To investigate the effects of iron on GM-CSF production by synovial fibroblasts, three different concentrations of ferric or sodium citrate (0.01, 0.1, and 1 mmol/l at final concentration) were added to synovial fibroblast 96 hour culture with three different concentrations of IL1β (1, 10, and 100 ng/ml at final concentration). Ferric citrate (1 mmol/l) but not sodium citrate significantly enhanced GM-CSF production by synovial fibroblasts on stimulation with 1 ng/ml of IL1β (fig 1A). Ferric citrate (0.1 mmol/l) enhanced GM-CSF production by synovial fibroblasts on stimulation with 10 ng/ml of IL1β (fig 1B). Also, concentrations of 0.01 or 0.1 mmol/l ferric citrate enhanced production of GM-CSF by synovial fibroblasts on stimulation with 100 ng/ml of IL1β (fig 1C). However, ferric citrate at any concentration tested did not enhance IL6 or IL8 production by synovial fibroblasts on stimulation with any concentration of IL1β including 100 ng/ml (data not shown).

To test whether iron regulates the transcriptional level of GM-CSF production by synovial fibroblasts on stimulation with IL1β, GM-CSF, IL6 and IL8 mRNA expression in synovial fibroblasts were examined semiquantitatively by reverse transcriptase-polymerase chain reaction (RT-PCR). The method used for RT-PCR using specific primers for cytokines and glyceraldehyde-3-phosphate dehydrogenase as a control has been described in detail previously.[5] The expression of mRNA encoding these cytokines in synovial fibroblasts was undetectable without IL1, and was dose dependent on IL1β reaching a plateau after three hours in culture. Ferric citrate (0.1 mmol/l) significantly enhanced IL1 induced GM-CSF mRNA expression in synovial fibroblasts but not that of IL6 or IL8 (data not shown).

GM-CSF is produced by T cells, macrophages, mast cells, endothelial cells, and fibroblasts in response to specific activating signals. GM-CSF gene expression is controlled by binding of transcription factors such as NF-GMa, NF-GMb, NF-κB, Elf-1, NF-AT/AP1 complex, and Sp1 related complex to their specific promoter regions.[6] It is unknown which transcription factors participate in the iron mediated regulation of IL1 induced GM-CSF production by synovial fibroblasts. Dlaska and Weiss recently showed that iron regulates the transcription of inducible nitric oxide synthase (iNOS) of macrophage-like cells stimulated with interferon γ (IFNγ) and/or lipopolysaccharide, and binding of NF-IL6 to its consensus motif within the iNOS promoter was reduced by iron and enhanced by an iron chelator.[7] We found that iron enhanced IL1 induced GM-CSF production by synovial fibroblasts. Taken together, the intracellular iron levels might control GM-CSF production induced by IL1 by mechanism(s) similar to the action of iron-responsive element binding proteins on ferritin or transferrin receptor synthesis or iNOS induction.[8]

We found that the effects of iron on GM-CSF production induced by IL1 were different from those on IL6 or IL8 production. Agro *et al* reported that PGE_2 enhanced IL6 and IL8 but inhibited GM-CSF production by IL1 stimulated synovial fibroblasts.[9] Previously, we showed that iron decreased PGE_2 production by synovial fibroblasts.[3] Iron might enhance GM-CSF production through down regulation of PGE_2 production. Yoshida *et al* found that gold compounds and divalent metal ions inhibited induction of IL6 and IL8 but not production of GM-CSF by IL1 induced synovial fibroblasts through inhibition of NF-κB binding to DNA.[10] Thus, there may be different signal transduction pathways among these three cytokines in IL1 stimulated human synovial fibroblasts.

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Clinical analysis of gouty patients with normouricaemia at diagnosis

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Gouty attacks usually occur in patients with hyperuricaemia, but patients with gout who have normouricaemia are believed to be in the minority.[1 2] This is a common conception about gout because monosodium urate crystals are formed at blood concentrations of uric acid over 420 μmol/l at a body temperature of 37°C.[3] Recently, two studies showed that the incidence of gouty attacks in normouricaemia was as high as 39–43%,[4 5] which is much higher than our traditional view.[1–3] However, few data on normouricaemia in gout have been reported so far.[6–8] The prevalence of patients with gout who have normouricaemia at diagnosis has not yet been determined, and even their clinical characteristics and laboratory findings have not been reported.

