STATUS OF HUMAN ONCHOCERCIASIS AND ONCHOCERCA VOLVULUS ANTIGEN-SPECIFIC IgA, igM AND igG CLASS AND SUBCLASS ANTIBODIES AND CIRCULATING EOSINOPHILS IN CLINICAL ONCHOCERCIASIS PATIENTS FROM KACHIA LGA OF KADUNA STATE, NIGERIA.

BY

MARCH, 1996

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BY

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B. Sc. (BENSU), EKPOMA).

A THESIS SUBMITTED TO THE POSTGRADUATE SCHOOL, AHMADU BELLO UNIVERSITY, ZARIA, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF

MASTER OF SCIENCE

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ZARIA.

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DECLARATION

I hereby declare that the work reported in this thesis for Master of Science degree in Microbiology was conducted by me under the supervision of Dr. Musa Galadima and Dr. L. E. Odama, both of the Department of Microbiology, Ahmadu Bello University, Zaria.

No part of this work has been presented for the award of a degree or any other qualification. Citation of other workers are duely acknowledged.



CANDIDATE'S SIGN.

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CERTIFICATION

This dissertation titled "Status of Human onchocerciasis and *Onchocerca volvulus* Antigen-specific IgA, IgM, IgG Class and Subclass Antibodies and Circulating Eosinophils in Clinical Onchocerciasis Patients from Kachia LGA of Kaduna State, Nigeria" by Hudu Okankhamame Osue meets the regulation governing the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to scientific knowledge and literary presentation.

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Dr. M. Galadima, Chairman — Date

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DEDICATION

To the cherished memories of Madam Rabietu Osue (Mum), Lt A.B. Lawrence (Maternal Uncle), Suadetu Hudu (My Duaghter), Mrs Aminetu Ahmed (Nee Ayoshi, Junior Sister), and Mallam Ahmed Momodu (Senior Brother). From Allah we come and unto Him we shall return. May the mercy, peace and blessing of Almighty Allah be with you and the Holy Prophet Mohammed (PBUH)

May Allah in His infinite mercy grant the blind poeple the world over the unshaken faith and forbearance to journey this world which is a temporary abode.

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ABSTRACT

From an estimated population of 1400 people in 6 villages made up of mostly non-migrant farmers, 531 of the 900 persons aged >15 years were skin snipped and examined for onchocerciasis related dermal and ocular lesions. 201 (37.9%) and 77 (14.5%) had microfilariae (mf) in one or both iliac crest skin snips and palpable nodule (N) respectively. The geometric mean mf load or community mf load (CMFL) per skin snip for those aged 20 years and above was 1.54 while the mean mf density (MFD) was 6.7±17.6 with a range of 0-345. The mf and N prevalence rates were 41.8% and 13.9% in the male cohort (n-237) and 34.8% and 15% in the female cohort (n-294). Infection intensity based on MFD were 7.4±16 and 6.2±18.8 respectively. Those with one or more dermal and ocular pathology were 10.2% and 5.8%, while papular onchodermatitis (6.9%) and optic nerve disease (OND) or pale disc (4.5%) were apparently the most predominant clinical disorders. Parasitologic and clinical manifestations increased with increasing age. The absence of obstructive lymphatic disease and low blindness rate, 7 (1.3%) in one eye and 2 cases involving both eyes could be an indication that the disease state is in a nascent endemic phase. Antibody responses to female adult worms of *Onchocerca volvulus* SDS-extracted crude antigens, recombinant DNA antigen coded Ov.1.9, and antigenic epitopes defined by monoclonal antibodies designated Cam8, Cam22 and Cam28 were tested in both indirect and competitive enzyme-linked immunosorbent assay (FLISA). Cases (n-95) with proven parasitologic or clinical evidences had the highest mean optical density (OD) value of 0.58±0.15, 0.38±0.12, 0.34±0.10 and 0.43±0.12 for IgG, lgG1, lgG3 and lgG4 antibodies against the

