Serum Immunoglobulins and IgG Subclasses in SS Patients with Saudi Arabia/India Haplotype

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Key Words

Serum immunoglobulins · IgG subclasses · Sick cell anemia · Saudi Arabia/India haplotype · Spleen function

Abstract

Objectives: This study investigates serum immunoglobulin fractions (IgG, IgA and IgM) and IgG subclasses (IgGScs; IgG1–4) in SS patients with the Saudi Arabia/India (SAI) haplotype. The influence of gender, Hb F levels, spleen function and coexistent α -thal trait were studied. **Methods:** Immunoglobulins and IgGScs were assayed using nephelometry. β^S -globin gene cluster haplotyping and α -globin genotyping were done using a combination of PCR and allele-specific oligonucleotide hybridization techniques. **Results:** Twenty-nine steady-state (i.e. not acutely ill) SS patients (23 SAI homozygotes and 6 SAI/Benin compound heterozygotes) and 34 healthy age- and sex-matched Hb AA and AS

controls were studied. Mean serum IgG and IgA values were significantly higher in the patient group, while there were no significant differences in the mean IgGScs. The patient group was further subdivided depending on whether the spleen was normally visualized (group 1), partially visualized (group 2) or not visualized (group 3) on ^{99m}Tc-labeled tin colloid scintigraphy. There was a significant sequential increase in mean IgG values from group 1 to group 3 but the reverse was true for IgM, while IgA showed no consistent trend. The mean levels of IgG1 and IgG3 were significantly higher in group 3 ($p < 0.01$). Hb F level did not influence immunoglobulins or IgGScs, but SS patients with coexistent α -thal trait tended to have lower IgG and IgG1 levels. **Conclusions:** Increased serum IgG is associated with poor splenic function and recurrent infections among SAI patients, while increased IgM is seen mainly in those with relatively normal splenic function.

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Introduction

Elevations of one or more of the different serum immunoglobulin fractions (IgG, IgA and IgM) have been reported in SS patients. This is due to reticuloendothelial stimulation from hematopoietic stress and recurrent infections. A direct relationship has been found between immunoglobulin levels (especially IgG and IgM) and spleen size and function [1–8]. However, most studies were carried out on American [1], Jamaican [2] or African [6, 8] patients who tend to have the severe variants [9, 10] of the disease (Benin and Bantu haplotypes with low Hb F). Studies of patients with the milder Saudi Arabia/India (SAI) haplotype (high Hb F) have been few [3]. Also, there is a paucity of information on IgG subclasses (IgGScs) in SS patients in general. We have quantitated immunoglobulin and IgGSc levels in a group of SS patients in Kuwait and correlated the results with age, sex, Hb F level, coexistent α -thal trait, spleen size and function.

Materials and Methods

The subjects were steady-state (i.e. not acutely ill) SS patients being followed up in the Pediatric Hematology Clinics of the Mubarak Al-Kabeer and the Al Amiri Hospitals in Kuwait. The diagnosis had been established from Hb electrophoresis, Hb quantitation using cation-exchange HPLC and family studies. Informed consent was obtained from the parents of all patients. A control group made up of apparently healthy Hb AA or Hb AS children (siblings of the patients) was also studied. Each individual in the study had a thorough physical examination and a review of clinical records to document any previous episodes of bacterial infections that required hospitalization.

The β -globin gene cluster haplotype and α -globin genotype were determined in all patients using standard methods [10, 11]. Liver/spleen scintigraphs were obtained with ^{99m}Tc -labeled tin colloid (Amersham) in the patients [11]. Radionuclide images of the posterior, left lateral and anterior views of the splenic area were

obtained. The results were graded as normal (group 1) if the splenic visualization was of the same intensity as the liver image, partial (group 2) if there was a decrease in the splenic image or no visualization (group 3). Serum IgG, IgA and IgM levels and IgGScs were quantitated using nephelometry (Beckman) [12, 13]. The quantitation was repeated at least a month after the last determination in any individual with an abnormally high or low level. In such cases, the average level was used for calculations.