Table 1 Comparison of the clinical and laboratory variables of normouricaemic gout and hyperuricaemic gout at diagnosis

	Normouricaemic gout (n=27)	Hyperuricaemic gout (n=81)*	p Value
Demographics			
Age (years)	60.6 (13.9)	54.2 (11.8)	0.02
First symptom to diagnosis (months)	32.2 (36.3)	54.2 (66.3)	0.03
BMI (kg/m^2)	23.8 (2.9)	23.1 (3.3)	NS
Associated conditions			
Hypertension	15	40	NS
Renal insufficiency†	5	28	NS
Heavy alcoholic intake	5	9	NS
Diabetes mellitus	2	11	NS
Precipitating events‡	11	35	NS
Associated diseases§	12	30	NS
Articular involvement			
First MTP	19	57	NS
Upper extremities	1	15	NS
Lower extremities	27	76	NS
Polyarticular involvement¶	11	45	NS
Documented tophi	4	36	0.006
Serum uric acid (μmol/l)	320 (80)	550 (90)	<0.001
24 Hour urinary uric acid excretion (mmol/day)	4 (1)	4 (5)	NS
BUN (mmol/l)	5.5 (2.0)	7.5 (1.5)	0.02
Creatinine (μmol/l)	100 (30)	140 (110)	0.002

BMI, body mass index; MTP, metatarsophalangeal joint; NS, not significant; BUN, blood urea nitrogen. *Patients with hyperuricaemia were randomly selected from the study group using a table of random sampling; †renal insufficiency was defined as a serum creatinine level above 130 μmol/l or a creatinine clearance below 1.00 ml/s; ‡precipitating events for an acute attack of gout are binge drinking, meat, surgery, infection, trauma, physical stress, and deterioration of underlying medical disease; §associated diseases are acute renal failure, chronic renal failure, renal transplantation, coronary artery occlusion disease, heart failure, stroke, and pulmonary tuberculosis; ¶polyarticular involvement was defined as more than one joint affected in a gouty attack.

We designed this study to determine the prevalence of normouricaemia in patients with gout at diagnosis, and to determine the natural course of normouricaemic gout and the differences between the clinical characteristics of patients with normouricaemic and hyperuricaemic gout at diagnosis.

We retrospectively reviewed 226 Korean patients who were newly diagnosed as having gout at the Severance Hospital, Yonsei University College of Medicine, Seoul, Korea, between January 1996 and May 2000. The diagnosis of gout was made during an acute attack of gouty arthritis and confirmed by either the presence of negatively birefringent needle shaped crystals or by satisfaction of the American College of Rheumatology criteria for gout.[9] Normouricaemia is defined as serum uric acid below 420 μmol/l in men and 360 μmol/l in women.[10] Patients taking urate lowering agents (allopurinol, probenecid) were excluded. Serum uric acid was determined in samples drawn when a gouty attack occurred at diagnosis by the uricase enzyme method.

Among the 226 gouty patients, 27 (12%) male patients showed normouricaemia at diagnosis. Table 1 summarises the clinical characteristics of the patients. Twenty one of 27 gouty patients with normouricaemia were followed up; among these patients, 17 patients (81%) developed hyperuricaemia at a median of one month after diagnosis (range: one week to 24 months) (fig 1), and only four patients (19%) still showed normouricaemia. These four patients were followed up at 11, 25, 28, and 34 months respectively, but showed no more gouty attacks. All these patients had gout proved by the presence of crystals and also had normouricaemia before diagnosis.

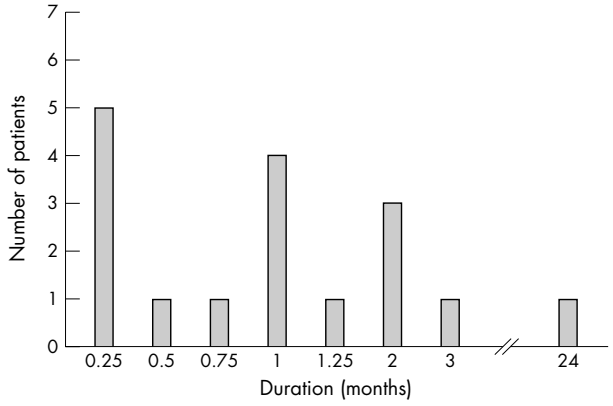

Figure 1 Time from the diagnosis of gout to the development of hyperuricaemia.

The mean age at diagnosis was higher in gouty patients with normouricaemia than in patients with hyperuricaemia, whereas the duration from the first symptom to diagnosis, and the prevalence of documented tophi, was higher in gouty patients with hyperuricaemia. Serum uric acid, blood urea nitrogen, and creatinine levels were lower in gouty patients with normouricaemia (table 1).