SDS extract compared to control group (n-7) with corresponding values of 0.12±0.03, 0.06±0.04, 0.06±0.05 and 0.04±0.05. Reactivity of sera varied with infection status. About 35%, 71% and 86% of infection sera inhibited binding of Cam8, Cam22 and Cam28 mAbs to SDS extract. Patients with chronic skin diseases (n-20) had more antibody levels in contrast to those manifesting OND (n-12): 0.65±0.14 vs 0.50±0.13 and 0.33±0.08 vs 0.28±0.10 for IgG and IgG3 response to SDS extract and 0.64±0.22 vs 0.48±0.25 for IgG against Ov1.9. A similar pattern was recorded for percentage Cam22 and Cam28 inhibitions. But for blood eosinophil levels the reverse was the case with 3.7±7.2 and 524.5*501.0 vs 11.3%±7 and 766.9±557.6 of differential and absolute counts. A combination of high eosinophil and low antibody levels that characterise OND group may not be an epiphenomenal occurrence. A follow-up of 32 patients 2 months post-initial ivermectin treatment showed a slightly remarkable change in IgG antibody against SDS-extract from 0.53±0.15 to 0.68*0.13 equivalent to mean percentage change of 20.5%±29.6 in patients with skin changes (n-18). Post-treatment antibody changes were more in N+ or low MFD (<19.5 mf/ skin snip) than N and high MFD (>20mf/skin snip). A percentage change >10% was recorded in 16 (50%) and 7 (21.9%) for IgG against SDS-extract and Ovl.9 while increase in inhibition was more in Cam22 followed by Cam8 and least in Cam28. Serodiagnostic sensitivity of IgG was by far higher than those of IgA and IgM, but only slight differences between the isotypes (IgG1, IgG3 and IgG4) with 98%, 37% and 36% respectively. Further evaluation of the specificity of the assays is recommended.

CHAPTER ONE

INTRODUCTION

Onchocerciasis afflicts over 20 million people in the tropics mostly in Africa, Central and South America and in Yemen. It is a debilitating disease causing various dermatological and ocular impairment which in extreme cases if untreated, manifest as hyperactive onchodermatitis (sowda) and blindness (WHO, 1987). Nigeria accounts for over one third of the global estimated 70 million people at risk of infection, with 360,000 projected to be blind (Abiose, 1992). The intervention measures depend on vector control and treatment of detected cases. Both are temporary but current dependable options.The negative impact of vector control (using larvicides and adulticides) on the environment limits its continued application. Current available drugs in use are only microfilaricidal and cause serious side effects. Diethyl carbamizine citrate (DEC) and suramin causes Mazzotti reaction and ocular damage respectively. Only ivermectin, recently introduced, has been found to be well tolerated (WHO, 1987). Effective control based on chemotherapy will depend on reliable diagnostic method. Practical demonstration of adult parasite through palpation of nodule (onchocercomata), or microfilariae in skin snip biopsies of onchocerciasis patients is definite diagnosis. Skin snip is more evasive but sensitive than the rapid assessment method which depends on palpation of nodules and presence or absence of depigmention of shin (Edungbola *et al.*, 1987; 1990; Taylor *et al.*, 1993). The need for an alternative diagnostic method to complement skin snip can not be overemphasized. Apparently the misdiagnosis of individuals with prepatent infection and low microfilarial load (MFL) particularly

in an area with ongoing control programme to break transmision cycle) calls for reliable detection methods (Taylor *et al.*, 1989; Weiss and Karam, 1989).

