Immunological Aspects of Sickle Cell Disease in the Eastern Province of Saudi Arabia

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The distribution of sickle cell haemoglobinopathy in various parts of Saudi Arabia was first documented in 1961[1]. It was soon realised that such patients from the Eastern Province of this peninsula usually have a relatively "benign" form of the disease[2]. The population of the Eastern Province is relatively homogeneous because of a community which hitherto has remained closed and in which consanguinity is the rule rather than the exception. In contrast, patients from the western part of the country which is nearer to the African subcontinent show the same severity as their counterpart in Africa and the USA[3,4].

Not only ß-globin gene haplotype in the Eastern province of Saudi Arabia is different from the western part[5], but there is also associated high level of HbF in these patients[4]. Some of these patients also have G6PD deficiency which along with high HbF is considered to have a beneficial effect[6]. Restriction-fragment length polymorphism study on these sickle cell patients who have HbF phenotype, shows a single base cytosine - thymidine (C-T) substitution at - 158 bp 5' to the cap (preinitiation) site, ie. the promoter region of the G-globin gene[7]. The authors believe that this mutation may be largely, if not uniquely, responsible for high HbF and therefore a milder sickle cell disease in the Eastern Province of Saudi Arabia. Nevertheless, these patients do suffer from a variety of diseases including respiratory tract infections, meningitis, osteomyelitis and septicaemia. Albeit, the incidence is 4 to 8 times less common than that in American or Jamaican blacks[8]. It is thus obvious that sickle cell patients in this part of Saudi Arabia are somewhat unique in their clinical presentation and the natural history of the disease. To our surprise, however, their immunological profile has not so far been fully investigated.

Review of the literature shows that sickle cell patients in other parts of the world have a variety of abnormalities in their specific and non-specific immune systems. These include functional hyposplenism, deficiencies in the alternative pathway of the complement system particularly factor B, and defects in neutrophil function including chemotaxis, chemokinesis, adhesions to bacterial and intracellular killing[9]. Total IgC and IgA levels are usually raised in these patients[10]. There are also reports of poor response to intravenous immunisation and of low levels of circulating tuftsin[11]. Poor skin reactivity to recall antigens, and altered helper: suppresser ratio (ie CD4: CD8) have also been reported[12]; while others have noted impaired in vitro function of B-Lymphocytes[13]. A poor lymphoblastic response to phytohaemagglutinin has also been documented[14]. There is also a report of zinc deficiency with poor NK cells activity[15].

Because of the unique nature of sickle cell disease in the Eastern region of Saudi Arabia and the paucity of information regarding the immunological profile, we have carried out this immunological study and have examined various humoral and cellular parameters in these patients particularly those who had chronic or recurrent infections.

METHODS

A total of 43 patients (Hb SS, = 26, Hb AS = 13, SB° Thalassaemia = 4) were included in this study, 20 were males and 23 females. Their ages ranged from 6 months to 12 years (average 7 years). Twenty-eight healthy control matched for age and sex were also studied for

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statistical analysis. Out of the 43 patients examined, 19 patients had evidence of infection which included osteomyelitis (6 cases), bronchopneumonia (3 cases), recurrent tonsillitis (2 cases), septicaemia (2 cases) and vaso-occlusive crisis precipitated by infections (6 cases).

Immunological parameters studied included serum immuno-globulins GAM and components of complements (C_3, C_4) factor B, sub-populations of B and T-lymphocytes, helper: suppresser ratio and in vitro lymphocyte response to mitogen.

Levels of complement, factors B and immunoglobulins were estimated by rate nephalometry using Beckman (USA) immunochemistry analyser. The population of B- and T-lymphocytes and their subsets were studied by indirect immuno-fluoresence using monoclonal antibodies to cell surface markers ie. cluster differentiation (CD) antigens (eg. CD19, CD2, CD3, CD4, CD8) obtained from orthodiagnostics (USA) and Dakopat (Sweden).

Lymphocyte function was examined by the uptake of radioactive thymidine in culture (set up in quadruplicate) when stimulated by phytohaemogglutinin (PHA). Transformation index was compared with those of the age and sex matched controls. The concentration of PHA used was based on a dose-response curve obtained in the beginning of the study.

Statistical analysis of the quantitative values obtained for the various immunological tests were carried out using Student's t-test. Skewness of data was accounted for and values were calculated. The differences in lymphocyte sub-populations were evaluated using chi-square test. SAS statistical package was used throughout this study.

