CORRELATION BETWEEN DIRECT AND INDIRECT TESTS FOR DIAGNOSIS OF SCHISTOSOMIASIS

THESIS

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BY

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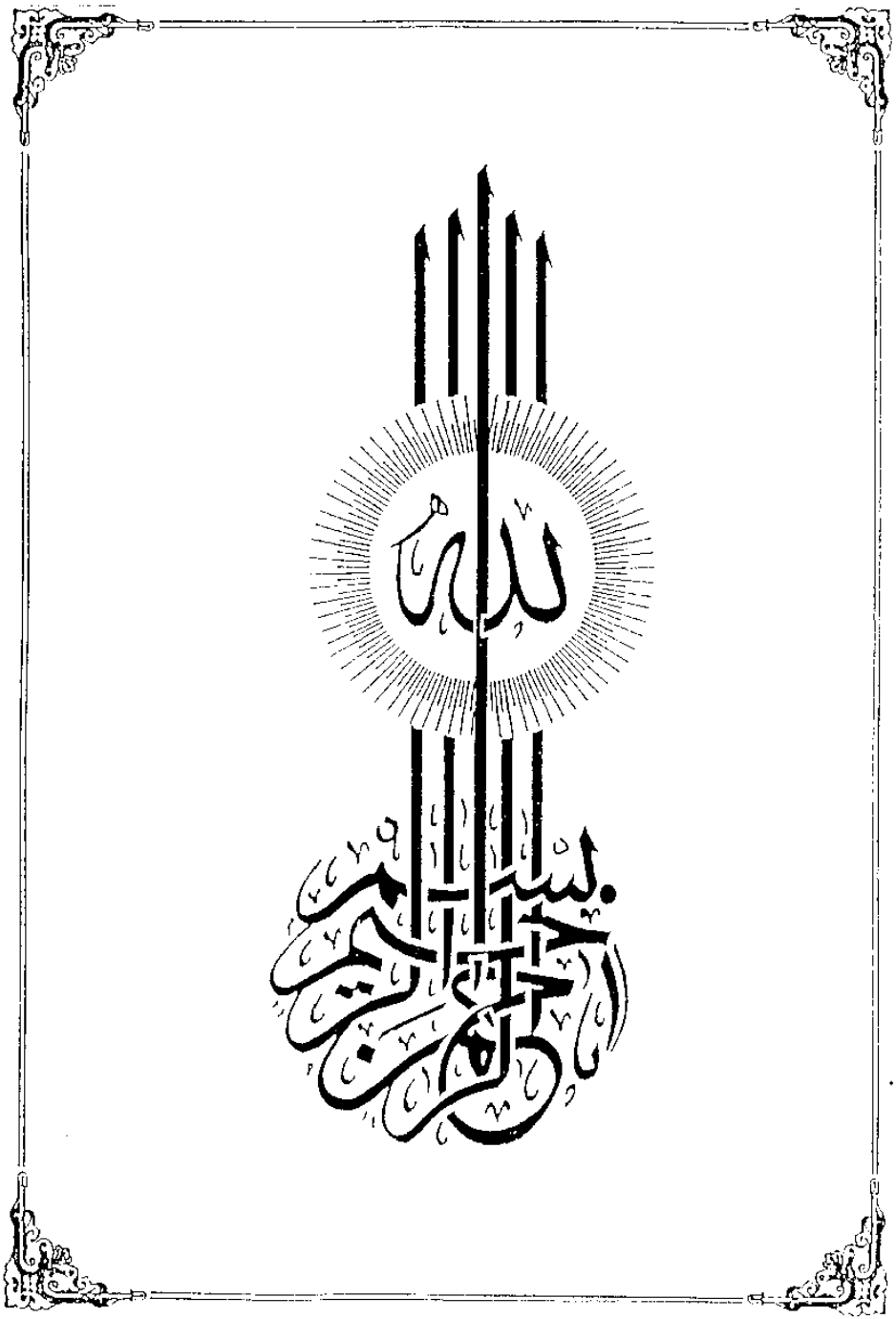

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AIM OF THE WORK

schistosomiasis leads to a reduction of about 30% of the agriculture economy (Moussa and Arfa, 1967).

The need for a sensitive, specific and quantitative techniques for diagnosis of bilharziasis is mostly required, not only for the diagnosis, but also for the assessment of chemotherapy and more importantly for the application to mass surveys and the evaluation of control measures.

Methods of diagnosis can be parasitological or immunological. Parasitological techniques are necessary for providing a definitive diagnosis of an active infection and species identification. Schistosoma ova are demonstrated in urine or stools, less frequently in tissues. Several sensitive methods of processing fecal specimens have been developed as the Kato thick smear (Martin and Beaver, 1968) and modified Ritchie formol-ehter concentration technique (MRCT) (Knight et al., 1976). However, failure to recover the eggs in stool by such reliable techniques does not exclude the possibility of infection.

The insistence on relying almost exclusively on the stool examination can reuslt in an erroneous evaluation of the control programms (Yogore et al., 1983 & Lewert et al., 1984), due to the insensitivity of stool examination.

