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Rheumatoid Arthritis Patients with Acute Myeloid Leukaemia

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The aim of this study was to examine the difference in serum immunoglobulin concentrations in the sera of rheumatoid arthritis (RA) patients, RA patients with acute myeloid leukaemia (AML) and to compare with those found in normal subjects. This study was performed in 43 of normal adult individuals (group 1), 40 RA patients (group 2) and 37 RA individuals with AML (group 3) were used for analysis of immunoglobulin levels by SDS polyacrylamide gel electrophoresis and ELISA. Measurements were made before treatment in the group with AML as well as when patients were both on and off chemotherapy. It was found that group 1 had a mean IgA level of 3.0 mg mL^{-1} and IgM level of 1.82 mg mL^{-1}. Group 2 had a mean IgA level of 5.5 mg mL^{-1} and IgM level of 1.91 mg mL^{-1}, while group 3 had a mean IgA level of 1.2 mg mL^{-1} and IgM level of 0.95 mg mL^{-1}. Significant increases in IgA level in group 2 ($p<0.00001$) while the levels of both IgA and IgM in group 3 were significantly decreased ($p<0.00001$). This decrease was significant during induction and maintenance treatment, with restoration to normal once the patient was off therapy. There was no significant difference in IgG level in both groups 2 and 3 compared to group 1 ($p<0.53$). The finding of normal IgG level at diagnosis suggests that the leukaemia process itself did not initiate the fall. In addition, the influence course of treatment on rheumatoid arthritis patients with AML has a direct relation to the level of IgA and IgM due to complex interactions of chemotherapy.

Key words: Rheumatoid arthritis, leukaemia, IgA, IgG, IgM

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INTRODUCTION

Rheumatoid arthritis is a serious medical problem, with approximately 1% of the people in the world affected. The disease is autoimmune in nature and characterized by chronic inflammation of the synovial tissue in multiple joints, which leads to joint destruction, although the etiopathogenesis has not been elucidated completely[1]. Acute leukaemia is characterized by a progressive infiltration of neoplastic cell into the bone marrow, lymph nodes and other organs, including the CNS[2,3]. Extensive use of combination chemotherapy and radiation therapy has resulted in increased long-term survival of cancer patients[4,5]. Continuous maintenance treatment from leukaemia may be more immunosuppressive than intermittent treatment[6]. Some of these differences may be due to the effects of treatment, where the chemotherapy may affect preferentially those B-lymphocytes that are in the process of maturing into IgG-or IgA-secreting serum cells[7]. B-lymphocytes play a major pathogenetic role by the generation of autoantibodies, such as Rheumatoid Factors (RF) and antinuclear antibodies[8].

An increase in circulating IgA concentrations is a generalized phenomenon among RA patients and RA complications are associated with a significant increase in serum IgA concentration[9]. However, acquired and usually transient IgA deficiency has been observed following drug therapy[10]. This hypothesis is supported by the fact that these drugs known to promote immuno-regulatory imbalance can induce selective IgA deficiency and autoimmune phenomena, either separately or in association[11].

None of the foregoing authors studied the serum immunoglobulin concentrations in RA patients with AML. However, long-term treatment with chemotherapy can produce or exacerbate immune-mediated complications. The purpose of this study was undertaken to investigate these differences in serum immunoglobulin levels in both RA and RA with AML patients compared to normal subjects, moreover to analyze the experience with immune-mediated complications in RA patients with AML undergoing treatment. The finding of normal IgG at diagnosis suggests that the leukaemia process itself did not initiate the fall. Similar arguments may be applied to the fall in IgA and IgM, which has an even shorter half-life. RA individuals have a high level of IgA while RA subjects with AML have a low level of IgA and IgM. In addition, the influence course of treatment on RA patients with AML has a direct relation to the level of IgA and IgM due to complex interactions of chemotherapy.

MATERIALS AND METHODS

Anti-human IgA (IgG, IgM) antisera (raised in rabbit), human IgA (IgG, IgM), rabbit anti-human IgA (IgG, IgM) conjugated to horseradish peroxidase (HRP) and tetramethylbenzidine were purchased from Sigma (Sigma-Aldrich Company Ltd, UK) and all other chemicals were supplied from BDH (VWR International Ltd, UK).

Subjects: The study population consisted of 43 normal adult male individuals (group 1) as a control group, 40 adult male patients with rheumatoid arthritis with recent onset of RA symptoms (group 2) and 37 rheumatoid arthritis male individuals with AML (group 3) were used for analysis of immunoglobulin levels by enzyme-linked immunosorbent assay [ELISA]. At the time of study, both group 1 and group 2 were evaluated for age and clinical examination showed no fever or sign of infection. No patients with rheumatoid arthritis in-group 2 had been treated with immuno-suppressant drugs at the time of immunoglobulin level determination.

