IgG and its subclasses responses to Ov-ASP-2/MBP protein as a target for diagnosis of Onchocerciasis infection.

By

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B.Sc. of Science

A Thesis Submitted for Fulfillment of the Requirements for the Degree of Master of Science (M.Sc.)

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March. 2004
Onchocerciasis, or river blindness, is one of the most dreadful parasitic diseases of mankind. It causes a great deal of misery as a result of the infection; and the most important complications of the disease are those of the eye. The current diagnoses of the disease depend on the detection of the parasites, either microfilaria in the skin or eyes or adult worms in subcutaneous nodules; but these methods are not always satisfactory. Ocular lesions occur primarily as a result of host inflammatory response to dead and/or dying microfilariae in the eye. In the cornea, this response is manifested by the development of new blood vessels (neovascularization) followed by corneal opacification. Different immunological diagnostic trials used for development of diagnostic tests with different type of Onchocerca volvulus antigens have shown variable degrees of cross-reaction with other nematodes co-endemic in the same area.

The study had identified the immune responses to Ov-ASP-2/MBP recombinant antigen of O. volvulus, which has been shown to play an important role as an early mediator of the ocular pathology. It is the only member of the Ov-ASP family expressed in microfilariae. Ov-ASP-2 was cloned and produced in E. coli as a maltose binding protein (MBP), fusion protein, to study the mechanism by which Ov-ASPs induce their angiogenic activity. As part of a collaborative study, Ov-ASP-2/MBP was provided to evaluate activity in diagnosis of Onchocerca volvulus infection in general by using ELISA method for screening sera of specific antibodies. Patients were classified into two main groups; (i) Endemic Positive Individuals (EP) and (ii) Onchocerca Negative Control (ONC) group; which include healthy controls as well as patients of schistosomiasis, tuberculosis, malaria which constitute co-endemic infections in most of the onchocerciasis endemic areas in the Sudan. In all serum immunoglobulins tested, there were significant differences between Ov-ASP-2/MBP reactivity to infected individuals compared with the control groups. This type of serological test can also be used, to quantify the amount of antibody response; and its correlation with different clinical presentations of the disease.

In the present study, Ov-ASP-2/MBP recombinant antigen was very specific in detection of onchocerciasis patients and the fusion partner does not contribute to its recognition power. Ov-ASP-2/MBP showed a high specificity and sensitivity in recognition of parasite-specific IgG and its subclasses IgG3 and IgG4. Most importantly, Ov-ASP-2/MBP it had discriminated sera from blind onchocerciasis patients and showed a positive correlation between blindness or severely impaired vision and clear or mild skin disease. Ov-ASP-2/MBP represents a novel class of antigen that might be used as a biomarker for the ocular disease of O. volvulus.

* * *
I would like to thank my supervisor Dr. Elamin Elnima, Dean /Faculty of pharmacy, University of Khartoum for his interest, guidance and encouragement throughout the course of this study. My gratitude to my co-supervisor, Dr. El Sheikh El Obeid, Tropical Medicine Research Institute (TMRI) for his remarks and valuable advice, which assisted me to completing this study.

Special thanks to Dr. Tarig Higazi, Division of Geographic Medicine, University of Alabama, U.S.A. for kindly providing us with the antigen.

I am greatly indebted to Dr. Atif A. Elagib, Tropical Medicine Research Institute (TMRI), for his great help, guidance and continuous interest during the development of this work. I would also like to express my acknowledgement to Dr. Amna El Subki, Department of Microbiology and Molecular Biology, Faculty of Science and Technology, Al Neelain University; for her generous and unlimited help.

Deep thanks extended to all the staff and my colleagues at the Tropical Medicine Research Institute for offering me all the facilities of the institute and every means to conduct this study. Very particular thanks are due to Prof. Suad Suliman, Dr. Galal Moustafa, Ms. Misk Elyemen, Ms. Asma Elassad, Mr. Zafeir Babekir, Ms. Somia Sadig and Ms. Tahani Najm Eldeen for their assistance and infinite help whenever needed. My thanks are extending to Mr. Mahadi and Mr. Salah Gumaa for their co-operation during the collection of samples and the fieldwork.

My deep appreciation goes to Ms. Zenab Shomo and Mr. Omaima Salih at the Malaria Research Unit and Ms. Wegdan Abd-Alla, Ms. Ameira Suliman, Department of parasitology (Natural Health Laboratory) for their at most help that allows me to fulfill this work.

Special recognition, and appreciation for my parents who surrounded me with love, supporting and care me in every possible way. Wishing to have succeeded to achieve some of their great hopes for me.

The UNDP/ World Bank/WHO Special Programme supported this work for Research and Training in Tropical Diseases (TDR) project ID no. 950208RSG; Therefore, Iam thankful for those responsible, for their support.

* * * * *
Dedication to…

My beloved father…
For his kind support, encouragement and unlimited attention
for me always, that shined so gracefully upon my life.
I am forever greatly indebted …

My beloved mother…
For her great unbound, tender love and moral support in
every possible way …

My dear brothers; and sisters…
Hopping that we will never separate …

The sweety kids…
Roaya & Jiddo.

My friends…
For their real and warm heartily love…

To all those who suffer from
The “River Blindness”

Thank you all, for what you have done….

Ever…

Nada
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<tr>
<td>ADCC</td>
<td>Antibody-Dependent Cell-mediated Cytotoxicity.</td>
</tr>
<tr>
<td>ASP</td>
<td>Ancylostoma Secreted Protein</td>
</tr>
<tr>
<td>B-cell</td>
<td>lymphocyte, which matures in bone marrow.</td>
</tr>
<tr>
<td>CD</td>
<td>Cluster of Differentiation.</td>
</tr>
<tr>
<td>cDNAs</td>
<td>Copy or complementary DNA.</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>Interferon-Gamma (also IFN-β, IFN-α)</td>
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<tr>
<td>Ig</td>
<td>Immunoglobulin.</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G (also IgM, IgA, IgD, IgE).</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin.</td>
</tr>
<tr>
<td>IL-2</td>
<td>Interleukin-2 (also IL-4, IL-5, etc.).</td>
</tr>
<tr>
<td>kDa</td>
<td>kiloDaltons (units of molecular mass)</td>
</tr>
<tr>
<td>L3</td>
<td>3rd stage infective larvae</td>
</tr>
<tr>
<td>LPS</td>
<td>Lipo-polysaccharide</td>
</tr>
<tr>
<td>MBP</td>
<td>Maltose binding protein.</td>
</tr>
<tr>
<td>Mf</td>
<td>microfilariae</td>
</tr>
<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
</tr>
<tr>
<td>mL3</td>
<td>molting third stage larvae.</td>
</tr>
<tr>
<td>NK cell</td>
<td>Natural killer cell</td>
</tr>
<tr>
<td>PBMC</td>
<td>Peripheral blood mononuclear cell.</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
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<tr>
<td>Te</td>
<td>Cytotoxic T-cell</td>
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<tr>
<td>T-cell</td>
<td>Thymus-derived lymphocyte.</td>
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<td>T-helper cells</td>
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* * * * *
1.1 General Introduction:
Filaria is a morbid condition produced by certain nematode worms (filariae) that cause the important filarial disease in the tropical area. The adult worms of both sexes live in the lymphatics, skin, connective tissues or serous membranes. They produce live embryos (microfilariae) which find their way into the bloodstream, or skin, where they are capable of living, without developing further, for a period varying from less than a week in the case of *Loa loa*, and *Wuchereria bancrofti* to three years in *Onchocerca volvulus* and *Dipetalonema perstans* (Manson, Apte, 1982). Thus, *Onchocerca volvulus* is one of the important helminthic nematode worm belonging to the family: Filaridae and super family Filarioidea which is the causative agent of onchocerciasis disease, regarded as the most dreadful parasitic disease of mankind as intermediate hosts (Steward, 1986). In 1875, O’Neil, first described the association of the microfilariae of *O. volvulus* with an irritating papular dermatitis called craw-craw in Ghana. Kean et al. (1978) and Michael (2002) mentioned that Leuckhart, (1893) was the first to describe the adult worm of onchocerciasis in the subcutaneous nodules. Michael, (2002) also stated that the black fly of the genus *Simulium* was the disease vector first demonstrated by Blacklock, (1923).

Onchocerciasis is common in many parts of the world. But about 96% of all cases are in Africa and mostly in western Africa. WHO, (1995) reported that the African countries showing high %age of this disease; Include Kenya, Uganda, Tanzania, Zimbabwe, Malawi, Sudan, Ethiopia, Senegal, Liberia, Upper Volta, Ghana, Nigeria, Cameroon, and Zaire. It is also detected in Yemen (Adams, Maegraith, 1984), along with six countries in Central and South America. Indeed, important foci exist also in Mexico, Guatemala, Venezuela and Ecuador (WHO, 1995). The prevalence and severity of Onchocerciasis, as well as, the magnitude of the associated social and economic effects, vary widely in different geographical areas where the disease occurs. WHO committee stressed, that Onchocerciasis can be a devastating disease, producing intolerable misery as a result of it’s effects on the eyes and skin, and that it can lead to the disintegration of social structure and the abandonment of homes and land.

However, the disease is often called river blindness, because, the most extreme manifestation and the black flies *Simulium damnosum*, that transmit the disease, are found in river side areas, where they breed in fast-flowing waters. The clinical features of onchocerciasis may
be divided into two main groups: ocular and non-ocular manifestations. The African *Simulium* species tend to bite the hips and lower limbs, whereas South American *Simulium* species prefer the head and upper body. Due to this reason the symptoms of the disease vary significantly among the different geographical areas and among different foci. Symptoms vary from mild skin irritation and oedema, to gross dermal atrophy and blindness. Subcutaneous nodules usually occur as another symptom of the disease over bony prominences (Michael, 2002).

Blindness is the worst complication of this disease, when it occurs it cannot be treated since it is an irreversible condition. The number of people suffering from vision loss is estimated as 270,000 and an additional 500,000 have severe visual impairment (Michael, 2002). The development of eye lesions is considered to be a consequence of heavy infection or infection of long duration. It correlates with the density of microfilariae in dermal and ocular tissue (Remme et al., 1989). Microfilariae can be found in ocular tissues at the early stage of the disease and entry into the eye occurs by direct invasion from the conjunctiva through the sclera or cornea (Michael, 2002). Degeneration of the microfilariae in the soft ocular tissue initiates the disease in both the anterior and posterior segments of the eye. In case of the anterior segment, permanent sclerosing keratitis develops, gradually and as for the posterior chamber, lesions include optic atrophy, chorioretinitis and optic neuritis. Sclerosing keratitis and optic nerve disease are the major causes of blindness (Abiose, 1993).

On the other hand, many of these manifestations of the disease appear to be caused by immunopathological changes in response to the death of microfilariae in the tissues, so that the observed clinical spectrum may be in part a reflection of the host immunological spectrum as a result of the ability of the host to mount an inflammatory immune response. The histopathological studies showed that perivascular cellular infiltrates were nearly always present in onchocercal skin in which that cellular infiltrate around the vessels consisted of varying numbers of eosinophils and mast cells together with mononuclear cells, such as macrophages, lymphocytes and plasma cells (Connor, 1974; Burcard, Bierther, 1978). These cells are common and involved in producing IgG & IgE immunoglobulins (David, 1982). The main classes of antibodies are (IgG & IgE) reported to play primary roles in immunity against filariasis (Aime et al., 1984; Hussain, Ottesen, 1986). The antibody responses were either against the parasite as IgG antibodies bound to dead microfilariae (Bryceson et al., 1976), or against products liberated upon the death of the parasite. IgE is usually associated with early infection and its level usually drops as it binds to the surfaces of basophiles and mast cells where it acts as a receptor for specific antigens.
Although the mechanisms underlying the host inflammatory responses in ocular onchocerciasis have been examined, the role of particular parasite proteins in this process remains largely unexplored (Hall, Pearlman, 1999). Recent study has evidenced that the parasite proteins designated Ov-ASP-1, Ov-ASP-2 and Ov-ASP-3, which are homologues of vespid venom antigen 5, are an important allergen to humans. The study hypothesized that these proteins may play a role in the development of ocular onchocerciasis. It was shown that Ov-ASPs homologues are capable of inducing angiogenic responses in naive mouse corneas, suggesting that these proteins may play a direct role in the pathogenesis of ocular onchocerciasis (Tawe et al., 2000). The potential importance of the Ov-ASP family members in establishing infection by the infective larvae and in the development of the nodule by the juvenile adult parasite, suggests that these proteins might be a potential target for immunotherapeutic or chemotherapeutic attack against O. volvulus infection (Wilson et al., 1994).

At the present time, the current mainstay of treatment is ivermectin which is a potent microfilaricidal, recommended by the world health organization, for control programme for use in community in hyper and meso-endemic areas (WHO, 1995). Although Ivermectin was found to be effective microfilaricidal, different studies showed that it has no macrofilaricidal effect on adult worms. In this regard there are no effective vaccines or chemoprophylactic drugs, while the prevention in endemic areas is performed by the three recommended ways: vector control, mass treatment with ivermectin and education of people in endemic areas.

1.2 Parasite and Life cycle:
The filarial nematode O. volvulus, that is the causal agent of onchocerciasis disease, was originally discovered in Ghana and subsequently named Filaria volvulus by Leuckhart (1893). Duke et al., (1966) mentioned that Blanchard, (1899) was the described to the parasite lay in a lymphatic space in a tumour, and then Brumpt classified the worms as distinct species, O. volvulus and O. caecutiens. Strong et al., (1934), demonstrated that they were morphologically identical. Duke et al., (1966) also, reported In spite of the fact that there are no morphological differences between strains of Onchocerca from different parts of the world, it is clear that there is considerable Onchocerca-simulium adaptation and that there can be a high degree of incompatibility between parasite strains and vectors from different geographical areas; It has been suggested that there are six main Onchocerca-simulium complexes, each associated with a characteristic pattern of the disease.
Generally, Human are only the definitive host and *Simulium* black fly, which releases the infective stage larvae during a blood meal, is the obligate intermediate host (Fig.1.1). Usually in the human host, the larvae molt twice to become male and female adult worms, consequently these adult worms can live up to 14 years, during which the females liberate large numbers of minute microfilariae (Mf). This process occurs in a subcutaneous nodule and takes 6-12 months before the mature female can produce these microfilariae (Michael, 2002).

The female worm length ranges from 30-80 cm, and more than one worm may be coiled in a subcutaneous nodule. The male adult worms are usually 3-5 cm in length and migrate between various subcutaneous nodules to inseminate females, thus the females produce 700-1900 mf per day (Michael, 2002). Microfilariae are unsheathed, with body nuclei that do not extend to the tail tip, and are 221 - 287µm long (Plaisier et al., 1991). A small percentage of microfilariae, reach the insect’s thoracic muscles after several molts then they become third-stage infective larvae (L3) in a 2-3 week period and migrate to the insect’s salivary glands where they are ready to be transferred during the next blood meal. Consequently, after entering the skin of the human host through the bite of an infected black fly, the infective larvae (usually 2-6 larvae) migrate through the subcutaneous tissues. There, over the next 12 months, each larva will mature into an adult male or female worm. Before a heavy load of adult worms and pathogenic microfilariae build up in the human host, this sequence has to be repeated many times over, and many years of exposure are usually required (Daniel, 2003).