Many factors may contribute also to the difficulty of detecting schistosome ova in excreta, as in mild infection oviposition may be too minimal to be detected

by direct methods (Abdel-Magied et al., 1984). The intense fibrosis around the eggs that may occur in chronic bilharziasis may hinder the passage of ova through the mucosa during their trial to reach the lumen of the bowel (Shaw and Ghareed, 1938).

The immunodiagnosis is required for supporting diagnostic information in the analysis of inidividual cases of suspected infection. Even more important are the immunodiagnostic tests in the field of epidemiological surveys. The immunodiagnosis may include complement fixation test (CFT) (Yoshimoto, 1910), intradermal test (IDT) (Fairley and Williams, 1927), cercarial hüllen reaction (CHR) (Vogel and Minning, 1949 a) indirect haemagglutination test (IHAT) (Boyden, 1951), circumoval precipitin test (COPT) (Oliver-Gonzales, 1954), indirect immunofluorescence test (IFAT) (Sadun et al., 1960), counterimmunoelectrophoresis (CIEP) (Philips and Draper, 1975), enzyme-linked immunosorbent assay (ELISA) (Huldt et al., 1975), radio-immunoassay (RIA) (Pelley et al., 1977), detection of schistosomal antigen in urine (Okabe and Tanaka, 1958), in the serum (Nash, 1974), and identification of circulating immune complexes (CIC) (Bout et al., 1975 & Santoro et al., 1976).

Ruiz-Tiben et al. (1979) reported greater sensitiviyty of serology, based on COPT, for detection of S. mansoni infection than single stool examination by MRCT. In the Philippines, serology based on ELISA test has also been found superior to routine stool examination by the Kato technique in determing the incidence and prevalence of infection with S. japonicum (Yogore et al., 1983 and Lewert et al., 1984).

This study aimed to correlate the direct and the indirect methods applied in the diagnosis of schistosomiasis, with special interest in Schistosoma mansoni infection by estimating the reactivity of such immunological tests as the intradermal test, the circumoval precipitin test, the counterimmunoelectrophoresis test, the double counterimmunoelectrophoresis test, the indirect haemagglutination test and the enzyme-linked immunosorbent assay test in the different clinical and parasitological stages of S. mansoni infection, in order to compare the reactivity of these different tests in each clinico-parasitological stage of S. mansoni infection, and to study the reactivity of S. mansoni infected cases by the different tests in comparison with that of S. haematobium and mixed bilharzial infected cases.

REVIEW OF LITERATURE

A) IMMUNITY OF SCHISTOSOMIASIS

Patients with schistosomiasis respond to infectin immunologically, either humoral (Makled , 1972) and/or cellular immunity (Ekladios et al., 1978).

S. mansoni cercariae while penetrating the skin secrete preacetabular gland material, sufficiently immunogenic to induce IgG and IgE antibodies (Minard et al.,1977). These antibodies, in the presence of complement, are responsible for the direct damage of schistosomules, (Clegg and Smithers, 1972 & McLaren et al., 1975).

Verwaerde et al. (1985) stated that the diffusable soluble material of the membrane and metabolic products of the schistosomula initiate an immune response directed against further homologous infections. This initial antibody response is then further amplified by antigens released by the adult worm. Concurrent with the first infection, specific lymphocytes are primed by schistosomula-released products (SRP), followed by clonal expansion of the memory cells after further infections.

The major immune reaction seems to occur after deposition of eggs in tissues (Warren, 1976). This reaction influences egg deposition, and egg extrusion (Parag et al., 1981).

Cell mediated immune response to living bilharzial ova is essentially responsible for the main pathological changes and complications of infection (Von Lichtenberg, 1967).

Harrison and Doenhoff (1983) found that in groups of mice subjected to different immunosuppressive measures

at the time of infection with S. mansoni, there was a reduction in the mature worm burden and the number of eggs produced by the surviving worms during early patency was also significantly reduced.

The most substantial retardation in parasite egg production was achieved by hydrocortisone acetate, which also caused a substantial reduction in worm burden. Cyclophosohamide, beta-methasone and T-cell deprivation (achieved by means of a combination of adult thymectomy and anti-thymocyte serum injections 40 days before infection) also inhibited S. mansoni egg-laying.

It was found that for the immunosuppressants to have their deterious effects on S. mansoni worm maturation, they have to be administrated around the time of infection. Alternatively, as in mice which have deprived of their T-cells, the host must be in a state of relatively permanent immunosuppression.

They stated that the effects of immunosuppressants on tissue egg number could be a reflection of delayed worm maturation rather than being due to a direct effect of the reagents on worm reproductive physiology. Thus T-cell deprivation of the host or cyclophosphamide treatment may in some way only cause a delay in the migration and maturation of S. mansoni worms, while hydrocortisone may have more potent actions in this respect which result in the death of a proportion of worms.

However, the experimental results do not indicate why hosts which have been immunosuppressed are less effectively parasitized by S. mansoni than are immunologically intact controls. One possibility is that

during the early stages of schistosome infection in normal hosts the inflammatory reactions, which are induced in tissues through which the larvae migrate cause a breakdown of normal intercellular organization, and thereby facilitate the migratory process.