In description of patient's in-group 3 who infected with leukaemia, 4 patients with newly diagnosed AML were tested before treatment, 10 patients during induction therapy, 19 patients during maintenance therapy and 4 achieved a complete remission. The age at presentation was 19-65 years, with a median age of 40 years. The rheumatoid arthritis patients with AML were treated with different therapeutic protocols, depending on the policy of the hospital they were reported to. Complete remission was evaluated according to the normal clinical features, normal blood cell counts and bone marrow smears containing less than 5% of blast cells for more than a month. All sera were collected within three months and stored in small aliquots at -20°C until tested under code.

Analysis of serum samples by SDS-polyacrylamide gel electrophoresis: Human serum samples (2 μL) were diluted with 50 μL of 1x PBS (phosphate buffered saline; 0.25 M NaCl, 0.0268 M KCl, 0.081 M Na_2HPO_4 and 0.0146 M KH_2PO_4), mixed with 20 μL of 2x Laemmli sample buffer (0.125 M Tris-HCl pH (6.8), 4% (w/v) SDS, 20% (w/v) glycerol, 10% (v/v) β-mercaptoethanol, 0.005% (w/v) bromophenol blue) and 20 μL of water. Samples were incubated at 95°C for 5 min. A fraction of this mixture (20 μL) was electrophoresed overnight on a discontinuous 10% polyacrylamide gel containing 0.1% (w/v) SDS at a constant voltage of 45 V at room temperature[12]. Low molecular weight markers (Dalton mark VII-L, Sigma) contained BSA (66 kDa), ovalbumin (45 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa),

carbonic anhydrase (29 kDa), trypsinogen (24 kDa), soybean, trypsin inhibitor (21 kDa) and α-lactalbumin (14 kDa). Following electrophoresis, proteins in gel were visualized by staining with coomassie blue staining[13].

Immunoglobulin measurement by ELISA: Coating antibody [anti-human IgA (IgG, IgM) antiserum] was diluted 1 in 1000 in 1x coating buffer (0.02 M Tris-HCl, 1.5 M NaCl pH 9.0) and 100 μL was added to each of the wells of a microtitre plate[14,15]. After overnight incubation at 4°C the plate was washed 4 times with PBST20 (0.1% (w/v) [Tween 20 in 1x PBS (phosphate buffered saline; 0.25 M NaCl, 0.0268 M KCl, 0.081 M Na_2HPO_4 and 0.0146 M KH_2PO_4)]. Sites unoccupied by antibody were blocked by addition of 5% (w/v) Marvel (dried skimmed milk) in PBS for 1 h at room temperature followed by washing 6 times with PBST20.

The human serum samples were initially diluted 1 in 2000 in 1x PBS and 2 fold serial dilutions subsequently performed on the plate. Diluted samples were allowed to bind to the first antibody and the plate was then washed 6 times in PBST20. Rabbit anti-human IgA (IgG, IgM) conjugated to HRP [second antibody] was diluted 1 in 1000 in 1x PBS, 100 μL was added to each well of the microtitre plate, incubated at room temperature for 1 h and then washed 6 times in PBST20. The amount of bound second antibody was determined by adding 200 μL of the substrate solution [tetramethylbenzidine 6 mg mL^{-1} in 0.1 M sodium acetate buffer pH 6.0] to each well. After incubation in the dark at room temperature for 20 min, the reaction was stopped by adding 50 μL of 10% (w/v) H_2SO_4 to each well after which the absorbance of each well was measured at 450 nm. The concentration of IgA, IgG and IgM (mg mL^{-1}) was calculated from the standard dilution series. A standard curve was constructed by plotting absorbance against concentration for the standard solutions and the concentration of immunoglobulin in the samples was determined.

Statistical analysis: After tabulating the data, the arithmetic mean for each group was calculated. The variation or variability in each group was represented by the Standard Deviation (SD). The means of the groups were compared to see if the differences were significant. Student's t-test was used to assess the significance of the difference between groups.