It is worth mentioning here, that the mean reproductive life span of the adult worm is between 9 and 11 years, with maximum reproduction during the first 5 years of potency (Plaisier et al., 1991). If the adult worms die, the (Mf) live in the skin for between 1 and 2 years and they are normally found in the dermis and are rarely seen in blood, sputum, or urine. Microfilariae can live for 6-30 months, and most of them die without completing their life cycle. Their death causes an inflammatory response in the surrounding tissues, which is responsible for disease morbidity (Michael, 2002).
1.3 Vector and disease transmission:

Black flies of the genus *Simulium* are the only vectors of *O. volvulus*. They are tiny ferocious biters and measures about 3-4 mm long. *Simulium*, often called the buffalo gnat, only the females transmit the worm of disease parasite (Adams, Maegraith, 1984). At least 15 different species of vector *simuliids* can transmit onchocerciasis (Eezzuduemhoi, 2002) from one individual to another but the most important species, known to transmit the infection, are *Simulium damnosum* (west Africa), *S. neavei* (East Africa) and *S. ochraceum, S. metallicum, S. callidum, and S. exiguum* (America). Some of the latter may be brightly coloured or have a metallic sheen (Adams, Maegraith, 1984), and the different in the behaviour of these species accounts for many differences in the epidemiology of the infection in the above areas (Bell, 1999).
The *Simulium spp.* requires hot moist shady conditions and fast-running rivers for breeding grounds. The larvae become attached to stones or vegetation, or sometimes crustaceae, in fast-running, well-oxygenated water to mature; and are thus especially frequent during rainy seasons, often in streams running over rocks which may be dry and exposed in the dry seasons. Although the black fly is a poor flyer and has a short flight range, (Onchocerciasis, 2002a) the adults emerging, after 8-12 days following egg production, have the ability to travel hundreds of kilometers in flight on wind currents and their life span is about 4 weeks (Eezzuduemhoi, 2002). When the *Simulium* takes up the microfilariae, they penetrate to the insect guts wall and migrates to the thoracic muscles of the insect, molts first to form the sausage shaped L2, and later to form the filari-form L3, infective stage which is transmitted to the next host when the fly feeds again (Onchocerciasis, 2002b).

### 1.4 Clinical aspects of pathogenesis:

There is a wide spectrum of clinical manifestations associated with onchocerciasis disease. In the early aspects of the disease; the infected persons usually appear within one to three years after the injection of infective larvae (Ottesen, 1995). The prepatent period, between infection with infective larvae and the production of detectable microfilariae by fertilized adult female worms, varies from 7 months to more than 2 years; most commonly it is 12-15 months. While the clinical incubation period, from invasion by infective larvae to the development of clinical symptoms or signs, is generally longer and more variable than the prepatent period. It may last for many years and is normally longer for ocular than for dermal disease (WHO, 1987).

Furthermore, the degree of pathogenicity appears to be directly related to both microfilarial numbers and the intensity of proinflammatory responses to them, and inversely related to the effectiveness of specific mechanisms to suppress this inflammation (Ottesen, 1995). All infections may be divided into two types, mild infections, may produce no symptoms, and worms may be free in the tissue: whereas, in heavy infections, major disease manifestations including dermatitis, onchocercomas (that is subcutaneous nodules containing adult worms), lymphadenitis, and blindness (Lynne, David, 1997). Small numbers of (Mf) can be found also, in other organs but neither the significance of the systemic manifestations of *O. volvulus* infection is clear, nor the possibility to assess their public health importance (WHO, 1987).

#### 1.4.1 Dermal Onchocerciasis:

Dermal onchocerciasis or onchodermatitis skin lesions is one of the most important clinical aspects of the disease, which presents a spectrum of manifestation from low to high reactivity
against microfilariae, and the majority of microfilariae are found in the upper dermis, but they can also be found at all skin levels. In some parts of the body the microfilariae may be found immediately beneath the skin epithelium without any local reaction in the tissues. The distribution of lesions and microfilariae in the body, in a given case, depends on the intensity of the infection and, possibly on, the site of the original biting (Adams, Maegraith, 1984). However, the clinical signs of the disease vary significantly among different geographical areas. In Africa serial skin snips, taken from individuals of selected areas, have shown that lesions and microfilariae are concentrated in the region of the calf and hip and to a lesser degree in the thighs and legs. The upper parts of the body are affected if the infection is relatively heavy. But in America the reverse was observed, where the upper half of the body including the eyes had being most affected (Adams, Maegraith, 1984).

Generally, the skin lesions may be divided into generalized and localized forms. In the generalized form, gross lesions develop all over the body producing pigmentary changes, atrophy and loss of elasticity. Prolonged infection is also characterized by depigmentation of skin that may result in a condition commonly known as “leopard skin”, these white areas lack melanin and the prevalence of skin depigmentation has been proposed as a marker for endemic onchocerciasis (Fawdry, 1957). In addition, the chronic hyper-reactive, localized onchodermatitis, often called Sowda, which is characterized by a severe papular dermatitis, usually localized to one limb, typically a leg, with darkening of the skin.

On the other hand, the individuals with onchocerciasis may have clinically normal skin, whereas others may have pruritis and disfiguring skin lesions. Pruritis is not necessarily related to microfilarial density and some people do not complain of any itching despite the fact that they have a significant microfilarial load in their skin. Other patients may develop sub-acute relapsing or chronic inflammatory skin disease. Severe itching and scratching may lead to superficial excoriations and crusts. Secondary bacterial infection may develop, some times leading to ulceration and, rarely, sepsis (Lynne, David, 1997). After years of chronic infection, with or without visible inflammatory lesions, atrophy of the skin develops. In severe cases the thin epidermis has a shiny fragile appearance that has been described as Lizard skin (WHO, 1987). Some foci, dermal atrophy may be seen, and different forms of inflammatory and atrophic skin disease may be combined with varying severity. Chronically infected skin loses its elasticity and becomes hyper-trophic or thickened. As a result of atrophy and loss of skin elasticity, patients may develop premature exaggerated wrinkling of the skin (Lynne, David, 1997).
1.4.2 Nodules (onchocercomata):
The classic lesion of onchocerciasis is the onchocercoma, a firm, painless nodule in the subcutaneous tissue. Onchocercomata are formed predominantly on the head, face and torso, but they may be found on the pelvic girdle and lower extremities deep-seated against the bones or near the joints. This nodule usually is composed of 2-3 females and daughter microfilariae encapsulated in a fibrous coat. Palpable nodules are found on various sites of the body either lying subcutaneously or occasionally attached to the skin (WHO, 1987); and the distribution of it on the body varies in different geographical regions; Such as in Central America, the nodules are usually found above the waist, whereas in most endemic areas in Africa they are mainly found below the waist and they are prominent when they occur over bone joints or on the skull region (Onchocerciasis, 2002). However, the average number of palpable nodules on adults living in hyper-endemic areas in Africa is 5-10, and an additional 3-4 nodules may be found deeper in the body during Nodulectomy. Individual nodule loads may be much higher, and cases of individuals with more than 100 nodules have been reported too. There are many people with microfilariae, especially children, in whom no nodules can be detected; conversely there are a small number of individuals with nodules but no detectable microfilariae (Manson, Apted, 1982).

Dead worms may, otherwise, calcify within the nodules (Eezzudemhoi, 2002) and the reactions to dead microfilariae around these nodules can lead to several unpleasant conditions. In the skin there is destruction of the elastic tissues and the formation of redundant folds. There is also often a loss of pigmentation and the histological appearance of advanced cases often resembles the skin of very old normal subjects. Moreover, the immature worms certainly stimulate immunological responses, and pruritis has been reported in the prepatent period. Even though there is an inflammatory reaction that causes the formation of a fibrotic capsule (onchocercoma) around the adult worms, the main pathology appears to be directed against the microfilariae (Mackenzie et al., 1987).

1.4.3 Lymphatic Onchocerciasis:
In onchocerciasis patients, lymph nodes draining areas of filarial dermatitis may be affected, the inguinal and femoral nodes being those most commonly involved. Most of these lymph nodes contain small numbers of microfilariae and in long standing infections severe fibrosis develops (WHO, 1987). Clinically they differ little from the lymph nodes of uninfected persons, and has several causes including infections of the legs and feet. Besides, after treatment with a microfilaricidal drug the regional lymph nodes usually become larger, soft, tender, often painful, and contain increased numbers of disintegrating microfilariae.
Furthermore, the antigens released from the Mf, can be found in the glands where they lead to a deposition of immune complexes leading to inflammation, perivascular fibrosis and obstructive lymphadenitis with “hanging groins” and elephantiasis (Gibson, Connor 1978). “Hanging groin” in males and “Hottentot apron” in females are the result of formation of a pendulous sac containing enlarged lymph glands with a prediposition to inguinal and femoral hernia (Manson, Apted, 1982).

1.4.4 Ocular lesions:
Other microfilariae may invade the eye by passing along the sheaths of the ciliary vessels and nerves, from under the bulbar conjunctiva directly into the cornea, via the nutrient vessels into the optic nerve, and via the posterior perforating ciliary vessels into the choroids. Eye manifestations are caused by an inflammatory reaction to the microfilariae as they migrate through the eye. The inflammatory reaction intensifies when microfilariae die (Michael, 2002). Hence, this heavy infection often leads to progressive blindness as a result of a variety of lesions that affect different parts of the eye, and the most important causes of disease include sclerosing keratitis and retinal damage.

Ocular onchocerciasis has been classified geographically into a savanna form and a rain forest form (Duke et al., 1966). The savanna form of the disease is characterized by more predominant sclerosing keratitis and iritis than the rainforest type, and there are fewer nodules (Anderson et al., 1974). Although, the prevalence of blindness depends on the prevalence of disease within an area, the degree of eye involvement depends on the duration and severity of infection (Lynne, David, 1997). Accordingly, the insidious nature of the disease means that blindness develops slowly with peak incidence occurring after 40 years, where by up to 40% of adult population in endemic areas is blind after the forties, in Africa. A slightly different pattern of blindness is seen in Central America, where it occurs an earlier age (Dev Medicine, 2003).

Moreover, the development of *O. volvulus* mediated corneal inflammation (keratitis) results from the temporal recruitment of neutrophils and eosinophils to the cornea through a network of newly synthesized blood vessels (Pearlman, 1996; Hall, Pearlman, 1999), known as neovascularization, resulting in loss of corneal function and blindness (Hall, Pearlman, 1999). Recently, it was found that a little understanding of the mechanisms by which the parasite is capable of inducing corneal neovascularization, the seminal event in the pathologic process (Tawe et al., 2000).
1.5 Immunopathogenesis:
The pathogenesis of filarial disease is characterized by acute and chronic inflammation; and the inflammatory responses were thought to be generating by the parasite, the immune response, or opportunistic infection (Taylor et al., 2000). Onchocerciasis is generally viewed as an immune-mediated disease, in which the host response to the parasite, particularly microfilariae in the skin and eye, leads to tissue damage. The diversity of clinical responses to *O. volvulus* infection is considered to reflect the intensity and type of immune response to the parasite and its products (Ottesen, 1985). It has been recently found that adult parasites release factors that are angiogenic (Smith et al., 1988) and probably contribute directly to the development of the rich vascularity in *Onchocerca* nodules, especially that ensheathing the adult worms (Edgeworth et al., 1993).

Local and systemic immune mechanisms to contain inflammation, such as blocking antibodies and down-regulating cytokines are prominent in infected patients, and their delineation is crucial to understanding the pathogenesis of onchocercal disease in the skin, eye, and elsewhere (Ottesen, 1995). In fact, it is only around dead parasites (Mf or adult worms) that there appear to be inflammatory responses, which came first, whether the parasite death or the inflammatory response can only be debated. The inflammatory process is dependent on development of a systemic Th2 type response to the parasite antigens (Pearlman et al., 1998; Hall, Pearlman, 1999). Monocytes /macrophages represent a link between the innate and acquired immune system and are candidate cells to promote inflammatory and anti-inflammatory processes (Brattig et al., 2000). In spite of the fact that immune responses may play a part in the degree of infectious disease, the present understanding of the cellular and humoral responses to *O. volvulus* infection in man and of the immunopathology of the disease is quite limited (Rubio de Kromer et al., 1995).

1.5.1 Cellular responses:
Cell-mediated immune responses have been clearly documented. The prerequisite for an adherence of effector cells to parasite’s larval stages is the chemotactic attraction and activation of the effector cells. For instance, Eosinophils have an important role in the immunopathology of onchocercal dermatitis and keratitis. The most compelling evidence is the consistent presence of eosinophils and eosinophil granule proteins at the site of tissue damage, either after parasite death or as an immediate response to direct injection of parasite antigens (Pearlman, 1997). Cellular migration is essential for extravasation of eosinophils from blood vessels into inflammatory tissues to interact with invading parasites by adherence reactions and then subsequently release their toxic granular effector molecules and cause
larval damage (Greene et al., 1981; Medina-De la Garza et al., 1990; Strote et al., 1990; Brattig et al., 1991; Wildenburg et al., 1994). Though, clearly, there are anti-inflammatory host molecules and mechanisms that serve to limit the tissue damage induced around each of these dying parasites. The astounding fact is that, even in lightly infected persons, microfilariae die at rates of 10,000-20,000 per day, and in heavily infected patients, at rates up to half a million per day (Duke, 1993).

On the other hand, the published information on cytokine responses in patients with onchocerciasis is minimal. Peripheral Blood Mononuclear Cell (PBMC) from patients with generalized microfiladermia, after stimulation with onchocercal antigens, also appear to produce more IL-4 than do PBMC of nonendemic normals (Soboslay et al., 1992) and more IL-5 but equivalent amounts of IL-10 compared with cells from endemic normals persons (EN/P), which are also defined, as those having lived in endemic areas and thus exposed to infection for many years but who are seemingly free of infection. Elson et al., (1995) from studies comparing mitogen-induced cytokine responses among these clinical groups, it appears that IL-2 and IFN-γ responses are equivalent in the microfiladermic and endemic normal groups (Ward et al., 1988; Elson et al., 1995) and that IL-5 responsiveness is greater in the microfiladermic patients (Elson et al., 1995). Mitogen–driven IL-2, IL-4, IL-5, and IFN-γ responses were variably enhanced during the first 6 months after treatment (Soboslay et al., 1992; Steel et al., 1994), persisting for 12-24 months for IL-2 and IL-4 (Freedman et al., 1991).

Generally, The skin pathology in onchocerciasis is a mix of a cute localized inflammatory reactions and chronic tissue damage such as atrophy and hypo-pigmentation (Mackenzie et al., 1985; Mackenzie et al., 1987; Ackerman et al., 1990). In the early skin lesions, microfilariae are found primarily in the dermis and appear to cause no observable reaction as long as they remain a live; whereas around dead or dying mf, Collections of eosinophils, eosinophil granules, neutrophils and macrophages can be seen. The skin itself remains relatively free of chronic changes, probably until the microfilarial death and concomitant inflammatory infiltration such as lymphocytes, histocytes, plasma cells, mast cells, and some times eosinophils reach the point at which hyper-keratosis, focal parakeratosis, and acanthosis develop along with pigmentary changes, lymphatic dilation, increased fibrosis, and increased numbers of dermal fibroblasts. While the severe onchocercal dermatitis has two common forms: first, the chronic Sowda picture of extensive inflammatory cell infiltration (plasma cells, eosinophils, occasional lymphocytes, and histocytes) that forms broad cuffs around dermal vessels and appendages, greatly thickens the dermis, and causes sclerosis, edema, and
hyper-pigmentation. The other form is the acute inflammatory reaction of persons who are usually visitors to an endemic area and are immunologically hyper-responsive to the parasite and its antigens. In the latter, the result is prominent intraepidermal and dermal edema, dilated lymphatics, and perivascular inflammatory cell infiltrates consisting predominantly of eosinophils, with a few lymphocytes and plasma cells. Although these histologic pictures have been extensively described, there is essentially no enough information about immunologic mechanisms involved in the tissue damage (Eezzuduemhoi, 2002). The composition of the nodules encasing adult onchocercal worms has also been described as including an outer capsule of fibrous tissue, an inner dense inflammatory cell infiltrate surrounding the enclosed adult worm, and a less dense layer of chronic inflammatory cells between the outer and inner layers (Parkhouse et al., 1985; Edgeworth et al., 1993). The character of the infiltrate appears to vary in terms of the presence or absence of eosinophils, but macrophages are almost always the predominant cell type. Lymphocytes are most abundant at the very periphery of the nodule and surrounding the inner core of a dense inflammatory cell infiltration composed primarily of macrophages around the adult worm itself. It is possible that the density and character of this infiltrate depend on the viability of the parasite, with living parasites inducing much less of an inflammatory cell response.