RESULTS

The mean values of IgC, IgA, IgM in patients with sickle cell disease treated as a single group were 14.638 gm/litre, 1.478 gm/litre and 1.448 gm/l respectively. The levels of IgC were significantly higher than age and sex matched control ($p=<.01$). There were no differences in levels of IgM but IgA was somewhat raised ($p<.02$). When the levels were analysed however, according to the subtype of sickle cell disease, IgC was found to be higher in sickle ß°-thalassaemia and those with HbSS. ($p<.001$, $p<.05$), whereas those with Sickle Cell Trait did not show any significant difference (Fig 1).

The level of complement (C_3, C_4) and factor B were comparable to the control value except in 4 cases of HbSS with G6PD deficiency in whom C_4 was marginally low normal 0.27 gm/l and patients = 0.22 gm/l $p< 0.5$) (Fig 2).

On the whole there was no difference in the number of B and T-lymphocytes. Suppresser-T cells were also

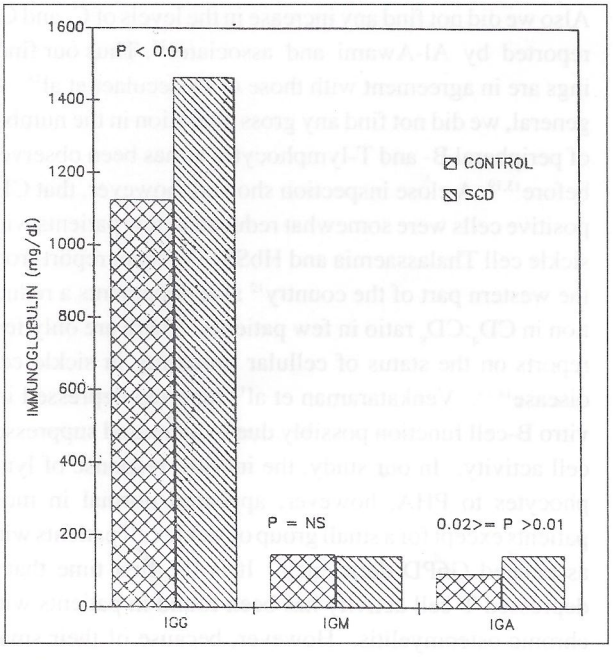

Figure 1: *Immunoglobulin level in SCD & control*

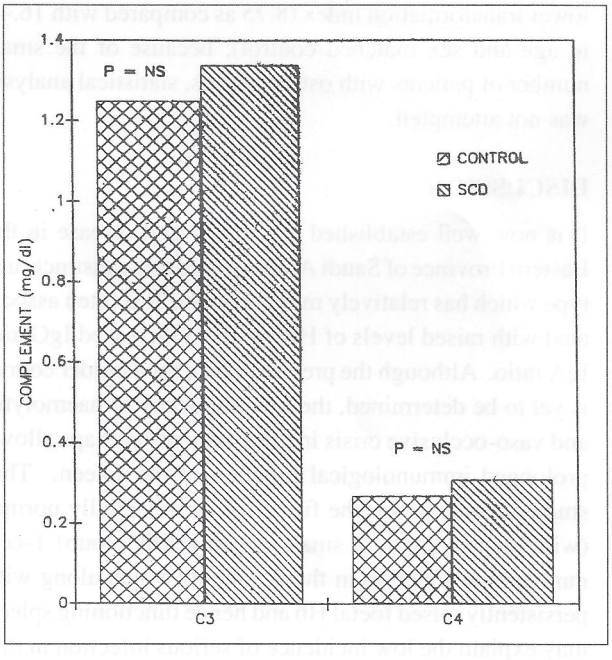

Figure 2: *Serum complement levels in sickle cell disease*

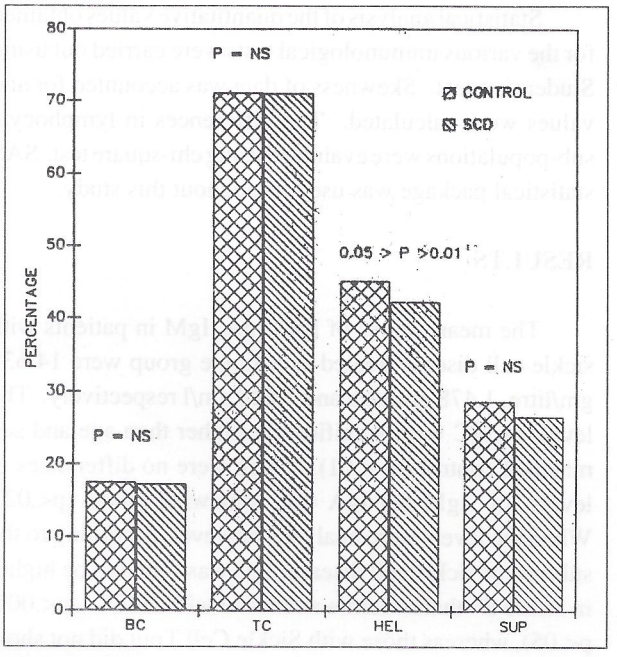

Figure 3: *B & T-cell subsets in SCD & control*

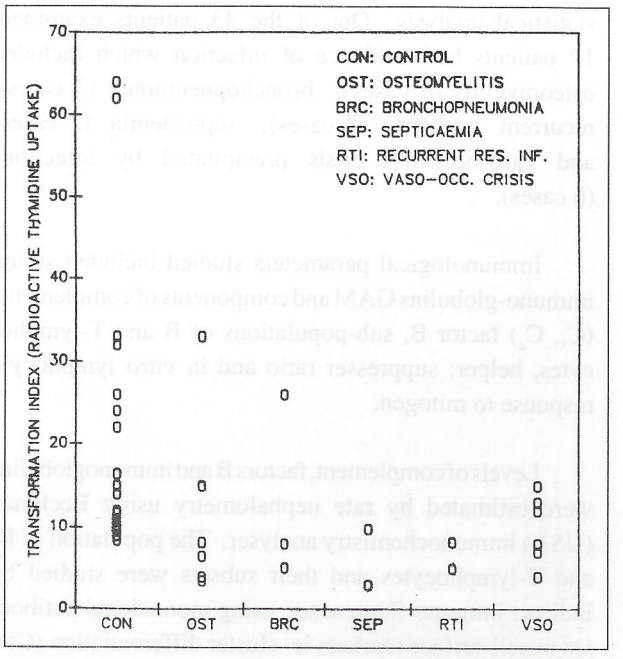

Figure 4: *T-cell function in SCD with infection & control*

within the normal range but the Helper-T cells were slightly reduced in patients with Sickle Cell Trait with G6PD deficiency ($p < .05$) (Fig 3).

The study of T-cell function in term of transformation index showed a wide scatter as depicted in Fig 4. It was of relevance however to note that mean values were slightly lower in patient with HbSS but it was most interesting to note that all 4 patients with osteomyelitis had almost 50% lower transformation index (8.75 as compared with 16.41 in age and sex matched control); because of the small number of patients with osteomyelitis, statistical analysis was not attempted.

DISCUSSION

It is now well established that sickle cell disease in the Eastern Province of Saudi Arabia represents a distinct subtype which has relatively milder course. It is often associated with raised levels of HbF with an increased IgG and IgA ratio. Although the precise reason for a milder course is yet to be determined, the low frequency of haemolytic and vaso-occlusive crisis in these patients perhaps allows prolonged immunological function of the spleen. This study documents for the first time an essentially normal (with the exception of small osteomyelitis group) T-cell number and function in these patients which along with persistently raised foetal Hb and hence functioning spleen may explain the low incidence of serious infection in this geographical region.