RESULTS

The present study was designed to evaluate the levels of serum immunoglobulins and the effects of other associated diseases included rheumatoid arthritis and acute myeloid leukaemia. Serum samples from a number of individuals were reduced with β-mercaptoethanol, denatured in 10% SDS, electrophoresed on a polyacrylamide gel and stained with coomassie blue to visualize the polypeptides present in the samples (Fig. 1). Comparison of the stained polypeptides with molecular weight markers allowed identification of those bands derived from the immunoglobulin heavy (H) and light (L)

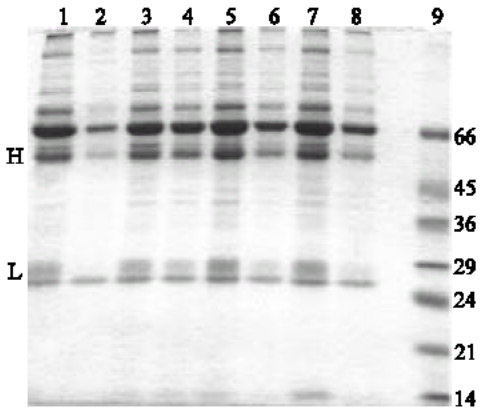

Fig. 1: Polyacrylamide gel electrophoresis of serum samples
Eight serum samples were mixed with 2x Laemmli sample buffer, heated at 95°C and then electrophoresed on 10% polyacrylamide gel. The proteins present were visualized by coomassie blue staining. Lanes 1, 3, 5 and 7 contain samples from rheumatoid arthritis individuals (each lane is from a separate patient). Lanes 2, 4, 6 and 8 contain serum samples from rheumatoid arthritis individuals with acute myeloid leukaemia. On the right are shown the position occupied by molecular weight markers; lane 9 (Dalton mark VII-L). H and L indicate positions of immunoglobulin heavy and light chains

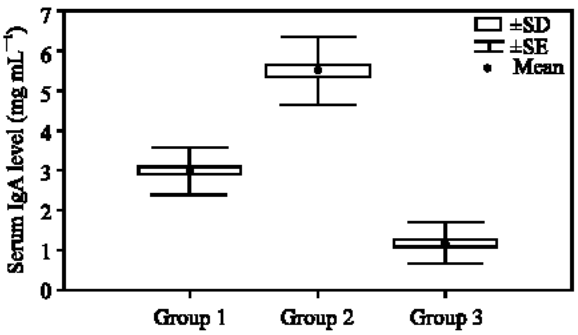

Fig. 2: Serum IgA levels in groups. Comparison of average serum IgA (mean±SD)

Table 1: Statistical analysis of IgA, IgM and IgG measured by ELISA. Group 3 were studied during treatment from AML

Study group	Case	Serum IgA level (mg mL^{-1})	Serum IgM level (mg mL^{-1})	Serum IgG level (mg mL^{-1})
1 (n=43)	Normal	3.0±0.58	1.82±0.68	9.37±1.29
2 (n=40)	RA	5.5±0.83*	1.91±0.54$^{\#}$	9.61±1.83^{+}
3 (n=29)	RA with AML	1.2±0.51**	0.95±0.30$^{\#\#}$	9.23±1.59^{++}

Values are mean±SD: *, **p<0.00001; $^{\#}$p<0.28 (NS), $^{\#\#}$p<0.00001; $^{+}$, $^{++}$p<0.53 (NS) compared with normal individuals

Table 2: Immunoglobulin levels measured by ELISA in RA patients with AML. Samples 1, 2, 3 and 4 were tested before treatment from AML. Samples 5, 6, 7 and 8 were tested after all chemotherapy had been discontinued

Sample ID	Serum IgA level (mg mL^{-1})	Serum IgM level (mg mL^{-1})	Serum IgG level (mg mL^{-1})
1	6.12	1.91	10.11
2	5.51	1.78	9.34
3	5.82	1.81	9.91
4	5.35	1.76	9.21
5	3.21	1.89	9.58
6	2.55	1.78	9.35
7	2.96	1.88	9.55
8	2.72	1.79	9.49

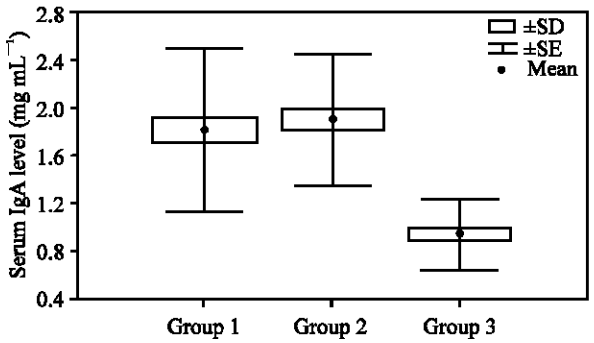

Fig. 3: Serum IgM levels in groups. Comparison of average serum IgM (mean±SD)

chains. Visual examination of the intensities of these bands showed that the level of immunoglobulin in rheumatoid arthritis serum samples was higher than that seen in rheumatoid arthritis with acute myeloid leukaemia serum samples. Therefore, ELISA then quantified the immunoglobulin concentrations.