Furthermore, the hypersensitivity responses of the ocular lesions in humans play a similar critical role in its pathogenesis. Larval death induces an inflammation that results in corneal opacification and neovascularization. Lymphocytes and eosinophils migrate to the peripheral cornea where the infection is denser. Sclerosing keratitis follows, which may involve the visual axis over time and the corneal neovascularization and opacification with interstitial keratitis lead to corneal blindness. This development of keratitis is dependent upon the previous immunization and the presence of sensitized T lymphocytes. It is associated with predominance of T-helper types II (Th2) response (Eezzuduemhoi, 2002). It is worth mentioning, that eosinophils are predominant inflammatory cells in the cornea after injection of the parasite antigen, and the neutrophils are prominent early in the inflammatory response and mediate keratitis in the absence of eosinophils (Eezzuduemhoi, 2002). The inflammatory cells initiate the anti-parasitic responses, such responses are the secondary reactions to otherwise damaged or naturally dying parasites, it is not clear (Ottesen, 1995).

1.5.2 Immunoglobulin and Antibody responses:
As a rule, the specific antibodies belonging to the immunoglobulin IgM, IgG, IgA, and IgE classes have been found, indicating, the body’s recognition of the parasite antigens (Lynne, David, 1997). In almost all reported studies, patients with generalized microfiladermia have
significantly higher specific IgG and IgG subclass responses to onchocercal antigens than do the endemic healthy individuals, living in the same region; while nonendemic normal individuals have minimal or no antibody reactivity of any isotype to the parasite antigen (Boyer et al., 1991). The degree of skin pathology also appears to be proportional to the level of the specific IgG and IgG subclass response to onchocercal antigens (Dafa’Alla et al., 1992). Specific IgE antibodies are clearly present in patients with onchocerciasis too (Ward et al., 1988; El Khalifa et al., 1991); this immunoglobulin classes are commonly involved in filarial infections.

Immunoglobulins may play a protective role, preventing host immunological attack on microfilariae stages. For instance, IgG seems to be involved in destruction of these parasites and thus immunologically mediated active killing of microfilariae and the development of micro-abscesses, may only occur when a specific IgG antibody is present (Ghalib, et al., 1985). Nevertheless, immune complex levels were still not readily relateble to the degree of either acute or chronic disease conditions, in spite of the fact that antigen-specific complexes were more likely to be present in sera from those showing any chronic signs of infection.

1.5.3 Immunologic responses to ocular pathology:

In the ocular tissues, where the disease is more important symptom, all degrees of pathology appears, directly related to both microfilarial numbers and the intensity of proinflammatory responses to them, and inversely related to the effectiveness of specific mechanisms to suppress this inflammation. The microfilariae elicit little or no pathologic responses when a live, but an inflammatory reaction develops when the parasites die either by natural attrition or after administration of chemotherapy (Garner, 1976; Taylor et al., 1986). In spite of the eye damage that makes onchocerciasis the important disease, very few immunologic investigations have focused on this specific organ, because a relatively small amount of ocular tissue has been available for study of the inflammatory cell infiltrations in ocular onchocerciasis (Ottesen, 1995). This condition is manifested as stromal opacification and corneal neovascularization; otherwise the histologic examination of corneas and conjunctival tissue from chronically infected individuals’ shows mononuclear cells, neutrophils, and eosinophils (Garner, 1976; Chan et al., 1989); Coupled with the observation, that persons with onchocerciasis have elevated Th2 responses (Elson et al., 1994; Steel et al., 1994), these histologic and clinical findings provide circumstantial evidence that stromal keratitis is due to host immune-mediated inflammatory reactions.
In the more acute reactions, the particular presence of eosinophils around dead mf has been noted in specimens of conjunctiva, cornea, iris, and anterior chamber. And the more chronic inflammatory reactions in these tissues, as well as reactions associated with atrophic and pigmentary changes in the retina, are characterized primarily by lymphocyte and macrophage infiltration (WHO, 1987; Mackenzie et al., 1987). Autoimmunity has also been suggested to contribute to ocular pathology and autoantibodies may have resulted from activation of T and B cells seen in parasitic infections (Eezzuduemhoi, 2002).

1.6 Angiogenic proteins of *O. volvulus*:

In previous studies, it has been demonstrated that *O. volvulus* homologues of the *Ancylostoma* Secreted Protein family (*Ov*-ASPs), have pronounced angiogenic activity (Tawe, et al., 2000; Higazi et al., 2003). Angiogenesis is an important manifestation of onchocercal corneal disease, and new vessels mediate recruitment of inflammatory cells from the blood to the cornea (Hall, Pearlman, 1999). Although the molecular basis for corneal opacification has been explored in some detail (Hall, Pearlman, 1999), the mechanism of *O. volvulus*-induced angiogenesis has not been fully investigated.

In the cornea, immune responses to microfilaria are manifested first by, the development of new blood vessels (neovascularization), which provide the vehicle for recruitment of host immune cells. The angiogenic response is shortly followed by corneal opacification (Hall, Pearlman, 1999). The finding that members of *Ov*-ASP protein family are capable of inducing new blood vessel formation in mouse corneas, suggested their involvement in the pathologic process of corneal neovascularization. Availability of a mouse model for *O. volvulus* corneal disease permits screening for anti-*Ov*-ASP factors, which inhibit or reduce the angiogenic activity of *Ov*-ASP, which would have significant implications in the treatment of ocular onchocerciasis (NYBC, 2003). The consequence of abnormal angiogenesis is either excessive or insufficient blood vessel growth. Excessive blood vessel proliferation may favor arthritis, blindness and tumor growth and dissemination. The manipulation of new vessel formation would present new therapeutic options for treating a vast array of angiogenesis –dependent diseases or conditions (NYBC, 2003).

1.6.1 *O. volvulus- Ancylostoma* Secreted Protein (*Ov*-ASP):

The filarial genome project has developed a library of Expressed Sequence Tags (EST) from *O. volvulus* (Filarial genome project, 1999). Lu et al., (1993) stated that one of the most abundant cDNAs identified in infective larvae encodes proteins that demonstrate a significant degree of similarity to the vespid venom antigen 5, and to the major secreted protein of
*Ancylostoma caninum* infective larvae (Hawdon *et al.*, 1996). Vespid venoms (bee and hornet venoms) are important allergens of humans. Furthermore, vespid venom antigen 5 has similarities to the testis-specific protein (Tpx) and cysteine-rich secreted protein (CRISP) family of proteins (Foster, Gerton, 1996). These proteins include a major autoantigen of the mammalian sperm acrosome and are found in many other vertebrate tissues (Foster, Gerton, 1996; King, Lu, 1997). Three *O. volvulus* homologues of the *Ancylostoma* Secreted Protein family (*Ov*-ASPs) have been identified as some of the most abundant cDNAs of infective stage larvae (L3) (Tawe *et al.*, 2003). *Ov*-ASPs exhibit distinct patterns of expression in the life cycle of *O. volvulus* (Tawe *et al.*, 2000). *Ov*-ASP-2 transcript was found to be present in all stages, while the *Ov*-ASP-3 was L3 stage specific, and the *Ov*-ASP-1 transcript was confined to the L2, L3, mL3 and adult female stages (Tawe *et al.*, 2000). Recombinant proteins expressed from full length cDNAs, encoding two members of the *Ov*-ASP family (*Ov*-ASP-1 and *Ov*-ASP-2), were found to induce angiogenic responses after injection into corneas of naïve mice, and vessel formation was associated with only minor inflammatory cell infiltration (Tawe *et al.*, 2000).

Wilson *et al.*, (1994) reported that *Ov*-ASP-2 was expressed in *E.coli* as soluble fusion protein with Maltose Binding Protein (MBP) and purified to homogeneity by amylose affinity chromatography for use to investigate its angiogenic mechanism. Higazi and Coworkers reported that *Ov*-ASP-2 acts to promote new blood vessel formation through an indirect mechanism that might involve processing of the protein by host factors (Higazi, *et al.*, 2003). The first 105 amino acids of the recombinant protein (full length = 229 amino acids) were as active as the full-length protein in inducing neovascularization (Higazi, *et al.*, 2003). Accordingly, the most active domain of *Ov*-ASP-2 was mapped to the first 105 amino acids with less active domains encoded downstream the open reading frame of the protein. It has long been known that *O. volvulus* parasites from the forest and savanna regions of west Africa differ in their ability to induce ocular disease and that this difference might be an inherent property of the parasites themselves (Higazi *et al.*, 2003).

However, a comparison of the *Ov*-ASP-2 genomic sequences from several parasites from the blinding and non-blinding strains; indicated that no sequence polymorphisms existed in the portion of the gene that encoded amino acids 1-105 that would result in a change to the derived amino acid sequence. Thus, it is not likely that the differences seen in ocular pathogenic potential between the forest and savanna strains is a result of qualitative differences in the *Ov*-ASP-2 protein in the two strains (Higazi *et al.*, 2003). It is still possible that differences exist between the forest and savanna strain with respect to *Ov*-ASP-2 but that
these differences may be at the level of expression and not in the protein sequence itself (Higazi et al., 2003).

1.7 Endosymbiotic *Wolbachia* spp. in relation to onchocerciasis:

*Wolbachia* Endosymbiotic bacteria are present in filarial nematodes (Bazzocchi et al., 2000). *Wolbachia* are related to Gram-negative rickettsiales, in being obligate intracellular organisms with putative LPS-like molecules in the outer membrane and known to be abundant in the hypodermis and the female reproductive organs of *O. volvulus* (Brattig et al., 2000). Vast majority of filarial species examined so far have been found to harbor these endosymbionts (Bazzocchi et al., 2000). *Wolbachia* has been reported to incite a severe inflammatory response, contributing to blindness and serious skin disorders associated with *O. volvulus* infection (Hoerauf, Volkmann, 2002).

Recent reports indicate that the bacterium *Wolbachia* may be required for growth and reproduction of *Onchocerca volvulus* and has a major role in the pathogenesis of *O. volvulus* infection (Hoerauf, Volkmann, 2002; Saint Andre et al., 2002). Utilizing a murine model of corneal inflammation (keratitis), to investigate the immune and inflammatory responses associated with river blindness, has recently demonstrated an important role for endotoxin-like products from endosymbiotic bacteria and for activation of Toll-like receptor 4 (TLR4) (Saint Andre et al., 2002). The authors have implicated the Lipo-polysaccharide (LPS), released by an endosymbiont of the genus *Wolbachia*, in the pathogenesis of filariasis and ocular onchocerciasis (Saint Andre et al., 2002). The pathogenic effects of LPS and those induced by the *Ov*-ASP homologues are strikingly different, while LPS induces corneal opacification in injected corneas; neovascularization is not seen (Saint Andre et al., 2002). In contrast, while the *Ov*-ASP proteins induce neovascularization, corneal opacification is not generally seen, a part from a small non-specific effect seen at the injection site (Tawe et al., 2000). These data, when taken together, suggest that the *Ov*-ASP proteins and LPS are involved in eliciting two different pathways; that together contribute to the development of ocular onchocerciasis (Higazi et al., 2003).

Another study showed that products of microfilarial (*O. volvulus*) and adult (*O. volvulus* and *O. ochengi*) parasites affect monocytes *in vitro*. Partial blocking of monocyte modulation by neutralizing antibodies to CD14 indicated the engagement of CD14, a dominant membrane receptor on monocytes and major binding protein for lipopolysaccharides. Lipopolysaccharides-like molecules were detected in sterile products of *O. volvulus* stages, which could originate from *Wolbachia* spp. the results indicate that the monocyte/macrophage may
be a major target cell for immunomodulatory parasite-derived and intraparasitic, bacteria-derived molecules, thereby contributing to the host’s cellular hypo-responsiveness (Brattig et al., 2000). These observations have led to a new understanding of the pathogenesis of the disease (Pearlman, 2003; Hise et al., 2004).

Furthermore, depletion of Wolbachia endosymbiont by tetracycline (Hoerauf et al., 2000) blocks female worm development as well as early embryogenesis. Wolbachia represent an attractive target for macrocidal drugs in contrast to the currently used microfilaricidal Ivermectin that block only the last stage of embryogenesis (Hoerauf et al., 2000). Standard dose of 100 mg of doxycycline given to onchocerciasis patients in Ghana, treatment for six weeks depleted Wolbachia from filarial worms and rendered the worms long-term sterile (Hoerauf et al., 2001). Current follow-up studies, covering a post-treatment interval of almost 18 months in humans, suggested that the depletion of Wolbachia has probably led to an irreversible sterility. In accordance, the microfilarial loads in the skin are 0 in 90% of the patients (Hoerauf et al., 2001). This raises the hope that this new strategy might help to eliminate the disease.

1.8 Diagnosis methods:
On the basis of the detection of onchocerciasis, infection is dependent mainly on the identification of parasites or parasite products. Several methods are available for diagnosis and the criterion standards, are documentation of microfilariae on skin biopsy and demonstration of the adult worm from subcutaneous nodules. Skin snips has been the gold standard diagnostic technique by using a scleral punch, that is the tool of choice to obtain samples of epidermis and dermis (Eezzuduemhoi, 2002) without drawing blood because in a bloody biopsy, other filarial pathogens may contaminate specimen (Michael, 2002). Microfilariae that have migrated from the snip are then counted by microscopy, which allow for relatively, precise quantification of microfilarial load per milligram of tissue. The endemicity of an area can be classified also, according to the microfilarial prevalence in the community, (Bradley, Unnasch, 1996). Skin snips, although, the most conclusive, but the microscopic diagnosis cannot detect prepatent infections or low-level infections by using this method (Tawill et al., 1995), which exhibits low sensitivity, particularly in areas of low transmission and severe skin disease; in addition skin snipping is invasive and hazardous.

Otherwise, a presumptive diagnosis can be made based on physical examination of dermatitis, subcutaneous nodules, or ocular findings. For instance, the degree of ocular pathology can be easily assessed by standard tests of visual acuity and ophthalmological examination of the
eye. Slit lamp examination is necessary for determining the free-floating intraocular microfilarial load in the eye. Parasites may be detected relatively easily swimming in the anterior chamber, but are less easy to detect in the cornea, as they are less motile and relatively transparent (O’Day, Mackenzie, 1985). However, for some purposes, such as epidemiological surveillance to define the endemicity of a given area, a rapid cheap method of diagnosis is sufficient with clinical diagnosis. This clinical diagnosis of onchocerciasis in areas of endemicity is not difficult when individuals with clinical manifestations such as hanging groin, leopard skin, skin atrophy, or subcutaneous nodules are presented. The other manifestation is blindness, which is the most severe in long-standing cases of infection. Thus, this method offers a cheap, rapid, non-invasive method for roughly defining areas that would most benefit from any control programme (Bradley, Unnasch, 1996). In addition, another type of these diagnostic techniques is often called Mazzotti test; this test relies on the intense pruritic response induced by microfilariae after treatment with Diethylcarbamazine (DEC), used in a minute quantity, it can be associated with untoward effects, ranging from mild discomfort, fever, headaches, and itching.