A major setback in using crude antigen extract in the immunology of onchocerciasis is cross-reactivity particularly with coendemic parasites. Moreso, there is variation from one batch of extract to another (Marcoullis *et al.*, 1978). More inportantly, phosphorylcholine (PC), an ubiquitous immunodorminant molecule whose epitope is found in bacteria, fungi and other parasites is a common source of cross-reactivity (Lal and Ottesen, 1989). Hence the advocate for the use of more defined antigens becomes imperative. This could be achieved by either purifying native antigens with monoclonal antibodies or using synthetic or recombinant DNA biotechnology to produce desired molecules (Philipp *et al.*, 1984; Connor *et al.*, 1988; Lucius *et al.*, 1988; Engelbrecht *et al.*, 1992). Another approach is exploring restriction of antibody isotype response to certain class of antigens previously reported by Natvig and Kunkel (1973). Specificity is known to vary among antibody isotype in filariasis patients including onchocerciasis (Lal and Ottesen, 1988; Weil *et al.*, 1990). Various degrees of diagnostic efficiencies of defined antigens have been reported (Lucius *et al.*, 1988; Weiss and Karam, 1989; Chandrashekar *et al.*, 1991; Engelbrecht *et al.*, 1992; Lucius *et al.*, 1992). One common problem limiting the serodiagnostic usefulness of individual antigens is their lack of commensurate high specificity compared to high sensitivity thus far reported. There is great improvement in the sensitivity and specificity of IgG4 serology in the diagnosis of filariasis, including onchocerciasis (Weil *et al.*, 1990; Kwam-Lim *et al.*,

1990; Ogunrinade *et al.*, 1992, Egwang *et al.*, 1993). The level of serum IgG4 also shows a positive correlation with parasite load, skin microfilarial density, and increase with age (Egwang *et al.*, 1989; Engelbrecht *et al.*, 1991; Egwang *et al.*, 1993; Boyer *et al.*, 1993). Severity of the diverse clinical manifestations depends on infection intensity, and it vary within one organ and from one host to another (Mackenzie, 1987). The involvement of host-parasite interaction in the aetiology of skin and eye lesions has been suggested (MacKenzie, 1985). Similarly, cellular and humoral immune responses vary from one host to another. The immunological mechanisms (immediate hypersensitivity, delayed hypersensitivity, immune complexes etc.) known to initiate inflammatory responses that result in the expression of clinical pathology remains unclear (MacKenzie, 1985). One hypothesis tend to implicate direct or indirect involvement of humoral responses elicited by live parasites (adults and microfilariae) or their antigens released at death as responsible for triggering and/or exacerbating ocular tissue damage (Braun *et al.*, 1991). The level of intraocular antibodies against native parasite antigens was reported to be more compared to ocular autoantigens (Van der Lelij *et al.*, 1991). This support direct host-parasite humoral reaction in the pathogenesis of the ocular lesions. On the contrary, reports by Semba *et al.*, (1991) on experimental inoculation of monkeys with life and dead microfilariae indicated that ocular lesions appeared even before serum antibodies could be detected.

Granulocytes; eosinophils, neutrophils and mast cells do not interact directly with living microfilaria except after treatment or natural attrition (MacKenzie, 1980). Eosinophilia is a common

feature in parasitic nematode infection and diseases including onchocerciasis. Comparably high level eosinophils are found in the blood and skin. Pronounced eosinophilia and strong antibody reactivity coupled with low skin microfilariae characterised the polar form of the disease, sowda (Bartlett *et al.*, 1978, Buttner *et al.*, 1988). Only mild inflammatory lesions and large numbers of living undamaged microfiliriae are seen in the generalized form. An implicating role for eosinophil involvement in *in vivo* microfilariae clearance and tissue damage has since been postulated by Green (1981). Based on *in vitro* analyses, mf clearance and or tissue damage seems to support the postulated mechanisms of eosinophil action through combined cyto-adherence and degranulation leading to the release of major basic proteins which are deposited on microfilariae or tissue. Cyto-adherence is either antibody dependent or independent (Brattig *et al.*, 1989). These facts reinforce possible association of eosinophil and parasite specific antibody in immunopathology.

Detecting existing correlation of humoral and cellular immune responses elicited by parasite antigen(s) with certain clinical manifestation(s) is a pre-requisite needed to critically appraise any role host-parasite interactions might play in the aetiology, pathology and pathogenesis of the disease.