Results showed that group 2 had a high level of IgA while group 3 had a low level of both IgA and IgM (Fig. 2 and 3). Statistical analysis of the immunoglobulin A and M (Table 1) revealed a significant increase in IgA level in group 2 ($p<0.00001$), while the levels of both IgA and IgM in group 3 were significantly decreased ($p<0.00001$). Non significant difference was found in IgG level in both groups 2 and 3 compared with group 1 ($p<0.53$). Immunoglobulin concentrations returned to normal when patient achieved a complete remission from AML (Table 2).

DISCUSSION

The results of this study have shown that rheumatoid arthritis patients with acute myeloid leukaemia on chemotherapy have reduced immunoglobulin A and M levels compared to normal individuals. Furthermore, total IgG concentrations were not significantly reduced in rheumatoid arthritis patients with AML, suggesting that the reduction in specific antibody was not reflection of the total immunoglobulin levels. The levels of IgA and IgM fell further during treatment, but recovered to normal values when treatment was discontinued. The underlying disease (AML) might cause a decrease in antibody production by bone marrow infiltration, where tumor cells provoke several alterations to normal regulatory T cells, which in turn, can impair the correct maturation of B cells. In addition, the direct inhibitory effect of B-leukaemic cells on immunoglobulin-secreting plasma cells may account for the humoral immunodeficiency[16], while cytotoxic treatment would produce defects in the cell-mediated response[17,18]. Present results suggest that these rheumatoid arthritis patients with AML have an impaired antibody production response and were less suffering from rheumatoid arthritis or autoimmune arthritis symptoms. This defect may provide a clue to reinterpret the events of immunodeficiency and autoimmunity.

Previous studies have shown that the mortality was lower in rheumatoid arthritis patients treated with methotrexate than in those not so treated, although maintenance therapy is usually carried out with methotrexate[19,20,21]. In accordance with these data, we suggest that the inhibitory effects of chemotherapy on both RA and AML may be bi-functional. Therefore, these findings bring us closer to achievement of remission and repair of structural damage in rheumatoid arthritis.

Furthermore, clinical data demonstrated that the immune system plays an important role in the control of hematological malignancies. An increased frequency of hematological malignancies is observed in immunodeficiency states. Reversal of the immuno-suppression is sometimes sufficient to induce tumor regression[22]. A number of humoral immune factors are present in the circulation in the early stages of RA patients and are thought to play an important part in the complex immunopathological interactions occurring during the establishment of the severe course of rheumatoid arthritis with systemic damages[23,24,25]. Rheumatoid arthritis patients with serum IgA level beyond the normal range were more likely to be positive for the autoantibodies[26].

The results of the present study pointed out that serum IgA levels were significantly higher in the rheumatoid arthritis group when compared with normal group since no patients with rheumatoid arthritis in-group 2 had been treated with immuno-suppressant drugs at the time of immunoglobulin level determination. Moreover, rheumatoid arthritis patients, had no clinical or laboratory findings indicating infection or other associated disease. Hence, the increased serum IgA concentration in the sera of rheumatoid arthritis suggests a possible role of IgA in the pathogenesis of the vascular complications. These results also indicate that high serum IgA levels reflect for disease activity of RA. This may reflect the increase production of autoantibodies and then, lead to humoral immune abnormalities.

Results showed that rheumatoid arthritis patients with AML who achieved a complete remission have a normal immunoglobulin level. We suggest that this normal concentration of immunoglobulin is due to off treatment with antileukaemic therapy in the remission phase. Thus, results in this report concluded that the influence course of treatment on rheumatoid arthritis patients with AML has a direct relation to the levels of immunoglobulin. The possible explanation for group 3 may be related to their malignancy as well as their state of differentiation and activation or it is uncertain, whether group 3 represents a variant of normal haemopoiesis, or whether it is due the clonal expansion of an abnormal stem cell damaged at the time of antileukaemic therapy. If the uptake of chemotherapy by leukaemic cells were a critical parameter for cell killing, it would be of importance to increase the intracellular drug concentration, which in turn lead to increment of toxicity.

In conclusion, the major goals of both rheumatoid arthritis and leukaemia treatment are prolongation of disease free survival and reduction of treatment associated toxicity. Moreover, the development of more efficient and less toxic antileukaemic treatment strategies could give the opportunity to design new combination regimens and would be to modulate cellular drug metabolism through introduction of new agents. Thus should be more focused research to study these complex interactions of chemotherapy, because this will allow us to understand the pathology of the immune system.

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