Eventually, the studies attempted to improve diagnosis of onchocerciasis have been either immunological or DNA probe-based. A variety of immunological tests are based on the detection of parasite-specific antibodies in serum, or the use of specific antibodies to detect parasite products in body fluids. Such earlier tests are complement fixation (CF), gel diffusion, indirect haemagglutination and indirect immunofluorescence; then Radio-immunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA). Most of the earlier tests are no longer in use, either because they are too complicated or they are unreliable and less sensitive. ELISA test is ubiquitous biological technique using multiple recombinant antigens, since it depends mainly on the reaction between the parasite antigens and the specific antibodies produced in response to those antigens. The specificity of ELISA depends mainly on the type and the purity of the antigen used. The use of recombinant antigens for serologic diagnosis can help overcome the problems associated with poor sensitivity and specificity, particularly in areas where other human filarial infections overlap with those caused by *O. volvulus* but cannot reliably distinguish past infection from current infection (Tawill *et al.*, 1995; Michael, 2002). The polymerase chain reaction (PCR) method is highly specific and significantly more sensitive for detecting low-density infections than the routine microscopic method to detect microfilariae in skin snips and is more reliable in detecting active infections than serologic assays (Zimmerman *et al.*, 1994). The skin scratch PCR assay is minimally invasive and painless and does not present the risk of transmitting blood borne infections; besides, it requires meticulous laboratory technique to avoid contamination
and false-positive results. Thus these properties make the skin scratch an attractive alternative to the skin snip for detecting *O. volvulus* infection (Toe et al., 1998).

### 1.9 Treatment:

The fight to treat river blindness, in the world, started a long time ago, and one of the urgent objectives for successful development of better chemotherapeutic agents, is a sound knowledge of the different metabolic pathways of *O. volvulus*. The treatment can be divided into chemotherapy and surgical treatment.

#### 1.9.1 Chemotherapy:

Diethylcarbamazine (DEC) and Suramin were previously used for treatment of onchocerciasis, either singly or in combination. Both of them were reported that they could cause more damage than the disease, itself. Diethylcarbamazine (DEC) is an effective microfilaricidal drug, yet it may precipitate serious dermal and systemic complications, besides ocular damage in heavily infected patients. On the other hand, DEC has little or no effect on adult worms, while the Suramin is only macrofilaricidal drug; but severe systemic adverse effects have prohibited its use. Diethylcarbamazine or suramin should no longer be recommend for the treatment of onchocerciasis because of their toxicity.

Presently, the current in use effective of onchocerciasis treatment is Ivermectin, which was developed in the 1980s. It is a semisynthetic derivative of one of the group of the natural substances (Ivermectin) that are derived from the *streptomyces avermitilis* (onchocerciasis, 2002a). A single oral dose of 150 µg/kg produces a prolonged reduction in microfilarial levels and may be repeated 6 to 12 months depending on symptoms; for instance, given as a single dose, it reduces the number of microfilariae in the skin to 83.2% after 3 days and 99.5% after 3 months (Eezzuduemhoi, 2002). In addition, the side effects of this drug are generally very much milder than DEC in severity, both in terms of local and systemic reactions; moreover, no functional ocular deficiency has been reported. However, despite of the fact that the microfilaricidal agent Ivermectin is a safe, effective drug for reducing microfilarial burden, which affects several facets of the disease, but, this drug, does not kill the adult worms; it is believed that these used in multiple doses should continue for at least 12 years, the average of the adult parasite life span (Boussinesq, Gardon, 1999; Grant, 2000).

#### 1.9.2 Surgical treatments:

Surgical removal of the subcutaneous nodules is known as nodulectomy and practiced through a systematic denodulization in most endemic areas of Guatemala (Eezzuduemhoi,
This way is beneficial, not only for cosmetic reasons but also to reduce the production of microfilaremia and thus the chances of blindness.

Although the surgical method is the only way for treating the adult worms, which helps decrease the risk of disease complications (Connor et al., 1983); this sort of onchocerciasis treatment is expensive, as it requires trained personnel, because many of the nodules may be nonpalpable or the adults may be freely migrating, and the removal of nodules may have little effect on the total microfilarial or worm burden.

1.10 Control and Prevention:
The past ten years have seen a rapid and remarkable expansion of onchocerciasis control activities worldwide; these efforts are coordinated by three major regional programmes, one in Central and Latin America, the onchocerciasis control programme of the Americas (OEPA); and two in Africa, the onchocerciasis control programme (OCP) and the African programme for onchocerciasis control (APOC). Together these three regional programmes, cover more than 99% of all endemic populations and are based essentially on two strategies: simulium vector control and large-scale chemotherapy with ivermectin. Each may be used alone or in combination (Daniel, 2003).

Vector control is the chief strategy used in West Africa by (OCP) since 1974 (Daniel, 2003). The main goal in vector control is to interrupt transmission of *O. volvulus* by regular aerial spraying of all *simulium* larval breeding sites (Bell, 1999). Despite of the fact that the treatment of streams and rivers with insecticides has successfully reduced or eliminated transmission in areas where infections are endemic; a significant problem in vector control has been the development of resistance to larvicides (LeBerre et al., 1990; Greene, 1992). WHO is entitled to a solution for this problem with the rotational use of different larvicides during the treatment campaign (LeBerre et al., 1990; Greene, 1992). In this manner, the protection against biting is possible at some degree by (a) means of protective clothing designed to cover the lower legs and (b) use of insect repellents in areas where application of insecticides would be difficult (Adams, Maegraith, 1984).

Chemotherapy as a means of another control has not previously been used because of its severe side effects from microfilarial death (Greene, 1992). Recently, Ivermectin therapy could be used as a control measure to reduce transmission in areas of endemicity. Its mass distribution constitutes the main strategy for the other two regional programmes, APOC and OEPA (Daniel, 2003). There are two main uses of ivermectin in the treatment of
onchocerciasis; passive or clinic based, and active, as in large-scale or mass treatment of entire communities. The Community mass treatment is the method of choice in meso- and hyper-endemic areas of onchocerciasis (Boussinesq et al., 1995). The latest and most widely used of these community-based strategies is known as community directed treatment with ivermectin (CDTI). With this method considerable efforts are made to involve affected communities themselves in the planning, implementation and monitoring of treatment activities. CDTI is the preferred and official method used throughout Africa by OCP and APOC. The main challenge facing ivermectin-based control, therefore, is to develop and implement simple methods of ivermectin delivery that can be sustained by the communities themselves with the attractiveness of CDTI (Daniel, 2003).

1.11 Human onchocerciasis in Sudan:
There are 4 types of filarial parasites infecting humans in Sudan: *Loa Loa*, *Mansonella perstans*, *Wuchereria bancrofti*, and *O. volvulus*. The latter causes widespread infection in Sudan (Satti, 1985), and the occurrence was first reported by Bryant in Bahr El Ghazal in 1933 then Kirk (1947) reinvestigated the situation in Bahr El Ghazal, conducting Radom surveys in South and Southeast of Wau, where Cruickshank (1934) had indicated that the problem was especially severe (O'Day et al., 1984). These early records make it clear that the disease was known to well established on the Jur River and its tributaries over 50 years ago (Kaneene et al., 1985), where there is fast flowing water suitable for *S. damnosum sensu lato* breeding. Parasite populations and infection rates are highest in villages situated on or near the probable breeding sites (Ibrahim, 1987). *S. damnosum s.l.* is likely to be the main vector of the disease since it is the only true man biting *simuliidae* in Sudan and the only species properly implicated in human onchocerciasis transmission in the country (Dafa’Alla, 1989).

Generally, all studies have demonstrated that human infection in three main regions, known as the Southern, Northern and Eastern foci (Mukhtar et al., 1998), while the Southern focus, is the largest of the endemic areas, it includes all the Southern states from Bahar El Arab area (Radom) in the west to El Naser area of upper Nile state in the East (El sheikh et al., 1986). Raja area is especially proved to be hyper-endemic; its endemicity is 98 % and has one of the highest rates of Blindness in the world, about 11.7 % (NOTF, 2001).

On the other hand, the clinical patterns of onchocerciasis in Sudan vary remarkably between the southern and northern foci. Atypical blinding savanna form of the disease is common in the south and southwest, which is characterized by generalized onchocercal dermatitis, high
microfilarial loads and higher prevalence of palpable nodules (Mackenzie et al., 1987; Stingl, 1997). The infection in this region is a mild skin reaction although microfilarial loads in the skin are high (Mukhtar et al., 1998). In contrast, a non-blinding form of the disease is often associated with severe localized dermal pathology, very low microfilarial loads and few palpable nodules in the northern and eastern foci of the country. The eastern endemic region is reported to be a common focus of (Sowda) the asymmetrical hyper-reactive form of the skin disease (Ghalib et al., 1987). Furthermore, the other valuable insights into pathogenic mechanisms have come from immunocytochemical analysis of skin tissues from patients in Wau. These have shown that both IgE and IgG were associated with microfilariae in tissues. IgG, which has been shown to be involved in the killing of microfilariae through eosinophil-dependent mechanisms \textit{in vitro} (Mackenzie et al., 1985) is seen in association with dying microfilariae in the skin of patients too. In contrast the immunoglobulin on the surface of live, apparently healthy microfilariae, which are not stimulating any cellular responses in the tissues, is IgE and not IgG.

Sudan has always taken an active role in the development of chemotherapeutic regimes for parasitic diseases including onchocerciasis. Baker and Abdelnur (1985) have reviewed the distribution of onchocerciasis and its vectors in Sudan with particular emphasis on the blinding foci of the disease, i.e. the foci requiring priority control. Ivermectin is the drug now used to control onchocercal morbidity. It is a microfilaricide which is effective, well tolerated and accepted by patients, and which has no particular toxicity (APOC, 1999). In 1990 a higher national committee was established in the Sudan, it launched a small-scale control program in Bahr el Ghazal and Abu Hamad, assisted by Global 2000 Foundation (NOTF, 2001).

Accordingly, any control programme needs trained manpower, equipment and vehicles and it is vital that provision is made for training and the extra resources required to combat this disease.

1.12 Impact on communities:

In communities where onchocerciasis is highly endemic with more than 60% and often nearly 100% of persons are infected; socioeconomic problems occur as a consequence to associated morbidity and disability (Duke, 1990). \textit{O. volvulus} infection is a public health problem, which would not be tolerated in developed world. In less developed tropical countries, where people are subjected to the more devastating onslaugths of malaria, severe gastro-intestinal and respiratory infections, as well as to a welter of other infectious and parasitic diseases.
The ocular lesions of onchocerciasis that cause the true disablement and which, in the worst-affected villages, account for the socio-economic disaster of desertion and depopulation, rendering ‘river blindness’ is the number one public health problem in the community. Beyond the well-documented effects of blindness, onchocerciasis has other economic and social costs; onchocercal skin disease may reduce marital prospects, influence the course of pregnancy, shorten the length of breast feeding and disrupt social relationships (Amazigo, 1994). Among agricultural workers, onchocerciasis has been associated with increased time away from work and reduced productivity, leading to lower income (Workneh et al., 1993). The Communities afflicted by onchocerciasis to this extent cease to be economically viable after a few years. Their inhabitants drift away, or the village moves lock, stock and barrel, to a less dangerous area, deserting the fertile land near the rivers in exchange for less productive land further a way from the simulium breeding sites (Duke, 1990).

1.13 Objectives of the study:
This study was carried out to examine the diagnostic value of *O. volvulus* recombinant antigen *Ov*-ASP-2/MBP under study comparatively with MBP alone. This protein is believed to be involved in the formation of new blood vessels (neovascularization) in murine models (Tawe et al., 2000) and their angiogenic mechanism is underway (Tarig Higazi, personal communication). Hence, they might be implicated in the onset of the ocular disease. Sera were used from well-characterized onchocerciasis patients in an ELISA assay to explore possible roles of these antigens in the pathogenesis of the disease.

The following aims were considered as specific purpose for this study:
1. To measure the levels of IgG, and IgG subclasses (IgG3 & IgG4) in sera of onchocerciasis against *Ov*-ASP-2/MBP antigen.
2. To determine the sensitivity and specificity of *Ov*-ASP-2/MBP recombinant antigen in the diagnosis of human onchocerciasis and compared with MBP fusion partner alone.
3. To correlate the reactivity of *Ov*-ASP-2/MBP with ocular onchocerciasis and other clinical presentations of the disease.
CHAPTER TWO
MATERIALS AND METHODS

2.1 Study population:
Onchocerciasis patients from different regions of southern Sudan were selected in this study. All subjects were displaced people originally; coming to Khartoum from heavily endemic areas due to civil war and live in camps where displaced people are housed in Khartoum. In this work Subgroups of patients voluntarily attend to the Tropical Medicine Research Institute, in Khartoum for health care; including diagnosis and treatment of Onchocerciasis. Another group of individuals living in the Wad El Basheer Camp in Om-Bada province, which is situated about 27 km from Khartoum province - Khartoum state. People here live in primitive houses and lead a very simple way of life, like all other displaced camps.

The population under study comprises 102 subjects, belonging mainly to Denka and Moro tribes, and composed of males and females of all ages. They are classified into; (a) Eighty-two endemic positive subjects [EP] who had previously long lived in onchocerciasis endemic areas, and were parasitologically positive, (b) twenty subjects of Onchocerca negative control [ONC] which include malaria, tuberculosis, schistosomiasis patients as co-endemic diseases, (c) healthy individuals coming from an onchocerciasis-free area (Khartoum).

2.2 Endorsement of participant:
Ethical clearance was obtained from the Federal Ministry of Health in the Sudan before collection of the samples. Each patient was briefed and informed respectfully about the aims of this work explaining simply the procedure to obtain his/her consent to participate in the study. Baseline data on age, sex, origin, history of living in onchocerciasis endemic area, presence of nodules, presence and severity of skin lesions and degree of optical atrophy involvement were recorded in a questionnaire form (Appendix.I), relying on Rapid Epidemiological Mapping of onchocerciasis pattern.

2.3 Approaches to Onchocerciasis examination:
2.3.1 Parasitological examinations:
Bilateral bloodless skin snips approximately one to several milligrams were taken from the iliac crest using a Wasler punch. Biopsies were incubated for 2 hrs at room temperature in microtitre-plates containing 100µl/well of saline solution (150 mM NaCl, pH 6.9) as recommended by Gqmez-Priego et al., (1993) or 1640-RPMI media and the emergent
microfilariae were observed under the inverted microscope. The plates were further incubated for overnight if no Mf were detected.

2.3.2 Clinical examinations:
Clinical examinations were performed on all individuals under medical supervision. These data of clinical investigation were obtained from each patient in a designed questionnaire such as subcutaneous nodules, ocular and cutaneous lesions besides the level of all damage (Appendix.I).

2.3.3 Antibody detection:
2.3.3.1 Enzyme-Linked ImmunoSorbent Assay (ELISA):
A sensitive immunoenzymatic test, since it depends on the reaction between the antigens and the specific antibodies produced as a response to those antigens. The specificity of this technique depends mainly on the type and the purity of the antigen used. This assay system had been initially used to measure immuno-globulins of various classes (Engvall, Perlmann, 1971; Van weeman, Schuurs, 1971). Then, Voller et al., (1976) first reported the use of this technique to measure antibody response to specific infections.

2.3.3.1.1 Materials required for ELISA:

I. Equipment:
1- polystyrene microtiter plates.
2- Multi-channel micropipette.
3- Micropipettes capable of accurately delivering (0.5 - 1.5 µl, 0.5-20µl, 2-200 µl and 200 – 1000µl) volumes.
4- Tipes (white, yellow and blue).
5- Test tubes for serum dilution.
6- Measuring cylinder (100 ml).
7- Flask (1 liter).
8- Timer (0 to 60 minutes).
9- Towel.
10- Plastic wrapping.
11- Wash bottle (simple decantation).
12- ELISA microplate reader with 492 and 405 nm filters.
13- Disposal basin.
14- Disinfectant (70% Ethanol).
II. Reagents:

- **Buffers:**
  0.05M Carbonate-bicarbonate buffer as coating buffer; washing buffer (0.5ml Tween20 + 999.5ml phosphate buffer saline); substrate buffers which were used to match the type of substrate; such as, Citrate buffer, for OPD substrate and Glycine buffer, for p-NPP substrate; stopping buffer, such as 20% sulphuric acid and 3M sodium hydroxide, for OPD and p-NPP, respectively (Table 2.1).