This study is geared towards evaluating the sero-epidemiologic, immunopathologic and immunopathogenic significance of recombinant *O. volvulus* Ov1.9 cDNA clone, adult worm sodium dodecyl sulphate (SDS) extracted crude antigens and those epitopes defined by Cam8, Cam22 and cam28 designated monoclonal antibodies. Enzyme-linked immunosorbent assay (ELISA) was used to measure serum IgG class and IgG isotype

antibodies specific against these antigens in clinically characterised onchocerciasis patients before and 2 months after receiving first dose of ivermectin compared to controls. The specific aims of this study are outlined as follows:

a. To obtain baseline parasitological, clinical and serological data prior to ivermectin initial dosing of Gantan, Sabon Gantan, Kurmi Gwaza, Bomjock, Ungwa Shaho (K.Gwaza 11) and Gidan Tama villages in Kachia Local Government Area of Kaduna State.

b. To evaluate IgA, IgM, IgG, IgG1, IgG3, and IgG4 antibodies against *O. volvulus* antigens (recombinant Ov1.9, adult worm SDS-extract, and mAb defined antigenic epitopes) in onchocerciasis patients documented for age, gender, skin mf load, and onchocerciasis induceable dermal and ocular disorders. Both IgE and IgG2 assays will not be performed for obvious reasons; they are relatively found in low levels, hence requires very sensitive analytical test like radioimmunoassay (RIA) method i.e. radioallergosorbent test has been adapted for IgE measurement (Halminton *et al*, 1981). Their evaluation using ELISA will require enhancing tertiary antibody conjugated to either digoxigenin, streptavidin, biotin and phycoerythrin. Though these reagents are available commercially, they are expensive and out of reach within the project schedule.

Among others, the specific objectives to be addressed are:

a. To assess the endemicity and public health importance of the disease in the study areas.

b. To show if the levels of antibodies and circulating eosinophils show any clinical correlation.

c. To assess the impact ivermectin initial treatment and infection status has on the antibody changes.

d. To determine the reliability of IgG4 ELISA antibody measurement in the diagnosis of the disease.

What is the level of detectable parasitological and clinical cases within the sample population. Are the people presenting with severe skin changes, and is the level of blindness or ocular disorders very high. As a result of scarcity of human tissue materials and the attendant ethical limitations imposed by this approach (Chan *et al.*, 1989) has made correlative study an appealing option in basic research into immunopathology and immunopathogenesis of onchocerciasis. Moreso, there are no suitable animal model (Donelly *et al.*, 1988). Some of the hypotheses to be tested are: one, is parasite-specific antibody reactivity and/or eosinophilia showing predilection (i.e. found in high levels) for a particular clinical outcome of the disease? Secondly, is there demonstrable increase in ELISA antibodies in follow up sera compared to their paired pre-treatment sera. If in the affirmative, it indicate that previously recognized or unrecognized parasite antigens are released into circulation. This could serve as a useful tool in drug screening, for monitoring the efficacy of treatment control strategy (More and Copeman, 1991b; c) and as a measure of state of anergy. Thirdly, can IgG4 serology compared to others detect all proven cases and more of those missed by one or both standard parasitologic (skin sipping and nodule palpation) methods. Ability of IgG, IgG1, IgG3 and IgG4 serology to discriminate infected (positive) from non-infected (negative) is based on ELISA optical density (OD) values above their cut-off points.

CHAPTER TWO

LITERATURE REVIEW

2.1. *PARASITE BIOLOGY:*

Within the subphylum Nematoda, the parasite *Onchocerca* belongs to the class phasmidae (lack caudal sense organs), order Filariata and family onchocercidae. About 34 species have so far been described, and they are mostly infective to animals (Muller, 1979). Among them , *O. gutturosa*, *O. dukei*, *O. gibsoni*, and *O. lienalis* are common cattle infective species (Flockhart, 1982). Other cattle infective species are *O. dermatitis* and *O. ochengi* which are localized to West and East Africa respectively. *O. cervicalis* is infective to horse and the only human infective species is *O. volvulus*. Biometric data show that a male adult worm measures 3-5cm long and 270-400µm wide. The female is about 19-42cm by 130-210µm. An adult has a life span of between 9-16 years (Sculz-key *et al.*, 1988). The female has a subperiodic cycle and is ovoviviparous. After mating it can carry 200,000-400,000 microfilariae in the uterus, and is able to release between 1,000-3,500 mf per day (Raymond, 1969; Schulz-Key *et al.*, 1980; Engelbrecht *et al.*, 1984; Schulz- Key and Karam,1986). Adult worms are found inside nodules or onchocercomata from where they release mf which migrate to the epidermis. From here they are ingested by black fly (*Simulium*) during blood meal bites.