Data are presented as means $\pm$ SE except where otherwise stated. Student's *t* test or analysis of variance (ANOVA) was used, where appropriate, to test the statistical significance of differences between the mean values in different groups. Pearson's correlation coefficients were computed to examine the relationship between variables.

Results

Twenty-nine SS patients (20 males and 9 females) aged 2–16 years (mean $\pm$ SD 7.6 $\pm$ 3.4 years) were studied. The control group consisted of 34 individuals (18 males and 16 females) aged 1–15 years (mean $\pm$ SD 5.8 $\pm$ 3.4 years) of whom 25 were Hb AA and nine were Hb AS genotype.

Twenty-three (79.3%) SS patients were homozygous for the SAI haplotype and 6 (20.7%) were compound heterozygotes for this and the Benin haplotypes. Hb F levels ranged from 6 to 30.6% with a mean of 20.3 $\pm$ 6.6%.

The α -globin genotype was successfully determined in all SS patients. Seventeen (58.6%) had their full complement of 4 α -globin genes ($\alpha\alpha/\alpha\alpha$), 8 (27.6%) were α -thal-2 heterozygotes ($-\alpha/\alpha\alpha$), and 4 (13.8%) were homozygotes ($-\alpha/-\alpha$).

Among the SS patients, 10 (34.5%) had palpable spleens of 2–8 cm below the costal margin. Liver/spleen scintigraphy was successfully performed in 23 patients of whom 10 (43.5%) had normal visualization (group 1), 6 (26.1%) had partial visualization (group 2) and 7 (30.4%) had no visualization (group 3).

Table 1. Mean ($\pm$ SE) serum immunoglobulin values in SS patients

Group	IgG, g/l	IgA, g/l	IgM, g/l
SS (n = 29)	14.38 $\pm$ 0.7	2.25 $\pm$ 0.2	1.48 $\pm$ 0.1
Group 1 ¹ (n = 10)	12.06 $\pm$ 1.0	2.25 $\pm$ 0.4	1.82 $\pm$ 0.2
Group 2 ¹ (n = 6)	13.85 $\pm$ 1.8	2.08 $\pm$ 0.5	1.28 $\pm$ 0.2
Group 3 ¹ (n = 7)	18.60 $\pm$ 0.8	2.49 $\pm$ 0.6	1.23 $\pm$ 0.2
Controls (n = 34)	11.84 $\pm$ 0.5	1.42 $\pm$ 0.1	1.25 $\pm$ 0.1

¹ Grouped according to splenic scintigraphy results (see text).

Table 1 shows the mean immunoglobulin concentrations in the patients and controls. The individual values of IgG, IgA and IgM were within the normal range for our lab, except for 7 patients who had excessively high IgG values. There was no significant sex difference in the values except for IgM which was significantly ($p < 0.05$) higher in females (1.80 ± 0.3 g/l) than in males (1.30 ± 0.1 g/l). There was no significant correlation of the immunoglobulin classes with age or Hb F level. There was no significant difference in the mean values of the three immunoglobulin classes in those with or without palpable spleens, irrespective of (splenic) size. Also the individual values of IgGScs were within the normal range for the population. Among the control subjects, there were no significant sex differences in the mean immunoglobulin values, and no correlation with age could be demonstrated.

When the values were compared according to splenic scintigraphy, there was a striking linear increase in IgG from group 1 to 3 (table 1), with significant differences in the mean values of the three groups (ANOVA $F = 8.50$, $p = 0.002$). The difference between the means of groups 1 and 3 was also highly significant ($p < 0.001$). On the other hand, there was a linear decrease in mean IgM from group 1 to group 3, but the difference was not significant ($F = 2.37$, $p = 0.12$). IgA did not demonstrate a

linear pattern. Of the 23 patients who had splenic scintigraphy, 15 (65.2%) had never been hospitalized for a bacterial infection, 6 (26.1%) had had one episode and 2 (8.7%) who were both SAI homozygotes and who belonged to group 3 had had more than two episodes each. None of the remaining 5 patients who did not have scintigraphy performed had been hospitalized for severe bacterial infections. None of the controls had a palpable spleen and none had been hospitalized for severe bacterial infection.