On the other hand, Farag et al. (1981) found that the administration of hydrocortisone and methotrexate at the start of S. mansoni worm maturation in the mice has resulted in an increase in worm load, i.e. these drugs allowed survival of a greater proportion of the slowly developing schistosomules which would have otherwise succumbed by the developing immune mechanism in the host. Also the administration of these drugs had increased retention of ova and a decrease in egg extrusion due to imparing the role of immunity in egg expulsion.

Patients with intestinal and chronic hepatosplenic schistosomiasis show significant increase in the humoral immune respones such as increased serum levels of immunoglobulins IgG, IgM, IgE, and IgA (Makled, 1972 & Camus et al., 1977) , while on the other hand , the cell mediated immunity is depressed, which is manifested by diminished number of peripheral lymphocytes and depressed phagocytic power (Ricci et al., 1969; Makled et al., 1985 and Khalil et al., 1987a). These immunological disturbances are considered the basis of immunopathology, immunodiagnosis and immunoprotection in schistosomiasis (Warren, 1982).

Nash et al. (1978) found that IgM and IgG titers run together,both being higher in acutely infected and low in chronically infected patients.

Buzaki and Damian (1981) stated that the IgM antibodies appeared quite early, and thereafter fall to very low levels as IgG antibodies were still increasing after a relatively delayed appearance. They found also that the elevation of IgA antibodies generally paralleled IgG antibodies, with two differences, the IgA response was much weaker, and there was a tendency toward slow and gradual decay of the response after about a year.

Hassan et al. (1980) found that the humoral antibodies detected by most serological methods showed increasing values in the early and late S. haematobium infection, which is parallel to the increase of the antigenicity of the disease. A corresponding pattern is observed in S. mansoni infection in the early and hepatosplenomegalic stages, while in the late ascitic stages a depression of antibody levels is seen.

Salih et al.(1978)found that the immunoglobulin levels specially IgG were higher in hepatomegalic than in intestinal patients, and highest in hepatosplenic patients.

Bassily et al. (1972) and Ghanem et al. (1975) found that there was no significant change in IgA levels among bilharzial patients in different stages of the disease.

Hillyer (1969) and Ghanem et al. (1975) found that IgM showed significant progressive increase with the advance of the disease. However, Antunes et al. (1979) and Bassily et al. (1972) found elevated IgM in active S. mansoni infection only. Hassan et al. (1980) found higher levels of IgM in S. mansoni infection than S. haematobium due to reticuloendothial proliferation, due to enlargment of the liver and the spleen, while

lower values in ascitic stage of S. mansoni infection may be attributed to low viable egg count encountered in these cases, also might be due to impairment of the immune system.

A high level of IgE in the sera of patients with active schistosomal infection was reported by El Raziky et al. (1974), Jarret et al. (1976) and Carson et al. (1975).

Capron et al. (1977) postulated the incorporation of IgE complexes with macrophages and eosinophils in the destruction of schistosomulae in the sensitized host and as a mediator of protective immuntiy in schistosomiasis.

Ottesen et al. (1981) stated that there are significant differences between IgE responses seen during the acute and chronic stages of S. mansoni in man. Total serum IgE levels in chronically affected patients were significantly greater than those of acute patients, also the levels of parasite-specific IgE antibody were greater in chronically infected individuals. These findings are of interest as in the acute stage of schistosomiasis is characterized clinically by allergic manifestations such as high-grade eosinophilia.

El-Gindy et al. (1985) stated that there is an inverse relation between IgG and IgE levels and faecal egg count, which probably indicates a protective action against schistosomiasis. These immunoglobulins may have lethal effect on schistosomules and/or may inhibit ovulation in female parasite. Barth et al. (1966) suggested that IgE increases the passage of other immunoglobulins across the mucosal surface.

Hiatt et al. (1980) stated that 4-8 weeks after cercarial penetration, at the time of oviposition, a state of relative antigen excess creates the classic situation leading to the development of soluble immune complexes and a concomitant serum sickness like reaction. Immune complexes isolated from S. mansoni infection sera have been shown to contain anodally-migrating antigen (CAA) (Deelder et al., 1978), antigen A (Santoro et al., 1978), and antigen M (Carlier et al., 1980). The immune complexes have a role in the manifestation of acute schistosomiasis (Hiatt et al., 1980), and in the glomerular lesions and proteinuria in chronically infected patients(Lehman et al., 1975).

Schistosomiasis is characterized by a profound alternation of T-cell functions in the chronic phase of the disease, (Colley et al., 1977). This T-cell function impairment is reflected in a way of suppression of the immune reaction response to specific antigens (Ottesen et al., 1978).

Khalil et al. (1987a) studied the T-lymphocytes subpopulation in patients with bilharzial hepatosplenomegaly by means of monoclonal antibodies and found that there was a significant decrease in the total number of T cells and depressed cell mediated immunity.

Ekladios et al. (1978) suggested that the spleen plays a significant role in the immune-depression.

The T lymphocytes are formed at least of three separate T-cell subpopulation, each of them has a specific immunoregulatory function (Cantor and Boyse, 1975).