Series of moulting ensue through first stage larvae (L1) that move to the thoracic musculature where another moulting results in a sausage shape L2. After another moulting, the filariform L3 that are formed move to the labium from where they are injected

into a new host during feeding. The life cycle of *O. volvulus* in man and vector is depicted in Figure 1. In the human host, L3 develop into L4 which finally moult into adult worms. It takes between 6-12 days to complete the life cycle in the fly.

2.2. *EPIDEMIOLOGY AND TRANSMISSION*

Tropical regions particularly Africa, Latin (South and Central) America and the Middle East especially Yemen contain the well known foci. An estimated 70 million people are at risk of infection and there are 20 million estimated cases of which approximately 360,000 are blinded (WHO, 1987). The disease is found in 34 countries, and two forms of *O. volvulus* are suspected to exist; they are the forest and savanna strains (Duke *et al.*, 1967). The former is associated with more skin lesions while the later causes more ocular impairment and blinding rate. Observed difference in the pattern of onchocerciasis presentation and severity (Duke *et al.*, 1967) has been attributed in part to clinical, rate of blindness, transmission and development of parasite in forest and savanna vectors. Biochemical evidence was first provided by Omar *et al.*, (1982), and later allele frequency differences in isoenzyme analysis (Flockhart *et al.*, 1986); antigenic differences in immune responses (Lucius *et al.*, 1987) and even more recently *Onchocerca volvulus* DNA specific probes under strigent conditons are purported to be specific and capable of distinguishing forest and savanna forms through hybridization with native DNA of parasite (Erttmann *et al.*, 1987; Meredith *et al.*, 1989;). Both strain and species specific members of 150 base pair repeat DNA sequence family are faithfully amplified, allowing characterization of the polymerase chain reaction (PCR) amplification products to previously developed DNA probes. This

method is shown to be applicable to all diagnostically important forms of the parasites, including adults, infective larvae, and skin mf (Meredith *et al.*, 1989). Predominance of head nodules in Guetamala and Mexico as against pelvic girdle in Africa is attributed to sociocultural difference like wearing of cloth, in effect, could determine the level on the body at which the vector bite (Albiez *et al.*, 1988). Only the female *Simulium* bites man (anthropophilic) and animal (zoopophilic). Suspected zoonotic infections are beginning to emerge with female *Onchocerca* found in histopathological sections of a nodule removed from the foot of a 2-year old girl in Southern Japan (Beaver *et al.*, 1989). Cases from Switzerland, Crimea, Canada, and the USA, cited by these authors, showed evident morphological features of the worm resemble those of *O. gutturosa* and *O. cervicalis*.