When the immunoglobulin values were stratified according to the patients' α -globin genotypes, mean IgG values showed a linear decrease from group 1 to 3 (table 2), a reverse of which was seen when splenic scintigraphy was used to stratify the group. However, the differences among the mean values were not significant ($F = 1.07$, $p = 0.35$). There was no linear trend among the values for IgA or IgM in the three groups and the differences between the mean values were not significant.

IgGScs were determined in 17 SS and 21 control subjects. There was no significant difference in the mean values between the two groups (table 3). However, when the SS population was classified according to splenic scintigraphy, the values for IgG1 and IgG3 were significantly higher ($p < 0.01$) in group 3 when compared to group 1, 2 or the controls. The mean values of each IgGSc in group 1 or 2

Table 2. Mean ($\pm$ SE) serum immunoglobulin values in SS patients with different α -globin genotypes

Group	α -Globin genotype	IgG, g/l	IgA, g/l	IgM, g/l
1	$\alpha\alpha/\alpha\alpha$ (n = 17)	15.14 $\pm$ 1.1	2.23 $\pm$ 0.3	1.54 $\pm$ 0.3
2	$-\alpha/\alpha\alpha$ (n = 8)	13.03 $\pm$ 1.3	2.41 $\pm$ 0.5	1.56 $\pm$ 0.5
3	$-\alpha/-\alpha$ (n = 4)	12.83 $\pm$ 1.3	2.05 $\pm$ 0.4	1.06 $\pm$ 0.4

Table 3. Mean ($\pm$ SE) IgG (g/dl) subclasses in SS patients and controls

Group	IgG1	IgG2	IgG3	IgG4
SS (n = 17)	8.29 $\pm$ 0.6	2.21 $\pm$ 0.2	0.84 $\pm$ 0.1	0.68 $\pm$ 0.1
Group 1 ¹ (n = 8)	7.54 $\pm$ 0.4	2.08 $\pm$ 0.3	0.69 $\pm$ 0.1	0.45 $\pm$ 0.1
Group 2 ¹ (n = 4)	6.00 $\pm$ 0.8	2.14 $\pm$ 0.5	0.45 $\pm$ 0.1	1.07 $\pm$ 0.2
Group 3 ¹ (n = 5)	11.67 $\pm$ 0.6	2.42 $\pm$ 0.4	1.28 $\pm$ 0.2	0.88 $\pm$ 0.1
Controls (n = 21)	8.19 $\pm$ 0.5	2.21 $\pm$ 0.2	0.80 $\pm$ 0.1	1.49 $\pm$ 0.9

¹ See table 1.

Table 4. Mean ($\pm$ SE) IgG (g/dl) subclasses in SS patients with different α -globin genotypes

Group	α -Globin genotype	IgG1	IgG2	IgG3	IgG4
1	$\alpha\alpha/\alpha\alpha$ (n = 17)	9.61 $\pm$ 0.7	2.03 $\pm$ 0.3	0.89 $\pm$ 0.2	0.73 $\pm$ 0.1
2	$-\alpha/\alpha\alpha$ (n = 8)	7.45 $\pm$ 0.5	2.39 $\pm$ 0.3	0.69 $\pm$ 0.2	0.52 $\pm$ 0.1
3	$-\alpha/-\alpha$ (n = 4)	5.15 $\pm$ 1.2	2.51 $\pm$ 0.4	0.92 $\pm$ 0.1	0.81 $\pm$ 0.4

were not significantly different from the mean value in the controls. ANOVA showed that the differences in the mean values in the three groups were significant for IgG1 (F = 22.9, p = 0.00001), IgG3 (F = 6.65, p = 0.009) and IgG4 (F = 6.09, p = 0.01). There were no significant age or sex differences in both the patient and control groups, and the values among the SS patients were not influenced by splenic size or Hb F level.

Table 4 shows the mean values of the IgGScs when the SS group was stratified according to α -globin genotypes. A significant

linear decrease was noticed from group 1 to 3 for IgG1 (F = 5.7, p = 0.013). A similar relationship was not demonstrated for IgG2, IgG3 or IgG4.