- **Antigen:**
  *Ov-ASP–2/MBP O.volvulus* antigen used in this study, was provided by Dr. Tarig B. Higazi; University of Alabama; U.S.A. This protein was expressed in *E.coli* as Maltose Binding Protein (MBP), fusion protein, and purified by column chromatography (Tawe et al., 2000). MBP fusion partner was used alone as a negative control for the fusion protein.

- **Test samples (sera):**
  Venous blood samples (3-5 ml) were collected from all subjects. The sera were separated from whole blood following standard procedure by centrifuging the blood samples at 1000 r.p.m. for 10 minutes and kept frozen at −70 °C in cryotubes vials until used.

- **Enzyme– antibody conjugates:**
  A variety of enzymes have been used as labels for enzyme immunoassay, Goat anti-human peroxidase IgG conjugate, Mouse monoclonal anti–human IgG3 Biotin conjugate, Mouse monoclonal anti–human IgG4 Biotin conjugate and Avidin alkaline phosphatase conjugate which in tests were employing labeled antibodies (Table 2.2).

- **Substrates:**
  The enzyme label used governs the choice of substrate; thus, p-nitrophenyl phosphate (p-NPP) for alkaline phosphatase and O-phenylene diamine (OPD) for peroxidase was used (Table 2.3).

2.3.3.1.2 ELISA method:
The reactivity of IgG class and the predominance of IgG subclasses levels of all the individuals included in this study, were measured by indirect ELISA test, which was useful for screening antisera for specific antibodies, when purified antigen are available. ELISA
was adapted according to the method of Voller *et al*., (1979) and performed with some modifications from the general procedure. *Ov*-ASP-2/MBP antigen and MBP protein were tested, first, in more than one trial but at the same conditions for obtaining the best result to give optimum response of antigen. The recombinant antigen had responded in comparison with the fusion protein MBP and after observing the blank, which was used as a control to determine the specificity to binding of human antibody.

For the detection of total IgG, polystyrene microtitre flat bottom plates; 96-well configured in twelve 1x 8 strips; (Immulon 2, Dynatech laboratories, USA) were used. The appropriate concentration of antigens, sera and anti-human serum probes were determined by checkerboard titration (i.e. trials of serial dilutions) using pooled positive sera (the highest responders of Sudanese onchocerciasis patients) and pooled negative sera (healthy individuals coming from areas free of filariasis) to be as the minimum amount required yielding optimum color formation. The *Ov*-ASP-2/MBP antigen (1.96µg/ml) and MBP fusion proteins alone (3.12 µg /ml) were diluted in coating buffer (Table 2.1). Multichannel pipet was used to dispense 50µl antigen solution into each well of the plate, at concentration 1: 50 and 1:100 for *Ov*-ASP-2/MBP and MBP alone, respectively. Coated plates were wrapped with plastic wrapping and incubated overnight at 4°C. The plates were flicked out and washed three times, for five minutes, with washing buffer (Table 2.1). The wells free sites were blocked by 3% BSA and PBS+ T20 (100 µl/well) as blocking agent, and incubated for two hours at 37°C.

The blocking buffer was discarded and the plates were washed three times, with PBS+T20, for five minutes. One hundred µls of each individual’s diluted sera (primary sera) were loaded in duplicates at a dilution of 1:500 in diluent solution (3% BSA/ PBS+ 0.05% T20), and incubated for one hour at 37°C. Each plate contained about 4 wells as controls (pool positive and pool negative sera), plus four wells to be used as a blank; two wells left without sera (PBS/0.05% T20 only) and another two wells without Antigen (only coating buffer). The plates were washed as before, 100µl/well peroxidase conjugate anti-human IgG (Table 2.2) was added to each well with concentration of 1:10,000 µl (in PBS /0.05% T20) and incubated at 37°C for one hour. The plates was washed three times with (PBS/0.05% T20) for five minutes, each well was filled with 100µls of peroxidase substrate solution consisting of O-phenylenediamine dihydrochloride (OPD) (Table 2.3) dissolved in citrate buffer pH 0.5 (Table 2.1) with 0.01% (w/v) H2O2 (5µl) immediately before use (100µl/well). After the substrate solution was added, the reaction was allowed to commence by incubating the plates in complete darkness for 5 minutes at room temperature (21-25°C). The reaction was stopped by addition of 50µls of 20 % sulphuric acid (3M H2SO4) to each well. The optical density
(OD) was measured using ELISA reader laboratory system Multiskan plus (serial 3140) with filter 492 nm and the optical density of each well was recorded. The amount of test antibody was measured by assessing the amount of the colored end product by OD scanning of the plate i.e. the intensity of color is an indication of the amount of bound antibody.

The detection of the specific antibodies in the IgG3 and IgG4 subclasses (using the above mentioned procedure) was similar to that of the IgG while the concentration of Ov- ASP-2 /MBP and MBP protein were 0.2 µg/ml. Thirty-one samples were selected as the highly positive reactive samples in the total IgG screening. The sera for the detection of two the subclass IgG3 & IgG4 were added, at a dilution of 1: 50µl /well, in diluent solution (3%BSA and PBS /0.05%T20). The plates were washed by addition of PBS / 0.05%T20 buffer. The wells were covered by addition of Biotin conjugate, monoclonal anti-human IgG3 & IgG4 at concentration of 1: 3000 and 1: 10,000µl (in 1%BSA and PBS /0.05% T20) respectively. Following the flicked out the plates were washed to free unbound conjugates (secondary antibody). Another layer of Avidin – Alkaline phosphatase at concentration 1: 50,000 µl (in PBS / T20 only) was added, incubated for one hour at 37°C and wrapped with plastic wrap.

The plates were loaded with the p-NPP substrate (Table 2.3) and the reaction was stopped after 30-minutes incubation period for (IgG3) and twenty minutes for (IgG4) in a dark place at room temperature by adding 50 µl of 3M NaOH. The optical density was measured at 405 nm. The results were expressed as the mean of duplicate wells after deducting the blank values (background without sera). All optical density (OD.) readings were corrected by using correction factor recommended by Pessee et al., (1977).

\[
\text{Individual corrected reading} = \frac{\text{Fixed OD. of reference +ve}}{\text{Mean OD. of a plates reference + ve}} \times \text{individual reading}.
\]

Reference positive sera were used in all plates as pooled positive sera and only inter-assay variation of less than 10% between plates was accepted (Denmark, Chessum, 1978; Kurstak, 1985). The discrimination level between positive and negative individuals was set at the mean of normal controls plus two standard deviations from the mean (Richardson et al., 1983).
Table 2.1: Constitution of ELISA buffers.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>Ingredients</th>
<th>Formula</th>
<th>Weight /L (D.W.)</th>
<th>pH</th>
<th>Adjustment solution</th>
</tr>
</thead>
</table>
| Coating buffer: 0.05M Carbonate bicarbonate buffer. | *Sodium carbonate Anhydrous.  
*Sodium bicarbonate. | Na₂CO₃                   | 1.59 gm/L               | pH = 9.6 | NaOH or HCl        |
|                                     |                                                                               | NaHCO₃                   | 2.93 gm/L               |      |                    |
| Washing buffer: Phosphate Buffered saline (PBS)+Tween20 | PBS =  
*Sodium chloride.  
*Sodium phosphate dibasic monohydrate.  
*Potassium dihydrogen phosphate.  
*Potassium chloride. | NaCl                     | 8.00 gm/L               | pH = 7.4 | NaOH or HCl        |
|                                     |                                                                               | Na₂HPO₄. H₂O             | 2.80 gm/L               |      |                    |
|                                     |                                                                               | KH₂PO₄                   | 0.24 gm/L               |      |                    |
|                                     |                                                                               | KCl                      | 0.20 gm/L               |      |                    |
| Substrate buffer: Sodium Citrate    | *Citric acid.  
*Disodium monophosphate anhydrous. | H₃C₆H₅O₇.H₂O             | 7.30 gm/L               | pH = 5.0 | NaOH or Citric acid |
|                                     |                                                                               | Na₂HPO₄                  | 11.86 gm/L             |      |                    |
| Substrate buffer: Glycine buffer    | *Glycine.  
*Magnesium chloride.  
*Zinc chloride. | C₂H₃NO₂                  | 8.00 gm/L               | pH = 10.4 | NaOH or HCl        |
|                                     |                                                                               | MgCl₂.6H₂O              | 0.20 gm/L               |      |                    |
|                                     |                                                                               | ZnCl₂                    | 200 µl/L                |      |                    |
| Stopping buffer: (For IgG)          | *20%Sulphuric acid. | H₂SO₄                    | 20 mL/L                | ----- | -------- |
| Stopping buffer: (For IgG3 & IgG4)  | *Sodium hydroxide. | 3M NaOH                  | 120 gm/L               | ----- | -------- |

* D.W. = distilled water.
Table 2.2: Description of conjugates used for specific immunoglobulins determination:

<table>
<thead>
<tr>
<th>Conjugate Name</th>
<th>Product NO.</th>
<th>Type of conjugate</th>
<th>Dilution</th>
<th>Diluent Solution</th>
<th>Incubation Time</th>
<th>Temp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG : Goat Anti-human IgG (Y-chain specific) IgG fraction of antiserum.</td>
<td>A-8419</td>
<td>Peroxidase</td>
<td>1:10,000</td>
<td>PBS+T20</td>
<td>One hour</td>
<td>37°C</td>
</tr>
<tr>
<td>IgG3 : Mouse monoclonal Anti-human IgG3 Fraction of mouse Ascites fluid.</td>
<td>B- 3523</td>
<td>Biotin</td>
<td>1:3000</td>
<td>1%BSA+ PBS+T20</td>
<td>One hour</td>
<td>37°C</td>
</tr>
<tr>
<td>IgG4: Mouse monoclonal Anti-human IgG4 Fraction of mouse Ascites fluid.</td>
<td>B- 3648</td>
<td>Biotin</td>
<td>1:10,000</td>
<td>1%BSA+ PBS+T20</td>
<td>One hour</td>
<td>37°C</td>
</tr>
<tr>
<td>Avidin</td>
<td>A-7294</td>
<td>Alkaline Phosphatase</td>
<td>1:50,000</td>
<td>PBS+T20</td>
<td>One hour</td>
<td>37°C</td>
</tr>
</tbody>
</table>

* Temp. : Incubation temperature.

Table 2.3: Description of substrate types.

<table>
<thead>
<tr>
<th>Substrate Name</th>
<th>Product No.</th>
<th>Type of substrate</th>
<th>Substrate Buffer</th>
<th>Incubation time</th>
<th>Reaction color</th>
<th>Reaction Stopping Solution</th>
<th>Reading Filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>O-Phenylenediamine dihydrochloride (OPD).</td>
<td>P 9029 (Powder)</td>
<td>Peroxidase</td>
<td>Sodium Citrate</td>
<td>5 min. (For IgG)</td>
<td>Orange to Brown</td>
<td>Sulphuric acid</td>
<td>492nm (IgG)</td>
</tr>
<tr>
<td>Disodium p-Nitrophenyl phosphate (p-NPP).</td>
<td>N 9389</td>
<td>Phosphatase</td>
<td>Glycine</td>
<td>20min. (For IgG4) 30min. (For IgG3)</td>
<td>Yellow</td>
<td>Sodium hydroxide</td>
<td>405nm (IgG3 &amp; IgG4)</td>
</tr>
</tbody>
</table>
2.4 Statistical analysis:

Statistical package for social sciences (SPSS)-USA computer program was applied for data analysis. All the data were checked for normal distribution, a prerequisite for choosing adequate statistical analysis. Independent-student T-test, One-way ANOVA, Kruskal-Wallis, Mann-Whitney and correlation tests were used according to the type of data distribution and information required.

The readings of the reactivity of optical density (OD.) values of ELISA for IgG and its subclass (IgG3) were not normal distributed data, therefore non-parametric tests were used; While IgG4 subclass data were normality distributed. Correlation tests were applied to evaluate the relationship between the clinical symptoms of the disease and the obtained (OD.) values of the total immunoglobulin IgG and subclasses (IgG3 & IgG4).

*****
CHAPTER THREE
RESULTS

3.1 *Onchocerca volvulus* recombinant antigen [Ov-ASP-2 /MBP]:

3.1.1 Detection of IgG and IgG subclasses (IgG3 & IgG4) of Onchocerciasis patients and control groups by Ov-ASP-2/MBP and MBP alone:

Sera of onchocerciasis endemic positive patients (EP) and onchocerca negative control (ONC) were tested in the present study using *O. volvulus* recombinant antigen under study and MBP fusion partner making clear the activity of antigen. The highest dilution, which tested positive was called the titre, and was taken as a quantitative value of the amount of antibody in the original sample.

To determine the reactivity power of Ov-ASP-2/MBP recombinant antigen, against sera of *O. volvulus* infected individuals, ELISA was performed on 82 individuals sera from endemic positive (EP) patients and a 20 sera from onchocerciasis negative control (ONC) group representing healthy individuals as well as individual infected with other co-endemic diseases. Sera of onchocerciasis patients (EP) showed high antigen reactivity to Ov-ASP-2/MBP compared to MBP alone. In contrast, no difference was seen in MBP reactivity to onchocerciasis patients and control group when individual sera were tested simultaneously (Fig 3.1).

ELISAs also, were designed, to detect antigen-specific sera IgG, IgG3 and IgG4. Thirty-one onchocerciasis sera samples representing the most highly reactive sera for total IgG were screened for IgG3 and IgG4 reactivity. All immunoglobulins under study showed reactivity to Ov-ASP-2/MBP. However, total IgG was the most reactive antibodies compared to subclasses IgG3 and IgG4 (Fig 3.2). For all sera Immunoglobulins tested, there was a significant difference between Ov-ASP-2/MBP reactivity to infected individuals compared with control group, (P = 0.001, 0.01 and 0.001) for IgG, IgG3 and IgG4 respectively (Table 3.1). In contrast, a similar level of MBP reactivity was seen between the different groups under study.
Fig. 3.1: Antibody responses to *Ov*-ASP-2/MBP and MBP alone in onchocerciasis endemic positives (EP) and negative control (ONC) groups.
Fig. 3.2: Activity power response of recombinant antigen Ov-ASP-2/MBP with onchocerciasis patients for IgG immunoglobulin and its subclasses (IgG3 & IgG4).
Table 3.1: Comparison of mean antibody (± S.d.) response activity between the Ov-ASP-2 /MBP antigen and MBP protein for infected and uninfected individuals:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Onchocerciasis endemic Positive (EP)</th>
<th>Onchocerca negative Control (ONC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ov-ASP-2/MBP Mean ± S.d.</td>
<td>MBP Mean ± S.d.</td>
</tr>
<tr>
<td>IgG</td>
<td>0.741 ± 0.417</td>
<td>0.287 ± 0.1576</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.301 ± 0.0989</td>
<td>0.199 ± 0.0560</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.225 ± 0.0725</td>
<td>0.125 ± 0.0171</td>
</tr>
</tbody>
</table>

Mean ± S.d. = Mean Antibody levels ± Std. Deviation

3.1.2 Efficiency of diagnostic value of O. volvulus cloned antigen (Ov-ASP-2 /MBP) to explore the possibility of this antigen in the diagnosis of the disease:

ELISA testing of Ov-ASP-2/MBP and MBP alone showed that Ov-ASP-2/MBP could detect the intensity of antibodies range of onchocerciasis patients and differentiate between positive and negative individuals through binding human IgG, IgG3 and IgG4. In contrast, MBP protein does not react differentially to either group (Figs. 3.3, 3.4 & 3.5).