In the present series IgG and to some extent IgA were found to be raised. This is similar to reports from other parts of the world[9,10,12,16]. We did not find any appreciable rise in the levels of IgM as reported by Al-Awami and co-worker from this region[17]. The cause of this discrepancy is not known but it is not clear from their study if they had age and sex matched control. Normal levels of IgM have also been repeated by others[10,16].

Also we did not find any increase in the levels of C_3 and C_4, reported by Al-Awami and associates[17]. Thus our findings are in agreement with those of Dececulaer et al[18]. In general, we did not find any gross alteration in the number of peripheral B- and T-lymphocytes as has been observed before[13,19]. A close inspection showed, however, that CD^4 positive cells were somewhat reduced in few patients with sickle cell Thalassaemia and HbSS. A recent report from the western part of the country[12] also documents a reduction in CD_4:CD_8 ratio in few patients. There are only few reports on the status of cellular immunity in sickle cell disease[14,19]. Venkataraman et al[13] reported depressed in-vitro B-cell function possibly due to increased suppresser cell activity. In our study, the in-vitro response of lymphocytes to PHA, however, appeared normal in most patients except for a small group of sickle cell patients with associated G6PD deficiency. It is the first time that a depressed T-cell activity has been found in patients with chronic osteomyelitis. However, because of their small number a statistical analysis was not possible.

CONCLUSION

It is obvious from the present study that there was no single common immunological abnormality in our patients. Raised levels of IgG and IgA probably reflect mild grade of recurrent infection. A depressed T-cell function may be pathogenetically related to osteomyelitis. Thus it can be deduced that sickle cell disease in Eastern Saudi Arabia runs a milder or "benign" course not only due to persistently raised level of HbF (possibly as a result of mutation in the promoter region of G) and a prolonged functioning spleen but also that they do not have any gross deficiency of either humoral or cellular component of the immune system. We are studying the phagocytic function in these patients which will be reported in due course.

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