Discussion

The clinicopathological features of SS disease are heterogeneous, and several ameliorating factors have been identified, including the β^s -globin gene cluster haplotype [9, 14], coexistent α -thal trait [15], level of Hb F and

female gender [16]. The SAI and Senegal haplotypes, unlike Bantu and Benin, are associated with mild disease probably because homozygotes and compound heterozygotes have high Hb F usually in the range of 15 to more than 30%. Kuwaiti SS patients are mostly SAI homozygotes [10] and, in addition, they have a high prevalence of α -thal trait, estimated at about 30–40% [11, 17]. It is therefore not surprising that, as has been reported for SS patients from Eastern Saudi Arabia, our patients have a low incidence of severe bacterial infections and recurrent infarction. In the present study, the mean Hb F was 20.3%, and 41.4% had coexistent α -thal trait (either as homo- or heterozygotes).

It is interesting that the most important factor associated with a high IgG level in the present study is a poor splenic function as identified by nonvisualization on scintigraphy. This is also the group of patients who have had frequent severe bacterial infections. It would, therefore, appear that stimulation from recurrent infections is the real determinant of high IgG in SS patients with SAI haplotype. Conversely, IgM was lowest in patients with no splenic visualization, which is in keeping with previous reports that IgM is only increased when there is a demonstrable functional splenic tissue [1]. It is also low in individuals who have been surgically splenectomized [1]. The increased levels of IgG1 and IgG3 in group 3 in the present study are probably related to the overall increase in IgG. This finding corroborates the report by Hedo et al. [6] from Nigeria, who documented increased IgG1 and IgG3 among their patients with a high severity index.

A previous study from our center demonstrated that SS patients with coexistent α -thal trait are more likely to retain normal splenic function [11]. It is therefore not surprising that coexistent α -thal was shown in the present study to have an influence on serum

IgG and IgG1 levels. On the other hand, the Hb F level does not influence immunoglobulin and IgGSc levels in our SAI SS patients.

While most of our patients did not have severe or recurrent infections, 2 (aged 7 and 11 years, respectively) SAI homozygotes among them had had five episodes of pneumonia, 3 of acute osteomyelitis, 2 of pyelonephritis, 1 lung abscess and 1 septicemia complicated by disseminated intravascular coagulopathy. Both had no splenic colloid uptake on scintigraphy and had the highest levels of serum IgG. Interestingly, one of them had the lowest IgG2 value (0.76 g/dl) among the patients; the normal range for our lab being 0.74–3.42 g/dl. This particular patient has been followed up for 2 years since the study concluded and her IgG2 continued to fall.

It is interesting that the mean serum IgG, IgA and IgM values obtained in the present study were not significantly different from those in the previous studies [3, 8] of SS patients from Eastern Saudi Arabia (17.82 ± 4.2 , 2.19 ± 0.7 and 1.62 ± 0.4 g/dl, respectively) and America (13.9 ± 0.6 , 2.23 ± 0.3 and 1.7 ± 0.1 g/dl, respectively), even though the latter were mostly of the Benin chromosomal haplotype background. However, the corresponding values in a group of Nigerian SS patients [8] were 23.15 ± 1.0 , 2.60 ± 0.2 and 5.45 ± 1.5 g/dl, respectively, showing grossly elevated IgG and IgM. This pattern has been attributed to an environmental factor, possibly a chronic or a recurrent malaria since the malaria parasite is a known mitogen that stimulates the B cell system [18]. Indeed, a direct relationship was demonstrated between malaria antibody titers and immunoglobulin levels among Nigerian SS patients [8].

In conclusion therefore, physicians looking after SS patients with the SAI haplotype should realize that a subset of these patients will present with a severe clinical course and

fulminant bacterial infections. Investigations should include splenic scintigraphy, α -globin genotyping, serum immunoglobulin and IgG subclasses. It is only after all these investigations that the patient and his/her family can be adequately counseled about the likelihood of frequent bacterial infections.

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