We found that 27/226 (12%) of gouty patients at diagnosis had normouricaemia, which was a lower incidence than found in the two reports mentioned previously.[4 5] The discrepancy between our results and the results of those studies may be because the previous studies analysed the incidence of gouty patients with normouricaemia at the time of any acute attack,[4 5] whereas we analysed the incidence at the time of diagnosis.

Seventeen of 21 (81%) gouty patients with normouricaemia at diagnosis subsequently became hyperuricaemic. The median time for progression from normouricaemia to hyperuricaemia was one month. Our results corresponded with those of previous reports which showed that serum uric acid usually fell during an acute attack and rose during the interim.[4 5] Only four gouty patients with normouricaemia at diagnosis continued to have normouricaemia. This "genuine" normouricaemic gout was present in only a small proportion, and these patients followed a mild disease course without secondary attack.

These observations may help a doctor to decide whether a diagnosis of gout can be made when normouricaemia is present and may help in predicting the disease course of normouricaemic gout at diagnosis.

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HLA-DRB1*03 and DQB1*0302 associations in a subset of patients severely affected with systemic lupus erythematosus from western India

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Genetic factors are likely to be important both in determining the overall susceptibility to systemic lupus erythematosus (SLE) and in influencing the remarkable clinical heterogeneity in disease expression found in affected subjects. The more common clinical features seen in patients with SLE include, skin and joint diseases, renal disease, neuropsychiatric complications, and also some haematological abnormalities. Genetic factors, together with environmental factors, strongly influence the development of SLE. Multiple loci within the major histocompatibility complex (MHC) have been implicated in susceptibility as have HLA class II alleles, complement components, and tumour necrosis factor (TNF) loci.

Currently it is believed that some HLA alleles are in genetic linkage disequilibrium with certain disease related genes and they regulate the immune responses. Since 1969, when the first case of SLE was reported from India, the disease has been extensively studied in different regions of the country—namely, Chennai, Calcutta, Mumbai, and New Delhi. A statistically significant clinical correlation comparing the clinical variables from other racial groups of the world has been reported in Indian patients with SLE.[1] HLA association studies from Indian patients with SLE are considerably limited[2] and, furthermore, varying interethnic differences in the associations have been reported from UK, South African and Icelandic populations.[3]

Table 1 HLA alleles associated with severe SLE

HLA	Patients (%PF) (n=53)	Controls (%PF) (n=110)	OR	p Value	CI
A1	39.60	17.80	2.99	<0.001	1.06 to 2.08
A2	45.30	29.70	1.95	<0.001	0.52 to 1.02
A19	26.50	43.40	0.48	<0.001	9.62 to 10.72
B15	2.00	16.30	0.16	<0.001	5.07 to 7.23
B27	12.20	2.30	5.40	<0.001	1.42 to 2.54
DRB1*					
03	**50.00**	**6.70**	**9.67**	**<0.0001**	**8.41 to 13.56**
14(6)	12.50	40.00	0.25	<0.001	1.09 to 2.56
1001	6.30	30.00	0.21	<0.001	1.84 to 2.34
DQB1*					
0302	**56.30**	**10.00**	**8.02**	**<0.0001**	**12.88 to 18.45**
0203	12.50	50.00	0.17	<0.0001	1.32 to 2.56

%PF, percentage phenotype frequency; OR, odds ratio; CI, 95% confidence interval.

PATIENTS AND METHODS

We studied 53 patients with severe SLE exhibiting the clinical manifestations described by the 1982 revised American Rheumatism Association diagnostic criteria who had one or more organ affected, such as kidney, brain, heart, and lungs. One hundred and ten normal healthy subjects with the same economic status and ethnic background comprised the controls for this study over the same period. The autoantibody profiles among the patients with SLE were studied by immunofluorescence and enzyme linked immunosorbent assay (ELISA). HLA-A and B locus antigens were identified by a National Institute of Health two stage microlymphocytotoxicity assay using indigenous and commercial antisera. The HLA-DRB1 and HLA-DQB1 alleles were determined by a polymerase chain reaction with sequence-specific primers technique from the total genomic DNA extracted from the EDTA blood. The phenotype frequencies, odds ratio, probability value, and confidence intervals were estimated using our database and computer programs. The p value was corrected by the Bonferoni inequality method.

RESULTS AND DISCUSSION

A significant increase in the frequency of HLA-A1, A2, B27, DRB1*03, DQB1*0302, and DQB1*0601 was found among patients with SLE. HLA-A19, B15, DRB1*14(6), DRB1*1001, and DQB1*0203 were found to be decreased in the patient group compared with the controls (table 1). The high risk alleles DRB1*03 and DQB1*0302 were then compared with the findings for other populations of the world (table 2). The