The recorded titres of mean antibody values (Table 3.1) showed variance of efficiency between Ov-ASP-2 /MBP and MBP proteins in the endemic positive patients. IgG immunoglobulin had the highest titres of antibody response which found with the maximum and minimum values as another association to illustration immune response (2.26 for maximum and 0.17 for minimum) while IgG3 & IgG4 have fairly low titres (0.47 for maximum and 0.15 for minimum) and (0.31 for maximum and 0.09 for minimum) respectively (Table 3.2). It was clear the possibility of antigen in diagnosis of the disease when comparing with (maximum and minimum) values of ONC group.
**A- B- C- D- E- F- G rows:**

[At 1-6 columns] = samples of tested sera with Ov-ASP-2/MBP + only 2 wells as control which loaded with pool positive serum sample.

[At 7-12 columns] = the same samples tested sera with MBP alone.

**H row:** = [Nc.: blank (no coating); Ns.: blank (no sera); –Ve: pool negative serum sample).

**Fig.3.3:** Discriminating power of Ov-ASP-2/MBP to detect IgG antibodies by ELISA. Left half of the plate was coated with Ov-ASP-2/MBP and right half coated with MBP alone.
**A- B- C- D- E- F- G rows:**

[At 1-6 columns] = samples of tested sera with Ov-ASP-2/MBP + only 2 wells as control which loaded with pool positive serum sample.

[At 7-12 columns] = the same samples tested sera with MBP alone.

**H row:** = [Nc.: blank (no coating); Ns.: blank (no sera); –Ve: pool negative serum sample).

**Fig.3.4:** Discriminating power of Ov-ASP-2/MBP to detect IgG3 antibodies by ELISA. Left half of the plate was coated with Ov-ASP-2/MBP and right half coated with MBP alone.
**A- B- C- D- E- F- G rows:**

[At 1-6 columns] = samples of tested sera with $Ov$-ASP-2/MBP + only 2 wells as control which loaded with pool positive serum sample.

[At 7-12 columns] = the same samples tested sera with MBP alone.

**H row:** = [Nc.: blank (no coating); Ns.: blank (no sera); –Ve: pool negative serum sample).

**Fig.3.5:** Discriminating power of $Ov$-ASP-2/MBP to detect IgG4 antibodies by ELISA. Left half of the plate was coated with $Ov$-ASP-2/MBP and right half coated with MBP alone.
Table 3.2: Cloned antigen participation in the disparity values of antibody response:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>EP</th>
<th>ONC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum OD.reading</td>
<td>Minimum OD.reading</td>
</tr>
<tr>
<td>IgG</td>
<td>2.26</td>
<td>0.17</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.47</td>
<td>0.15</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.31</td>
<td>0.09</td>
</tr>
</tbody>
</table>

3.1.3 Sensitivity of Ov-ASP-2/MBP recombinant antigen:

The proportion of confirmed positive individuals who tested positive by this antigen determines the sensitivity of an antigen in a given test. The positivity was determined by cut-off values of optical density [cut-off value = mean of negative controls individuals (O.D.) + 2 S.D.]. For total IgG, 61 sera from a total of 82 were positive as determined by IgG cut-off point value [0.2] (Fig. 3.6). The sensitivity of Ov-ASP-2/MBP in detection of IgG antibodies was calculated as 74.4% (Table 3.3). In a similar manner, Ov-ASP-2/MBP sensitivity to IgG3 and IgG4 were 64.5 % and 70.9 % respectively (Tables 3.4 & 3.5), Which did, 0.07 and 0.04 cut-off point values represent for IgG3 & IgG4 respectively (Figs. 3.7 & 3.8).

To confirm this finding a statistical analysis using Mann-Whitney U tests was done on IgG and IgG3 Immunoglobulin while independent-sample T- test for IgG4 subclass ELISA readings of all individuals.
Fig. 3.6. Positive individuals were determined according to the ELISA cut-off value for IgG response.
Fig. 3.7. Positive individuals were determined according to the ELISA cut-off value for IgG3 response:
Fig. 3.8. Positive individuals were determined according to the ELISA cut-off value for IgG4 response:
**Table 3.3:** Ov-ASP-2/MBP sensitivity and specificity to total IgG immunoglobulins.

<table>
<thead>
<tr>
<th></th>
<th>EP</th>
<th>ONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +ve</td>
<td>61</td>
<td>4</td>
</tr>
<tr>
<td>Test -ve</td>
<td>21</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>82</td>
<td>20</td>
</tr>
</tbody>
</table>

Sensitivity = 74.4 %  
Specificity = 80 %

**Table 3.4:** Ov-ASP-2/MBP sensitivity and specificity to IgG3 immunoglobulins.

<table>
<thead>
<tr>
<th></th>
<th>EP</th>
<th>ONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +ve</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Test -ve</td>
<td>11</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>20</td>
</tr>
</tbody>
</table>

Sensitivity = 64.5 %  
Specificity = 65 %
Table 3.5: *Ov*-ASP-2/MBP sensitivity and specificity to IgG4 immunoglobulins.

<table>
<thead>
<tr>
<th></th>
<th>EP</th>
<th>ONC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test +ve</td>
<td>22</td>
<td>3</td>
</tr>
<tr>
<td>Test -ve</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>20</td>
</tr>
</tbody>
</table>

*Sensitivity* = 70.9 %

*Specificity* = 85 %

3.1.4 Specificity of *Ov*-ASP-2/MBP recombinant antigen:

The proportion of onchocerciasis negative sera that were identified as negatives by it determines the specificity of *Ov*-ASP-2/MBP. *Ov*-ASP-2/MBP showed specificity of 80 % for IgG, 65% for IgG3 and 85% for IgG4 (Tables 3.3, 3.4 & 3.5) respectively. IgG4 antibodies showed slightly higher specificity to *Ov*-ASP-2/MBP while IgG antibodies had the highest sensitivity to the recombinant antigen; In contrast IgG3 antibodies showed lower specificity and sensitivity to *Ov*-ASP-2/MBP (Fig. 3.9).

Control sera from malaria patients cross-reacted with *Ov*-ASP-2/MBP resulting in reduced specificity in all immunoglobulins under study. In contrast, none of the schistosomiasis, tuberculosis or healthy control sera reacted with the recombinant antigen. Furthermore the optical density values of ELISA showed highly significant association of *Ov*-ASP-2 /MBP antigen in detecting IgG and its subclasses IgG3 and IgG4 antibody responses to infected individuals (EP) compared with different control groups (P = 0.000) (Table3.6).
Fig.3.9: Sensitivity and specificity of Ov-ASP-2/MBP recombinant antigen to IgG and its subclasses IgG3 and IgG4.

Table 3.6: Comparison of antibody responses to Ov-ASP-2/MBP antigen between Onchocerca volvulus-infected and uninfected control groups:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Onchocerciasis Mean ± S.d.</th>
<th>Schistosomiasis Mean ± S.d.</th>
<th>Tuberculosis Mean ± S.d.</th>
<th>Malaria Mean ± S.d.</th>
<th>Healthy Individuals Mean ± S.d.</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IgG</strong></td>
<td>0.741 ± 0.417</td>
<td>0.157 ± 0.182</td>
<td>0.186 ± 0.109</td>
<td>0.300 ± 0.184</td>
<td>0.160 ± 0.077</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>IgG3</strong></td>
<td>0.301 ± 0.0989</td>
<td>0.076 ± 0.015</td>
<td>0.065 ± 0.01</td>
<td>0.112 ± 0.044</td>
<td>0.098 ± 0.032</td>
<td>0.000</td>
</tr>
<tr>
<td><strong>IgG4</strong></td>
<td>0.225 ± 0.0725</td>
<td>0.133 ± 0.0171</td>
<td>0.107 ± 0.007</td>
<td>0.144 ± 0.072</td>
<td>0.131 ± 0.023</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Mean ± S.d. = Mean Antibody levels ± Std. Deviation.
3.1.5 **Seroprevalence levels of Ov-ASP-2/MBP antibodies in the study population:**

The recorded titres of the immunoglobulin IgG and its subclasses are essential aspects for the detective antibody responses of onchocerciasis patients against the cloned antigen that to take an interest in this study.

*Ov*-ASP-2/MBP-specific IgG, IgG3 and IgG4 antibodies of 31 infected sera were determined by ELISA and titres were compared to determine the seroprevalence of these immunoglobulins in onchocerciasis infected individuals (Fig.3.10). IgG antibodies clearly predominated IgG3 and IgG4 subclasses. Optical density of ELISA reading for total IgG reactive sera of onchocerciasis patients had the highest proportion level of antibody response (74%), while IgG3 and IgG4 had fairly low responses 14 and 12% respectively.

![Proportion of Ov-ASP-2/MBP-specific IgG and IgG subclasses IgG3 and IgG4 antibodies in onchocerciasis patients.](image)

**Fig. 3.10:** Proportion of *Ov*-ASP-2/MBP-specific IgG and IgG subclasses IgG3 and IgG4 antibodies in onchocerciasis patients.
3.2 Association between *Ov*-ASP-2/MBP antibody responses and clinical profiles:

Individuals in the endemic positives (EP) group were investigated for association between antibody responses to *Ov*-ASP-2/MBP and different clinical presentations of the disease. In terms of vision, the group was classified into three subgroups: clear vision, low vision and blind. In terms of onchodermatitis, patients were divided into four groups those who had no lesions, mild lesions, severe lesions and Sowda. The same group was also classified based on presence or absence of palpable nodules. Included in these analyses was age of infected individuals (Appendix II, III & IV).

3.2.1 Seroprevalence of the antibody response in the vision status groups of study population:

Antibody responses to *Ov*-ASP-2/MBP were tested against the vision status of seropositive individuals. Patients with different vision status showed similar distribution of IgG and its subclasses IgG3 and IgG4 (Fig 3.11). Total IgG responses that represented the majority of immunoglobulins, showed increased association with the blind individuals subgroup Fig. (3.11).

Statistical analysis was then performed using Kruskal-Wallis test to investigate associations between vision status and *Ov*-ASP-2/MBP antibody responses for IgG and IgG3 subclass while One-way ANOVA test was used for IgG4 subclass. Statistical analysis showed significant correlation between IgG responses to *Ov*-ASP-2/MBP and patients with blindness due to onchocerciasis (P = 0.002) ; In contrast, no significant difference in IgG3 and IgG4 antibody responses of clear and low vision subgroups (P= 0.485 and 0.198) for IgG3 and IgG4 respectively (Table. 3.7). Fig. 3.11 shows the difference level of IgG and IgG subclasses between the eye condition groups.
Fig. 3.11: Seroprevalence of antibodies against Ov-ASP-2/MBP in patients with different vision status.

Table 3.7: Ov-ASP-2/MBP antibody responses of patients with different vision status:

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clear Mean ± S.d.</th>
<th>Low Mean ± S.d.</th>
<th>Blind Mean ± S.d.</th>
<th>P values</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>0.779 ± 0.421</td>
<td>0.679 ± 0.415</td>
<td>1.396 ± 0.319</td>
<td>0.002</td>
</tr>
<tr>
<td>IgG3</td>
<td>0.313 ± 0.0967</td>
<td>0.272 ± 0.107</td>
<td>0.337 ± 0.0139</td>
<td>0.485</td>
</tr>
<tr>
<td>IgG4</td>
<td>0.239 ± 0.0697</td>
<td>0.202 ± 0.0781</td>
<td>0.264 ± 0.0244</td>
<td>0.198</td>
</tr>
</tbody>
</table>

Mean ± S.d. = Mean OD ± Std. Deviation
3.2.2 Correlation of Ov-ASP-2/MBP activity of ocular onchocerciasis groups with other clinical presentations:

(a) Dermatological status and ocular conditions:
The correlation between Ov-ASP-2/MBP antibody responses in vision subgroups and dermatological stage of onchodermatitis is presented in fig.3.12. Onchocerciasis patients with different degree of eye disease were further classified based on their skin lesions [no lesions, mild, severe and Sowda] according to their skin condition, for the purpose of study immune response to Ov-ASP-2/MBP.

Blindness was found to correlate with clear skin, while negative correlation was seen between blindness and severity of skin lesions and Sowda Fig. (3.12). No correlation was observed between clear skin and low vision, whereas mild onchodermatitis was related higher response values in clear and low vision groups (Fig. 3.12). However no statistically significant differences (Kruskal-Wallis test) were showed between different vision and dermatological subgroups (P = 0.151) (Table 3.8).

![Fig.3.12: IgG responses of vision groups with dermatological status.](image-url)
Table 3.8: Correlation of mean antibody values (± S.d) of vision status groups with skin lesions type:

<table>
<thead>
<tr>
<th>Eye condition</th>
<th>No lesions Mean ± S.d.</th>
<th>Mild Mean ± S.d.</th>
<th>Severe Mean ± S.d.</th>
<th>Sowda Mean ± S.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>0.651 ± 0.204</td>
<td>0.849 ± 0.388</td>
<td>0.809 ± 0.522</td>
<td>0.484 ± 0.213</td>
</tr>
<tr>
<td>Low</td>
<td>** 0.734 ± 0.456</td>
<td>**</td>
<td>0.579 ± 0.306</td>
<td>0.751 ± 0.613</td>
</tr>
<tr>
<td>Blind</td>
<td>1.43 ± 0.34</td>
<td>1.15 ± 0.005</td>
<td>**</td>
<td>**</td>
</tr>
</tbody>
</table>

** Not observed

(b) Nodules presence and vision subgroups:

Ov-ASP-2 /MBP antibody responses in various vision subgroups on basis of presence or absence of palpable nodules are shown in fig. 3.13. Slight variation in Ov-ASP-2 /MBP antibody responses in patients with or without palpable nodules was seen in the three vision groups (Fig. 3.13). However, these variations were statistically not significant different within the subgroups (P = 0.412) (Table 3.9).

** Mean ± S.d. = Mean Antibody levels ± Std. Deviation

Fig 3.13: Ov-ASP-2 /MBP IgG responses of vision groups with or without palpable nodules.
Table 3.9: Correlation of mean antibody values (± S.d) of vision status groups with nodules presented:

<table>
<thead>
<tr>
<th>Eye condition</th>
<th>Absent Mean ± S.d.</th>
<th>Present Mean ± S.d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear</td>
<td>0.782 ± 0.409</td>
<td>0.748 ± 0.561</td>
</tr>
<tr>
<td>Low</td>
<td>0.703 ± 0.384</td>
<td>0.619 ± 0.510</td>
</tr>
<tr>
<td>Blind</td>
<td>1.410 ± 0.321</td>
<td>1.380 ± 0.390</td>
</tr>
</tbody>
</table>

Mean ± S.d. = Mean Antibody levels (OD) ± Std. Deviation

3.3. IgG titre with ranged age of onchocerciasis patients:

Only IgG responses to Ov-ASP-2/MBP were tested for correlation with patient’s age. Patients were divided into six age groups [10-20, 21-30, 31-40, 41-50, 51-60, and >60 years]. Mean of IgG titre of all age groups is showed in fig. 3.14. Kruskal-Wallis test was applied to study the correlation and rank IgG responses to Ov-ASP-2/MBP in all age groups (Table 3.10).

Younger age groups showed higher IgG responses to Ov-ASP-2/MBP. IgG responses decreases with to reach minimum vales in the > 61years age group (Fig. 3.14). However, a marked decrease in antibody responses was noticed in age group >60 which led to an overall statistically non-significant correlation (Fig. 3.14)(Table 3.10).
Table 3.10: Relationship between age and IgG antibody responses to Ov-ASP-2/MBP recombinant antigen.