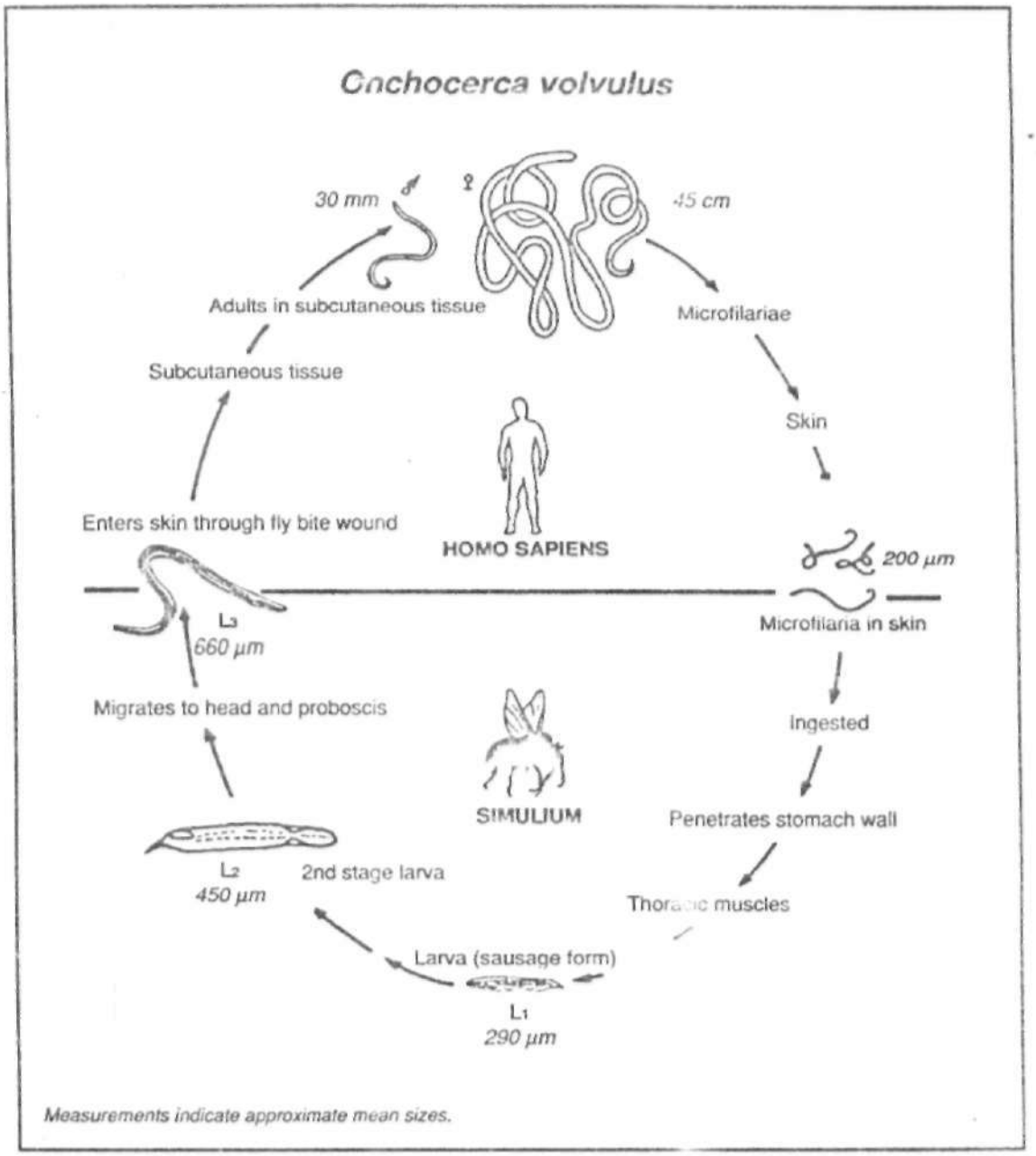


Fig. 1. Life cycle of *Onchocerca volvulu* adapted from WHO, (1990).

Incidence and prevalence of the disease in the community depends on proximity to vector breeding sites, fast flowing rivers, and their population dynamics, abundance and annual transmission potential (ATP). The *S. damnosum* migration propensities in wet season explains why black fly is found 400 km from the breeding sites (Davies and Crosskey, 1991). Migrant flies are often responsible for sporadic and low level community microfilarial load (CMFL). Several mf are ingested from the dermis of infected persons after blood meal bite. The blood is needed for the maturation of each batch of eggs laid. Most mf which arrive in her stomach are destroyed as they are trapped, digested and cleared in the peritrophic membrane. A few may pass through the intestinal wall and reach the abdominal cavity from where they migrate to the thoracic muscle for development (Davies and Crosskey, 1991). Infective larvae are usually less than 10 and in most cases from one to three in a black fly (Ramurez-Perez, 1977). The life span of a mf is about 1.5-3 years.

