Clinical and Immunopathogenetic Aspects of Sarcoidosis

Abstract

Background

Sarcoidosis is a multiorgan granulomatous condition of uncertain aetiology, in which the lungs are commonly affected. The disorder comprises important inflammatory along with immune components; however, the exact aetiology is unknown and the immunopathogenesis is not completely understood. Due to its similarities, both radiologically as well as clinically, and particularly in high tuberculosis (TB) burden regions, sarcoidosis is regularly misdiagnosed as TB.

Variation in disease prognosis, progression, presentation and susceptibility have been linked to various human leukocyte antigen (HLA) phenotypes, which are important in antigen presentation and involved in mounting an immune response to foreign antigens. Cell-mediated immunity play a central part in the sarcoidosis' immunopathogenesis. Humoral immunity changes, such as polyclonal gammopathy, presence of auto-antibodies, immunoglobulins, complement and immune complexes in the granuloma of sarcoidosis suggests that humoral mechanisms may well have a role in the pathogenesis. Micro-ribonucleic acids (microRNAs or miRNAs) are groups of non-coding, small, single-stranded RNAs. Their key role is to mediate the post-transcriptional silencing of target genes. The role of miRNAs has attracted attention, both in pathogenesis and as biomarkers for many disorders, including sarcoidosis. In sarcoidosis, the role of miRNAs is not well recognised, but their role is being unravelled by many workers. Chitotriosidase is a chitinase enzyme that acts as protection against

nematodes, fungi, and insects which are chitin-containing pathogens. It is selectively expressed by activated macrophages. Sarcoidosis' pathogenesis is known to involve activated macrophages through the release of various cytokines, chemokines and enzymes.

Aims

The current research was conducted in order to analyse and review clinical features of sarcoidosis patients in the South African population. Being a relatively uncommon condition, this would add to the profile of patients from other studies conducted in South Africa. In addition, HLA Class I along with Class II antigens in sarcoidosis patients were evaluated. Serum immunoglobulin (Ig) G subclass levels in newly diagnosed cases with sarcoidosis were assessed, noting changes in antibody levels in corticosteroid-treated patients and those undergoing spontaneous clinical response. The serum expression of approximately 800 miRNAs in sarcoidosis patients were compared with race-, age- as well as gender-matched healthy controls. Analysis of serum chitotriosidase activity in sarcoidosis and TB patient cohort, both granulomatous ailments of differing aetiology, was undertaken and compared with controls.

Methods

A five-year investigation was conducted among 102 patients with sarcoidosis attending the pulmonology services of the Charlotte Maxeke Johannesburg Academic Hospital. HLA-A, B, C (Class I) along with HLA-DQB1 and DRB1 (Class II) antigen typing in 51 patients from this cohort, was prospectively carried out by polymerase chain reaction (PCR), utilising sequence specific primers. These antigens were compared with 63

healthy control subjects. Serum IgG subclasses levels were assessed prospectively, both before and after treatment, or in cases with spontaneous recovery, in 17 newly diagnosed cases of sarcoidosis, using a laser nephelometric method with appropriate test kits. The RNA was extracted from stored plasma samples utilising the “QIAGEN miRNeasy Mini Kit®” and concentrated using a salt-ethanol precipitation. The RNA extracted was analysed using an nCounter® miRNA human v3 expression assay. Twelve samples (6 patient and 6 matched controls) were analysed. Data were analysed using the nSolver™ Analysis Software. Chitotriosidase activity was prospectively assayed in serum of 12 biopsy-proven sarcoidosis cases before treatment, 9 sarcoidosis patients after treatment, 10 confirmed pulmonary tuberculosis patients prior to treatment, and in 12 healthy controls. Plasma chitotriosidase activity was analysed utilising “4-methylumbelliferyl-β-D-N, N', N″-triacetylchitotriose” as a substrate.

Results

Out of 102 sarcoidosis patients, 59 were Black, 28 Indian, eight White and seven Coloured (mixed race). There were 69 females (67.6%) and 33 (32.4%) males. Majority of patients were non-smokers (85.3%). The group’s mean age was 44.6 years. Hypertension (32.4%), renal dysfunction (creatinine > 104 µmol/L) (19.5%), obesity (17.6%), diabetes mellitus (15.7%), asthma (11.8%), and gastro-oesophageal reflux (11.8%) were the most prevalent associated chronic comorbid diseases. Indian patients had significantly more dyslipidaemia than Black patients ($p = 0.02$). Almost 17% of patients had been treated for tuberculosis, based on the chest radiograph, prior to the diagnosis of sarcoidosis. Two cases developed active TB whilst being treated with corticosteroids for sarcoidosis. Four Black patients contracted human