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Immune response Mean ± S.d</th>
<th>R values [Mean Rank]</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>1.097 ± 0.809</td>
<td>7.00</td>
</tr>
<tr>
<td>21-30</td>
<td>0.755 ± 0.383</td>
<td>5.00</td>
</tr>
<tr>
<td>31-40</td>
<td>0.711 ± 0.408</td>
<td>3.00</td>
</tr>
<tr>
<td>41-50</td>
<td>0.521 ± 0.184</td>
<td>2.00</td>
</tr>
<tr>
<td>51-60</td>
<td>0.744 ± 0.269</td>
<td>4.00</td>
</tr>
<tr>
<td>&gt;60</td>
<td>0.219 ± 0.0180</td>
<td>1.00</td>
</tr>
</tbody>
</table>

P value = 0.423

Fig.3.14: Correlation between Ov-ASP-2/MBP IgG responses titres and Age of onchocerciasis patients:
CHAPTER FOUR
DISCUSSION

Onchocerciasis, or river blindness, is caused by the filarial nematode *Onchocerca volvulus*. When measured in terms of the socioeconomic impact on afflicted communities, onchocerciasis is one of the most important infectious diseases worldwide (WHO, 1995). As a result of the socioeconomic impact of the disease, several international programs are underway in Africa and the Americas have the goal to eliminate onchocerciasis as a socioeconomic and public health problem (WHO, 1995; Remme, 1995). However, onchocerciasis remains a major health hazard in many tropical countries, including Sudan.

River Blindness is characterized by corneal opacification and neovascularization, resulting in loss of corneal function and blindness (Hall, Pearlman, 1999). The microfilariae migrate through the skin of infected individuals, and can invade the ocular tissue. Corneal disease, as other disease manifestations, is primarily a result of host inflammatory responses to dead and dying microfilariae. In a recently developed onchodermatitis mouse model (Hall, Pearlman, 1999), the corneal disease was associated with infiltration of neutrophils, eosinophils and T cells to this site (Chakravarti et al., 1993; Hall, Pearlman, 1999).

Within any onchocerciasis endemic area there is a spectrum of clinical symptoms (King, Nutman, 1991). At one end of the spectrum, individuals show no evidence of infection; at the other, blindness is the most severe manifestation in long-standing cases of infection. There are also different manifestations of skin pathology, characterized as localized and generalized forms of onchodermatitis. This Pathology is almost exclusively associated with the death of the microfilariae but the actual mechanisms remain poorly understood. The spectrum of pathology may be due to differences in the host’s response to infection or differences in exposure levels between individuals (Bradley, Unnasch 1996). Furthermore the most important complications of the disease are those of the eye. Development of eye lesions is generally considered to be a consequence of heavy infection or infections of long duration and it correlates with the density of microfilariae in the dermis of the ocular region and in dermal tissues (Remme et al., 1989).

As part of an effort to understand the immunological factors involved in the development of ocular onchocerciasis, it has been demonstrated that a number of parasite proteins are capable of inducing an inflammatory reaction similar to ocular onchocerciasis in mice pre-immunized with parasite extracts (Pearlman et al., 1994). While conducting these studies, the authors
made the surprising discovery that one family of parasite proteins, designated the Ov-ASP family (for Ancylostoma’s Activation-associate Secreted Protein) induced an angiogenic response in naive animals injected in the corneal stroma with recombinant proteins expressed from the parasite open reading frames.

Corneal neovascularization is an important clinical feature of the ocular manifestations of *O. volvulus* infection. The highly specialized anatomy of the eye is such that highly vascularized and avascular tissues exist side by side. This requires tight regulation of the vascular growth process. When this balance is upset the unchecked development of new blood vessels, the eye can have a devastating effects. The corneal disease is primarily a result of host inflammatory responses to dead or dying microfilariae, and in mouse models is associated with infiltration of neutrophils, eosinophils and T cells to this site (Chakravarti *et al.*, 1993; Hall, Pearlman, 1999). Angiogenesis is an important early manifestation of onchocercal corneal disease, and new vessels mediate recruitment of inflammatory cells from the blood to the cornea (Hall, Pearlman, 1999). The fact that members of the Ov-ASP family are capable of inducing corneal neovascularization has implicated them as important potential mediators in the ocular disease (Tawe *et al.*, 2000) and probably the maintenance of adult-containing nodules. These nodules are highly vascularized (Smith *et al.*, 1988), and their survival may depend on production of angiogenic proteins in a manner similar to that described for tumors (Folkman, 1996).

Three *O. volvulus* homologues of the *Ancylostoma* secreted protein family (Ov-ASPs) have been identified as some of the most abundant cDNAs of infective stage larvae (L3) in express sequence tag (EST) database analysis. These proteins exhibit characteristics similar to vespid venom antigen 5 and members of the mammalian CRISP/Tpx protein families. They contain 6/10 conserved cysteine residues of the Tpx family and contain putative signal sequences at their amino terminal ends. The three proteins are roughly similar in predicted molecular weight (24-29 kDa) and share 43–58 % identity and 51–66% similarity (Tawe *et al.*, 2000). A survey of the expression pattern Ov-ASP proteins during the parasite’s lifecycle to identify those family members that were most likely to be expressed in microfilaria (the life stage responsible for ocular pathogenesis) indicated that Ov-ASP-2 was expressed in all stages of the parasite’s life cycle. For this reason, we focused on Ov-ASP-2 in all experiments of this study.

Injection of Ov-ASP proteins into corneas of pre-immunized mice resulted in massive corneal opacification and neovascularization accompanied by pronounced infiltration of neutrophils and eosinophils into the cornea. Similar responses were seen in other parasitic
antigens implicated in ocular pathology (Pearlman et al., 1997). However, none of the *O. volvulus* recombinant proteins have elicited any responses in naïve animals indicating that prior immunization is necessary for induction of corneal responses in ocular onchocerciasis mouse model. In contrast, two members of the *Ov*-ASP family (*Ov*-ASP-1 and *Ov*-ASP-2) have angiogenic activity in naïve mice, inducing corneal neovascularization (Tawe et al., 2000; Higazi et al., 2003). The new blood vessels were detected at day 3 post injection following minimal trauma-associated non-specific response. Blood vessel growth was maintained through day 7 before they regress and disappear. Furthermore, injection of naïve animals with *Ov*-ASP-2 resulted in a minimal inflammatory response that is characterized by mononuclear cells infiltrate (Tawe et al., 2000).

ASPs were originally identified as major proteins associated with the transition into parasitism by infective hookworm larvae (Hawdon et al., 1996). The infective larvae (L3) of *Anclystoma caninum* release several related cystiene-rich secretary proteins (AcASPs) *in vitro* when their culture media is supplemented with host serum fractions and glutathione analogues and undergo a phenotypic change characterized by resumption of feeding (Hawdon et al., 1996). At least six AcASPs (1-6) has been identified so far in adult and larval hookworms, representing single and double-domain proteins (Zhan, Yue, 2003). ASPs have been described from other nematode taxa, including both animal and plant parasites (Hawdon et al., 1999; Ding et al., 2000; Gao et al., 2001).

The functions of the ASPs remain unknown. Observations on *Ov*-ASPs and AcASPs suggested ASP role in the pathogenesis of human and animal nematode infections. However, the presence of at least 17 ASP-related genes in the free-living nematode *Caenorhabditis elegans* (Hawdon, et al., 1999), suggested that the ASP protein family might have a broader function in nematode biology in addition to parasitism. Moreover, nematode ASPs share significant similarities with a diverse family of CRISP proteins, pathogenesis-related proteins (PRPs) of plants (Fernandez et al., 1997), and glycoproteins found in mammalian testis (Tpx), suggesting that they serve diverse but critical functions. From these similarities, it is difficult to deduce a general function. The ASPs are of therapeutic interest because of their potential as vaccines candidates for human and animal nematode infections. Natural products and recombinant L3 are effective vaccine antigens in laboratory animals challenged with L3 of *A. caninum* (Hotez et al., 1999), *Haemonchus contours* (Schallig, et al., 1997), *Brugia malayi* (Murray, et al., 2001) and *Onchocerca volvulus* (Lustigman et al., 2002). Vaccination of mice with recombinant *Ov*-ASP-1 induced partial but significant reduction in the recovery of viable *O. volvulus* L3 larvae (Lustigman et al., 2002).
A definitive association between parasitic molecules and angiogenesis has not been documented; however, some studies have reported the presence of host- and parasite-derived growth factors and growth factor-like molecules. Unclear in *Trichinella spiralis* infection, L1 larvae are encapsulated in specialized host-derived nurse cells that are surrounded by a unique network of blood vessels within the striated muscles (Baruch, Despommier, 1991). In this context, vascular endothelial growth factor (VEGF) up regulation was reported in and around developing nursing cells and it has been suggested that *T. spiralis* might induce hypoxia, which in turn up-regulates VEGF production (Capo et al., 1998). Similarly, soluble egg antigen (SEA) of *Schistosoma mansoni* was shown to stimulate *in vitro* endothelial cell proliferation in a dose-dependant fashion (Freedman, Ottesen 1988). It was then demonstrated that the schistosomal egg granulomas produce a heparin-binding growth factor that shares characteristics with fibroblast growth factor-1 (FGF-1) (Prakash, Wyler, 1991). Furthermore, African trypanosomes and Leishmania were reported to synthesize their own FGF-2-like molecules (Morris et al., 1990; Kardami et al., 1992). In complicated *P. falciparum* infection, increased levels of FGF-2 were suggestive of an endothelial cell repair mechanism after parasite clearance (Burgmann et al., 1996). It tempting to note that growth factors are implicated in many physiological and pathological processes other than angiogenesis, including endothelial cell repair, wound healing and host-parasite interactions.

To our knowledge, Ov-ASPs are the only parasitic molecules with defined angiogenic responses characterized to date, representing a novel class of angiogenesis-stimulating molecules that deserve elucidation of their nature and antibody responses specially in patient with onchokeratitis.

For any disease, it is important to have safe and effective diagnostic methods available. The detection of microfilariae in the eye requires expensive equipment and highly trained personnel. Subcutaneous nodules may not always be palpable and other types of nodular tissue such as lipomas; lymph nodes and cysts can frequently be mistaken for being onchocercal in origin (Bradley, Unnasch, 1996). Otherwise, the widespread use of ivermectin as an additional control tool, the detection of microfilariae is no longer possible for this purpose so the detection of specific antibody in sera is ideal (Bradley, Unnasch, 1996). Unequivocal diagnosis of onchocerciasis, until recently, was dependent on the detection of parasites, either microfilaria in the skin or eyes or adult worms in subcutaneous nodules.

Traditional methods are not always satisfactory for a number of reasons. The collection and examination of skin snips, although the gold standard for most purposes, is of low sensitivity,
particularly in the areas of low transmission. It also does not allow for the detection of pre-patent infection important in the detection of reinfection post-vector control (Williams et al., 1985b). Besides the difficulties in the laboratory maintenance of many filarial species, coupled with the inherent complexity of this organism, have limited the rate of progress in our understanding of their development (Wu et al., 1995). Because of these shortcomings there is enthusiasm for development of immunological diagnostic tests for onchocerciasis. This enthusiasm resulted in the development of a number of parasite antigens for the diagnosis of infection, including a recent rapid-format antibody card test with high sensitivity and specificity (Weil et al., 2000) and recombinant hybrid antigens (Nde et al., 2002). Not long ago, the filarial genome project has begun to develop a library of expressed sequence tags ESTs from O. volvulus (Filarial genome project, 1999). Moreover there have been a number of advances in the methods available to investigate stage-specific protein synthesis and transcription in the larval stages of filarial nematodes. Patterns of gene expression in L3 larvae may therefore shed light on important questions concerning the acquisition of infectivity by pre-infective stages (Wu et al., 1995).

ELISA is a useful method for screening sera for specific antibodies when milligram quantities of purified or semi-purified antigen are available. Moreover, this type of serological test can be used to quantify the amount of antibody response. Earlier serological methods usually provide a ‘yes’ or ‘no’ answer to the question whether there are antibodies in the sample serum or not. With this method, quantification is achieved by diluting the serum sample (Giesecke, 1994). In this study the efficiency of diagnostic value of Ov-ASP-2/MBP recombinant antigen was explored (figs. 3.3, 3.4, & 3.5). The optical density values of IgG and its subclasses were blotted and cutoff-values defined positive and negative individuals (figs.3.6, 3.7 & 3.8). Therefore, antibody response in individual groups was studied. All these findings focused our attention on IgG immunoglobulins and the important role they play in the immunity against onchocerciasis.

Characterization of immune responses in onchocerciasis is an especially challenging goal. The antigenic complexity of the parasite, and the corresponding wide range of the host immunological reactions, make it extraordinarily difficult to dissect out those specific reactions which may be of diagnostic value, those which may serve in some protective capacity, and those which may contribute to the pathogenesis of the disease, whether during its natural course, or as a result of exacerbation produced by chemotherapy (Ghalib et al., 1985). In previous studies, high levels of IgG were correlated with severe inflammatory reactions (Lucius et al., 1986) and this might be attributed to the role it plays in killing of
microfilariae as reported in bancroftian filariasis (Subrahmanyam et al., 1978; Aime et al., 1984). Furthermore, *O. volvulus* specific IgG immune complexes were detected in the circulating blood of onchocerciasis patients (Sisley et al., 1987). For example, IgG promotes the killing of microfilariae *in vitro* by enabling eosinophils to adhere to the parasite surface; leading to the release from these cells of components toxic to the parasite (Mackenzie, 1980; Greene et al., 1981).

*Ov*-ASP-2 was chosen for this study, as it is the only member of the *Ov*-ASP family expressed in microfilariae (Tawe et al., 2000). *Ov*-ASP-2 was cloned and produced in *E. coli* as a maltose binding protein (MBP) fusion protein to study the mechanism by which *Ov*-ASPs induce their angiogenic activity (Higazi et al., 2003). As part of a collaborative study, *Ov*-ASP-2/MBP was provided to perform study of its activity in diagnosis of *O. volvulus* infection in general and its correlation with different clinical presentations of the disease using sera from infected individuals and various control groups from the Sudan. Patients were classified into two main groups; endemic positive individuals (EP) and Onchocerca negative control (ONC) group which include healthy controls as well as patients of schistosomiasis, tuberculosis, malaria which constitute co-endemic infections in most of the onchocerciasis endemic areas in the Sudan.

Since *Ov*-ASP-2/MBP is a fusion protein, the most important step was to determine its reactivity compared to the MBP fusion partner alone. All ELISAs performed in this study were done simultaneously on *Ov*-ASP-2/MBP and MBP alone for each individual serum. While MBP has similar residual activity to all sera under study, *Ov*-ASP-2/MBP had a highly significant reactivity to infected individuals IgG, IgG3 and IgG4 antibodies. This step was essential to exclude any specific antibody responses to the fusion partner alone and correlated responses between individual antigens (Trenholme et al., 1994). EP group had the highest reactivity of immune response values in the *Ov*-ASP-2/MBP than the ONC group. In addition, significantly higher levels of *Ov*-ASP-2/MBP antibodies were detected in onchocerciasis endemic positives compared to all other groups of endemic parasitic infections under study (P = 0.001, 0.01, 0.001) for IgG class and it is two subclass (IgG3 & IgG4) respectively. On the other hand, When *Ov*-ASP-2/MBP antigen was compared with the MBP protein no difference between the antibody responses in the ONC group by ELISA.

IgG antibodies predominated other IgG subclasses in the EP group in this study (table 3.1). IgG immunoglobulins and their immune modulation may be partly responsible for the
variable spectrum of the disease. It is also of paramount importance to define the association of these antibodies with defined parasite–specific antigens in diagnosis of the disease. For example, the differences in Ov-ASP-2/MBP recognition across the disease spectrum are of interest to evaluate if there are immunological markers for the blinding or non-blinding forms of the disease.

Comparison of the newer tests for the diagnosis of onchocerciasis showed that antibody testing may be more useful for screening populations for infection or exposure to *O. volvulus*, whereas PCR and antigen testing are potentially more useful for diagnosis of infections in individuals and monitoring success of therapy (Vincent *et al.*, 2000). The majority of studies have examined antibody responses specific for adult *O. volvulus* antigens. What is clear from these investigations is that the antibody response is strong and diverse with the immunoglobulin IgG 4 constitutes the predominant and most specific IgG subclass (Stwart *et al.*, 1995; Sobosaly *et al.*, 1997). Our results supported this observation; IgG4 was the most specific subclass in detecting Ov-ASP2/MBP with a specificity of 85% (table 3.5), while the highest sensitivity was attributed to IgG class at 74.4% (table 3.3). The fact that Ov-ASPs has homologues in various parasites might have lowered its specificity.