2.2.1 *MECHANISM OF TRANSMISSION:* Skin laceration is caused by biting mouth parts from which seeping blood is sucked. The short proboscis penetrates upto a depth of 0.4 mm in *S. damnosum* s.l. Blood with many mf from skin wound is imbibed by muscular pumping of the cibarium and pharynx passing rapidly from food-channel in the proboscis to midgut (Davies and Crosskey, 1991). Salivary anticoangulant is discharged from the hypopharnx during blood meal and infective *O. volvulus* larvae (L3) apparently escape from the fly's head into the skin at the same time.

2.3. *STATUS OF ONCHOCERCIASIS IN NIGERIA*

Onchocreciasis has been recognised as a public health problem since 1906 (NOCP, 1992). It was first reported in Nigeria by Parson in 1908 when four people from Lokoja were diagnosed of the disease. In the following year, Austen established the presence of the vector in areas now under Cross-rivers state as cited by Ogunba, (1990). The impact of the disease was not fully appreciated until the work of Budden was published in 1956. Human onchocerciasis has been reported in all the states of the federation including Abuja the Federal Capital Territory (Roger, 1973; Uchay, 1982; Ogunba, 1982; Edungbola, 1982; Edungbola *et al.*, 1987; Onwuliri, 1987; Okonkwo *et al.*, 1991). Prevalence of the disease varies from one place to the other, while some areas are free; upto 100% has been reported in some villages in Bauchi, Taraba, Benue, Borno, and Kaduna states (Akogun and Onwuliri, 1991; NOCP, 1992) which contain hyperendemic foci.

Both forest and savannah onchocerciasis are found in Nigeria which explains the high blindness rate in the nothern states in contrast to the more skin clinical manifestions seen in southern states (Edungbola *et al.*, 1990). Some areas with forest-savannah mossaic vegetation have both types of onchocerciasis (Okonkwo *et al.*, 1991). Nigeria is projected to account for about one third of global estimate with roughly 23 million of her people at risk of infection, with about 7.5 million cases and 120,000 are blinded (NOCP, 1992). This diease which is found mostly in rural areas no doubt inpoverishes the already pitiable socioeconomically weak peasants who forms the bedrock for the agrarian drive of the nation.

2.4. *CLINICAL ONCHOCERCIASIS*

Subsequent to infection a prepatent period lasting upto 9 months is followed by acute and later chronic phase. Ensueing clinical manifestation is diverse with various dermatological and ocular disorders that varies in severity from one individual to another, even within the same person symptom in different parts of the body are not bilaterally symmetrical (Buck, 1974; McKenzie, 1987). A person may present with one or more signs of the disease. Severity of clinical lesions depends on intensity of worm burden (Ghalib *et al.*, 1987; MacKenzie *et al.*, 1987) and vigorous immune responses correlate with lower skin mf densities.

2.4.1. *SKIN ONCHOCERCAL DISEASES*: Early or acute stage of the disease show papulitis, pruritus, hyperkeratosis, parakeratosis, follicular plugging and acanthosis (with some papillary elongation of the rete ridges). Fibrous substances are deposited round adult worms leading to the formation of nodules (onchocercomata) which lie free in the subcutis (Nnochiri, 1964). They are found commonly on bony prominences, pelvic girdle, head, thorax, and leg. Exemptions are those found on abdominal region or corium attached onchocercomata (Albiez *et al*, 1988). Some are hidden and not palpable. Lichenoid changes, hanging groin, dyspigmentation, atrophy, lymphadenitis and onchocercomata are signs of late or chronic stage (Buck, 1974). Two polar forms of skin presentation are recognised: hyper-reactive onchodermatitis (sowda) and generalized forms. Sowda is characterised by localized intense itching, assymetrical, well-circumscribed dermatitis with or without oedema, pachyderma and darkening and thickening of the skin (Buttner *et al.*, 1982). In

