation of B cells was regarded to be responsible for the IgG4 class switch. Also, augmentation of IgG4 in response to exogenous antigens supported the role of infectious agents in the aetiopathology.[26] IgG2 levels of RA serum measured by ELISA were not apparent.[27] However, this study revealed a rather unusual observation of IgG2 dwindling, which may be due to conformational differences in disease-specific IgG2.[27]

In contrast to the marginal elevation of IgE in RA than the healthy controls was not significant.[21] However, IgE level was relatively high in seropositives. This may be due to hindrance of IgE owing to the presence of complexed anti-IgE in sera. Although the typical explanation of IgE mediating inflammation in a chronic disease such as RA has been questioned, the enhanced expression of CD40 in rheumatoid synovium and CD40L by synovial T cells are believed to be responsible in production and isotype switching to IgE. Furthermore, it was shown that TNF-α acts synergistically with IL-4 in the induction of IgE.[21]

IgG-RF of seropositives was significantly higher than the OA and HN controls. In contrast to previous records[21] the disparity of IgG-RF was not pronounced between seropositives and seronegatives. IgM-RF was markedly elevated in seropositive RA than in seronegatives, HN and OA groups. The underlying reason for this elevation remains elusive.

A novel correlation of serum IgG with immune complexes in seropositive synovium has been observed.[28] Also, it has been evident that serum IgM, IgG and IgA strongly correlate with the disease activity of RA.[29] There were many disease-specific correlations of Igs and RFs in RA sera but

with the paucity of research the interpretation has become a challenge. It was suggested that the strong degree of concordance between Ig in patient sera reflected that heterogeneous antigens are involved in generating these responses.[8]

Subcutaneous nodules and erosions were exclusive to the seropositive group. The only major differences detected on blind assessment had been greater tendency to deformity, erosions and nodules in the seropositives.[30] Further IgM-RF correlations with clinical manifestations and severity of erosions were observed. Moreover, several studies identified IgM-RF as the "culprit".[31] It was suggested that a subpopulation of patients, diagnosed on clinical, radiological and pragmatic grounds, but with RF-negativity, represents a quite distinct clinical entity from seropositive RA.[30,31] With the observed degree of disparity in levels of Igs, RFs, disease-characterization and correlation matrices, seronegative RA can be identified as a different disease entity on immunological grounds.

As in previous reports[32] radiological erosions were not correlated with IgG, IgM, and IgA, except for a significantly weak concordance with IgE [$r = 0.277$, $P < 0.05$]. In contrast IgM-RF was significantly correlated with the presence of erosions, supporting previous observations.[33] It was surmised that the inverse correlation of IgG-RF with erosions revealed a protective mechanism in seronegative RA.

While augmentation of immunological data provides optimism in the development of successful RA therapies, investigation into environmental risk factors predicting RA offers new insights to disease management.[16] Familial clustering is a significant risk factor in Sri Lanka.[6] A positive family history imposes a risk of 3.95 in developing RA. This study further revealed a strong association between a positive family history and seropositive RA with a 4.8 fold risk of developing the seropositive form, over the seronegative form.

Though low socio-economic status, manual occupation, and lower level of education were pronounced in RA patients, mostly among seropositive individuals, this may be the selection bias of the type of patients reporting to this hospital. The postpartum period was identified as a risk factor of RA confirming previous observations.[16] The risk factor profile offers opportunities for primary and secondary prevention of RA.

This was the first study which investigated serology, disease characteristics and socio-economic aspects of RA in Sri Lanka. While C-reactive protein (CRP) measures disease activity, IgM-RF is one of a few parameters that are indicative of the severity of the seropositive disease. The sensitivity of ELISA is approximately 1000 fold higher than the widely used conventional RF agglutination assays.[3] Therefore, the use of IgM RF detected by ELISA as a prognostic tool is highly recommended. The in-house developed RF ELISAs are more cost-effective than those available commercially.

Studies from Asia on RA are scanty. Therefore, research has to be promoted in order to understand the interregional and intraregional disease variations.[2] Hence, prospective studies on sociodemographic, immuno/molecular-genetic factors of RA in Sri Lanka are decisive requirements. Further, investigating the scientific basis of the effectiveness of herbal drugs used in ayurvedic and indigenous health systems, using animal models is another possible facet of RA research in Sri Lanka.

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