Higazi and coworkers reported that Ov-ASP-2 acts to promote new blood vessel formation through an indirect mechanism that might involve processing of the protein by host factors (Higazi *et al.*, 2003). The first 105 amino acids of the recombinant protein (full length = 229 amino acids) were as active as the full-length protein in inducing neovascularization (Higazi *et al.*, 2003). Accordingly, the most active domain of Ov-ASP-2 was mapped to the first 105 amino acids with less active domains encoded downstream the open reading frame of the protein (Higazi *et al.*, 2003). It would be of interest to test a truncated contrast of the first 105 amino acids of Ov-ASP-2 in detecting specific IgG antibodies. Enhanced specificity and sensitivity might be achieved based on the reported angiogenic activity of this domain of the recombinant antigen.

The range of activity against microfilariae in the dermal and ocular tissues, extending from an absence of any clinical or histological activity to vigorous responses to organisms in these tissues has been shown in patients in many endemic zones in Sudan (Ghalib *et al.*, 1985). IgG class seems to be involved in destruction of these parasites and thus immunologically mediated active killing of microfilariae and the development of microabscesses. This may only occur when a specific IgG antibody is present. A proportion of patients have antibodies in their sera, which can sensitize microfilariae for eosinophil-mediated adherence. The
evidence for variations in host responses to the presence of *Onchocerca volvulus* parasites is striking, although the mechanisms behind these variations are not fully known (Mackenzie *et al.*, 1985). Hamilton, (1988) reported that IgG subclasses vary in their biochemical nature and function. This variability allows for diversity in function of the different subclasses and therefore in their roles in infection and immunity (Jassim *et al.*, 1987).

To investigate the relationship between *Ov*-ASP-2/MBP and the spectrum of the eye involvement in *O. volvulus* infection, antibody responses to *Ov*-ASP-2/MBP was correlated to vision status of infected individuals. All individuals entered in this study were classified into three main groups according to ocular changes due to the disease (clear, low vision and blind). Implication of *Ov*-ASPs as mediators of ocular pathology led to the assumption that *Ov*-ASP-2/MBP recombinant antigen may be play an important role in the ocular pathology. Interestingly, highly significant more IgG responses were seen in blind patients compared to low and clear vision individuals (P=0.002) while IgG4 subclass antibodies showed a trend towards blind patients (P=0.198) (Fig 3.11). The lower number of individuals tested might have obscured a positive IgG4 response in blind patients. However, no significant relationship was observed for IgG3 subclass antibodies and vision status of infected individuals (P=0.498). This result supports an essential role of *Ov*-ASP-2 in the ocular disease (Tawe *et al.*, 2000) and suggests a role for *Ov*-ASP-2 as a biomarker for the blinding disease. However, a comprehensive study including a larger sample size will be needed to confirm the relationship between antibody responses to *Ov*-ASP-2 and the blinding form of the disease.

It has long been known that *O. volvulus* parasites from the forest and savanna regions of West Africa differ in their ability to induce ocular disease and that these differences might be an inherent property of the parasites themselves. In a comparison of the genomic sequences encoding *Ov*-ASP-2 protein in several isolates from the two strains identified few polymorphisms that resulted in changes in amino acid sequence of the *Ov*-ASP-2 open reading frame (Higazi *et al.*, 2003). However, these polymorphisms were restricted to more or two of the individual isolates and it appears unlikely that the observed amino acid polymorphisms can explain differences in the ocular pathogenic potential of the two parasite strains, (Higazi *et al.*, 2003). However, it is possible that the observed amino acid polymorphisms do result in alterations in the angiogenic activity of *Ov*-ASP-2. Alternatively, it is possible that quantitative differences exist at the level of expression of *Ov*-ASP-2 in the two strains. Our results suggest differential host antibody responses to *Ov*-ASP-2, and it does not exclude quantitative difference in *Ov*-ASP-2 expression.
The findings that blind onchocerciasis patients have significantly higher IgG responses to \textit{Ov}-ASP-2 led us to use this relationship as a basis to study correlation between other disease manifestation and IgG responses to vision subgroups. The relationship between vision status and dermatological manifestations, classified as clear skin, mild, severe or localized lesions (Sowda), were investigated in terms of IgG antibody responses. Blindness was found to correlate positively with clear skin, and mild skin lesions and negatively with severe skin lesions and Sowda (Fig. 3.12). In contrast, no correlation was observed between clear or low vision with skin manifestations of infected individuals under study. This finding is supported by earlier observations that the non-blinding disease is often associated with severe skin disease and Sowda (Ghalib \textit{et al.}, 1987) while the blinding disease is associated with generalized and mild skin lesions. Furthermore, presence or absence of palpable nodules was compared to vision status of infected individuals on basis of \textit{Ov}-ASP-2 IgG response. No significant differences were seen between patients with or without palpable nodules. This could be attributed in part to the fact that nodules are not always palpable and sometimes hard to detect, especially in case of deep tissue onchocercal nodules (Homieda \textit{et al.}, 1986). Alternatively, \textit{Ov}-ASP-2 antibody responses might be independent of the immune responses leading to the formation and maintenance of nodules.

Analysis of \textit{Ov}-ASP-2 antibodies responses in relation to the age-profile of infected individuals showed elevated IgG responses in younger age group (10-20 years). The level of IgG antibodies decreased slightly and maintained throughout older age groups. Age of infected individuals doesn’t seem to have a significant effect on \textit{Ov}-ASP-2 specific antibody responses. Similar relationships were reported between IgG antibody responses and age in onchocerciasis patients (Faulkner \textit{et al.}, 2001).

The antibody response of individuals with different forms of pathology, particularly to specific antigenic fractions or cloned recombinant proteins, is extremely attractive as it may provide qualitative information on mechanisms of pathology or provide prognostic indications for disease progression. Besides the increased sensitivity of techniques to define infected individuals, it also provides better definition of the group of individuals who are exposed but “infection free. These individuals may be immune to the parasite, and are thus a valuable population for studying mechanisms of immunity (Bradley, Unnasch 1996).

In general, our results suggested that \textit{Ov}-ASP-2/MBP recombinant antigen could reliably be used in diagnosis of onchocerciasis patients. Malaria, schistosomiasis and tuberculosis are co-endemic with onchocerciasis in most the endemic regions of the Sudan. We found that
these infections do not seriously cross-react or interfere with the detection of *O. volvulus*-specific antibodies when tested with *Ov*-ASP-2/MBP recombinant antigen. However, we could not test for cross-reactivity of *Ov*-ASP-2 with other filarial and parasitic nematodes due to in availability of those control sera. Sisley *et al.*, (1985), reported in examination of serum samples from patients in Sudan by ELISA using crude extracts of *O. volvulus*; there was a high degree of antibody responses but the results have limited diagnostic value because many a symptomatic endemic individuals were seropositive. Moreover, filarial antigens share determinant with those of many other nematodes, which result in pronounced cross-reactivity. Cross-reactivity has been proven difficult to overcome in *O. volvulus* as many antigens are shared with other filariae and even other families of nematodes (Williams *et al.*, 1985a). Under these circumstances it is not surprising that development of specific immunodiagnostic assays has been difficult.

In conclusion, we have characterized the immune responses to *Ov*-ASP-2/MBP recombinant antigen of *O. volvulus*, which has been shown to play an important role as an early mediator of the ocular pathology. *Ov*-ASP-2/MBP was very specific in detection of onchocerciasis patients and the fusion partner does not contribute to its recognition power. *Ov*-ASP-2/MBP showed a high specificity and sensitivity in recognition of parasite-specific IgG and its subclasses IgG3 and IgG4. Most importantly, *Ov*-ASP-2/MBP discriminated sera from blind onchocerciasis patients and showed a positive correlation between blindness or severely impaired vision and clear or mild skin disease. *Ov*-ASP-2/MBP represents a novel class of antigens that might be used as a biomarker for the ocular disease of *O. volvulus*.

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REFERENCES


Foster J.A. & Gerton G.L. (1996). Autoantigen 1 of the guinea pig sperm acrosome is the homologue of mouse Tpx-1 and human Tpx-1 and is a member of the cysteine-rich secretory protein (CRIISP) family. Mol. Reprod. Dev. 44: 221-229.


* * * * *
Appendix (I): Questionnaire.

Sample No.: ☐

Name: ........................................

Age: ☐ Sex: ☐ Area: ☐ Tribe: ☐

Clinical Manifestations:

*Vision:
  Clear: ☐
  Low: ☐
  Blind: ☐

Note: ..............................................................................................................

*Nodules:
  Absent: ☐
  Present: ☐

Note: ..............................................................................................................

*Onchodermatitis:
  Clear: ☐
  Itchy Skin:
    Mild: ☐  Severe: ☐  Sowda: ☐

Note: ..............................................................................................................

Do you take treatment before by Ivermectin:
  Yes: ☐  No: ☐

NOTES:
..............................................................................................................
..............................................................................................................
..............................................................................................................
**APPENDIX (II):** IgG class responses of Endemic positive individuals with different Lesions groups.

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**APPENDIX (II)** Con.: IgG class responses of Endemic positive individuals with different lesions groups.

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**Vision** ➤ Clear (0)  Low (1)  Blind (2)

**Nodules** ➤ Absent (0)  Present (1)

**Dermatitis** ➤ Clear (0)  Mild (1)  Severe (2)  Sowda (3).
Appendix (III): IgG₃ subclass responses of Endemic positive individuals with different lesions groups.

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**Vision** ➤ Clear (0)    Low (1)    Blind (2)

**Nodules** ➤ Absent (0) Present (1)

**Dermatitis** ➤ Clear (0) Mild (1) Severe (2) Sowda (3).
**APPENDIX (IV):** IgG4 subclass responses of Endemic positive individuals with different lesions groups.

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**Vision** ► Clear (0)    Low (1)    Blind (2)

**Nodules** ► Absent (0)    Present (1)

**Dermatitis** ► Clear (0)    Mild (1)    Severe (2)    Sowda (3).
Appendix (V): Important note for ELISA technique.

► Use only distilled water and clean glassware.

► Microtiter plates made of polystyrene has a major advantage over other solid phase ELISA methods as the unreacted reagents can be separated easily.

► Do not let wells dry during assay; add reagents immediately after completing wash steps.

► If Washing steps are performed manually; wells are to be washed three times. Five wash cycles necessary if needed.

► Avoid cross-contamination of reagents. Wash hands before and after handling reagents.

► Air bubbles must not be trapped in the wells.

► All reagents must be at room temperature (21 to 25 ºC) before running assay. [Remove only the volume reagents that are needed] so, do not pour reagents back into vials as reagent contamination may occur.

► By addition of a nonionic detergent, such as Tween20, BSA, or Washing buffer. These treatments do not interfere with the antigen-antibody reaction but prevent or block the non-specific factors binding to the plastic surface.

► A variety of enzymes for conjugates has been used as labelled antibodies and the choice of it depends on the requirements of a particular assay. In this system, a specific antibody is labelled with biotin and enzyme-labelled avidin used as an indicator.

► Avoid contact of sulfuric acid with skin or eyes. If contact occurs, immediately flush area with water.

► Results can be read either by ELISA reader for quantitative measurements or with eye to determine the presence or absence of colour products as positive or negative results, respectively.
Appendix (VI): The global distribution of onchocerciasis disease.

Approximate Geographic Distribution of Onchocerciasis
(Parasites and Parasitological Resources)

APPENDIX (VII): Ethical clearance certificate.

In the Name of Allah the Gracious the merciful
Republic of Sudan

Federal Ministry of Health

Date: 5.6.2003

ETHICAL CLEARANCE CERTIFICATE

This is to certify that, the Proposal entitled (Immunoglobulin Response to Angiogenic Proteins of Onchocerca Volvulus in Sudanese Patients Sera detected by ELISA.), introduced by, Nada El Tigani Khalil, from Faculty of Pharmacy, University of Khartoum. has been approved by the Federal Ministry of Health to be carried out in the Sudan.

Dr. Sara Hassan Mustafa
Raporteur of the Ethical Review Committee
Appendix (VIII): National endorsement.

Date: 5/6/2003

NATIONAL ENDORSEMENT

This is to confirm that the Federal Ministry of Health is fully recommending the study of \textbf{Immunoglobin Response to Angiogenic Proteins of Onchocerca Volvulus in Sudanese Patients Sera detected by ELISA)}, which will be carried out by Nada El Tigani Khalil from, Faculty of Pharmacy, University of Khartoum

Dr. Sarra Hassan Mustafa
Director of Research Directorate
ملخص الدراسة

بعد مرض عمى الانهار من أكثر الأمراض الطفيلية المروعة للجنس البشري وذلك بما يسببه من
ثبوت حدوثها نتيجة أعراض مرضيه نتاجه للأصابات بالمرض. ومن أهم هذه المضاعفات الاكلينيكية التي
لرد الفعل المناعي قد تمت نتاجه لموت الديدان الطفيلية داخل جسم المصابة مما أدى إلى زيادة الأوعية
الدموية وهو العرض الذي يؤدي إلى العتمة ثم العمى.

وبرغم أهمية هذا المرض و احتياجه إلى الفحص الدقيق و المتخصص إلا أنه ما زالت طرق الفحص لهذا
المرض تعتزم كثيرا على تحديد وجود الطفل المسبب (Onchocerca volvulus) في الجلد أو العين و
ربما الاعتماد أيضا على وجود ما يعرف بالعيره (عده صغيره) تحت الجلد. إلا أن هذه الفحوصات لم
ثبت النتيجة المرضية للفحص وذلك لعدة أسباب أهمها عدم صدقها على تحديد الأصابات في مراكحها
المبكر. نتتيجه لما سبق انتهت الجهود البحثية حديثا لا مكانه وجود عاج و فحص دقيق عن طريق
المداواه بالتنقية وذلك بمهاولة الفهم للا ليات المناعية التي يمكنها أن تحدث كرد فعل إيجابي لمقاومة
المرض وبالتالي الوصول إلى معدل وقائي بعد من هذا الخطير الذي يهدد الكثير من المجتمعات خاصه
بلدان المناطق الحارة التي انتشر فيها بصورة واسعة بما فيها السودان.

اهتمت هذه الدراسة بالتشخيص المناعي بإجراء تجارب تشخيصية مصليه مختلفه وذلك باستخدام أحد
الانتريجنات للطفل المذاب البسيطه. وقد تم اختيار الانتيجين (Ov-ASP-2/MBP) للاستعمال لما
ثبت من ارتباطه مع الطور الأصابي للطفل (L3). تم اختيار العينات المصليه لمرضى المصابين بعِمٍ
الانهار باستخدام الانتيجين المختار ومقارنتها بما يعرف ب (MBP) بروتين و هو البروتين المستخدم في
اذابه الانتيجين و يعمل ككترون للكشف عن حساسية الانتيجين والمدى النوعي له. ذلك بالاضافة لاختيار
عينات لافراد اصحاء من المناطق غير متوطنه للمرض وذلك للكشف عن فعليه هذا الانتيجين في
طريقه تشخيصيه لدراسة معدل الاستجابه المناعيه للفيرونين المناعي IgG & IgG3 وخصائصه المناعيه مثل ال
ELISA.

تم تحديد اهميه هذا الانتيجين بما أظهره من درجات التباين الواضحه تمعدلات التفاعل في مرضى طفيل
عمى الانهار مقارنه مع المرضى الأصحاء و المصابين بأمراض طفيليه اخرى. كما اثبت الانتيجين
المختار اهميته في اظهار ردود الفعل المناعي للفيرونين المناعي مختلفه خاصه ال
التشخيصي و ارتبطها بالصفات السريريه للمرض مما يحدث أهميه للتوجه نحو العلاج المناعي للمرض
و استنبط الامصال العلاجيه.