immunodeficiency virus (HIV) infection whilst on treatment for sarcoidosis. The salient clinical manifestations were dry cough, the commonest presenting symptom in 82.4%, dyspnoea in 53.9%, cutaneous lesions, other than erythema nodosum, in 33.3%, and on lung examination crackles were noted in 37.3% of patients. The majority (48%) of patients had stage II chest radiographic changes. Cutaneous biopsies (28.4%), transbronchial lung biopsies (25.5%) and biopsies of mediastinal lymph nodes via mediastinoscopy (25.5%) were the most frequent sites for confirming granulomatous inflammation. Blacks were more likely to have non-erythema nodosum dermatological manifestations at presentation. In Blacks 67.8% had an elevated ACE level compared with 32.1%, 50% and 14.3% in Indians, Whites and Coloureds respectively. The mean ACE level was higher in Blacks than in Indians (157 vs. 58 IU/L). The initial mean serum calcium was elevated (> 2.5 mmol/L) in 22.7% of cases, the highest incidence being in Whites (37%). Raised gamma-glutamyl transferase (GGT) (> 78 IU/L) and alkaline phosphatase (AP) (> 128 IU/L) were observed in 31.8% and 21.6% of patients, respectively. Elevated creatinine (> 104 µmol/L) was seen in 19.5% of patients; Whites having the highest incidence of renal dysfunction at 37%. Overall, 21.2% of patients had obstructive airways disease on lung function testing. Systemic corticosteroids were indicated in 87.3% of patients and the relapse rate was 60.7%.

HLA class I along with class II phenotype analysis in 51 sarcoidosis patients found significant increased levels of HLA B15, C4, C7, C12, C15, C16, C17, DQ3, DR8 and DR11. A significant negative (protective) connotation with HLA A9, A28, B12, B17 and DR2 was observed.

In the IgG subclasses study, there were 17 Black patients of which six were men and 11 women. The mean age was 44 years (range 23-60 years). Raised initial IgG1 levels

were noted in 10 patients, IgG3 levels in 8, IgG2 levels in 4, and IgG4 in 2 patients. The mean IgG1 and IgG3 levels were significantly raised above normal control values. Fourteen patients were treated with corticosteroids with good clinical response, and three, in whom there was no indication for therapy, were asymptomatic. On follow-up there was a significant decrease in IgG subclass levels toward normal. IgG1 levels returned to normal in almost all cases, and IgG3 remained raised in two cases. The mean levels of IgG1 and IgG3 decreased significantly, as did the levels of IgG2.

Regarding miRNA, one pair of results was excluded due to cellular RNA contamination. Therefore, five patients and matched control samples were analysed. After excluding miRNAs that were below background levels, 145 miRNAs could be analysed. On applying a Bonferroni correction, the only miRNA that was significantly different was miRNA *let-7a-5p*, which was significantly overexpressed (141-fold change; $p < 0.0003$) in sarcoidosis cases compared with controls. Of the 145 miRNAs, many had been differentially expressed among patients compared with controls, although not reaching statistical significance.

Serum chitotriosidase activity has been shown to be significantly greater in sarcoidosis cases, both before ($p < 0.0001$) and after treatment ($p < 0.0039$), compared with controls. Sarcoidosis cases had significantly higher chitotriosidase levels than TB cases and chitotriosidase activity decreased with treatment in sarcoidosis. While in patients with TB chitotriosidase activity was noted to be lower than in sarcoidosis, it was higher than in controls.

Conclusions

In South Africa, sarcoidosis is often misdiagnosed as TB. In this study, the most frequent sites for biopsy for histological confirmation were skin, mediastinal lymph node and transbronchial lung biopsy. Comorbid illnesses were common and should be screened for and treated promptly to improve quality of life. Most of the cases in this cohort with sarcoidosis had an indication for treatment with corticosteroids.

The HLA study is unique to South African patients and indicates that genetic influences may play a significant part in the potential aetiology of sarcoidosis. There are some HLA subtypes that may be a predisposition to the development of sarcoidosis and other HLA subtypes that may be protective.

IgG subclasses, mainly IgG1 as well as IgG3 were elevated in a substantial proportion of sarcoidosis cases initially, and reduced with corticosteroid therapy and in those patients with spontaneous clinical resolution. These IgG subclass responses may be due to protein antigens that usually trigger B-cells leading to class switching to IgG1 and IgG3 via T-cell help through MHC-class II expression. Therefore, IgG subclasses may act as opsonins, bind to microbes and facilitate phagocytosis in sarcoidosis.

This is the first study of differentially expressed miRNAs in serum of sarcoidosis cases and matched healthy controls in South Africa. The results obtained indicate that miRNAs may have a role in sarcoidosis pathogenesis involved in regulating inflammatory pathways. Whether these molecules have diagnostic or prognostic implications, further research in larger cohorts are required.

This is the first study of chitotriosidase activity analysis in sera of sarcoidosis and TB cases in South Africa. The processes that result in the increase of chitotriosidase

activity are not yet understood, but in reaction to macrophage activation, this enzyme may be involved when there are chitin containing organisms implicated in the disease pathogenesis. To validate these findings, further studies with higher patient numbers are required. It remains to be determined if chitotriosidase is a diagnostic or predictive marker in sarcoidosis.