the generalized cases of the disease, apparently no visible inflammatory reaction is seen in view of existing large numbers of undamaged living mf in the skin (Bartlett *et al.*, 1978). This contrasting fact is a clear indication that direct parasite damage to tissue does not occur. Humoral and cellular responses to dead or dying mf is a likely aetiology of most skin pathology. Knaussmann's, (1988) ultrastructural studies report that microfilariae with intact appearing morphology were embedded in dense material containing granules from eosinophil granulocytes in various stages of disintegration. Other parasite with beginning morphological changes of cuticular elements are in addition surrounded by macrophages and multinucleated cells thus forming granuloma. A similar mechanism may be responsible for the eye as well where punctate keratitis and cornea opacities are due to granulocyte adherence to damaged or dead mf.

2.4.2. *EYE ONCHOCERCAL DISEASES:* Visual impairment and blindness are the most socioeconomically important aspect of the disease. It is the fourth major blinding disease in the world (WHO, 1992). Lesions accompany mf invasion of various parts of the eye. Sites where mf are commonly found include the cornea, anterior chamber, retrolental space and the vitreous, and the conjuctiva (Garner, 1976). Dead mf elicit inflammatory reactions resulting in anterior diseases involving the cornea (punctate keratitis and sclerosing keratitis) and iris (iritis, secondary glaucoma and cataract) or posterior segment diseases which are chorioretinal, optic neuritis and atrophy (Anderson *et al.*, 1976a; 1976b; Reme *et al.*, 1990; Semba *et al.*, 1990; Abiose *et al.*, 1993). Some eye lesions are not reversible i.e. are permanent. Those induced by onchocerciasis are sclerosing keratitis, iritis with synechae,

chorioretinitis and postneuritic optic atrophy. The blindness resulting from these lesions are not redeemable (Dadzie *et al.*, 1991). Chorioretinitis account for much of the blindness (Anderson *et al.*, 1976a; Abiose, 1990). Abiose *et al.*, (1993) found that optic nerve disease is the major cause of blindness in Nigeria based on the study carried out in Kaduna state.

Optic atrophy is usually associated with peripheral visual field loss and severe visual handicap (Abiose *et al.*, 1993). Severely infected patients often present with chemosis and epiphoral (WHO, 1987) which follows early conjuctival reactions. Many patients though have good central vision are reported to have visual loss and a poor night vision as a result of restricted visual field (Anderson *et al.*, 1976b). Hence, motility is made very difficult.

2.5. *IMMUNOPTHOGENESIS AND PATHOLOGY*

2.5.1. *SKIN CHANGES*

a. *ONCHOCERCOMATA:* Subcutaneous granuloma are formed round live or dead worms (Buttner *et al.*, 1984). It is thought to be influenced by parasite released angiogenesis factor (Folkman *et al.*, 1971) analogous to those produced by solid tumors (Smith *et al.*, 1988). Small nodules have extensive blood supply, diffusely distributed through out the nodule matrix, and in close association with the coils of worms. In bigger nodules the central area appeared more dense, and vascularization seems to be more peripheral. The cellular infiltration differs from one nodule to another depending on the number of degenerating mf (Buttner *et al.*, 1984). Often, they contain plasma cells, eosinophils, macrophages and giant cells. Calculated mean geometric number of live male and female worms are 1.1-1.2 and 1.6-1.8,

respectively (Schulz-Key and Albiez, 1977; Albiez *et al.*, 1988; Schulz-Key and Karam, 1988). Young worms are attracted to older worms forming conglomerate nodules. When partially attached they are referred to as satelite nodules, but when separated are called diaphanous capsule containing young female worm (Schulz-Key, 1988). Composite nodules have several centres, and caseous or milky centre is sign of beginning calcification process. Nodule consistency alters with age and carrier rate varies. An average of 10 per person of 40 years and above and cases with 100 have been reported.

b. *ONCHODERMATITIS*. Perivascular infiltrate in the skin of onchocerciasis patients consist predominantly of eosinopils and mast cells together with mononuclear cells; macrophages, lymphocytes and plasma cells. An extensive inflammatory cell infiltrate charaterises sowda (Knaussmann, 1988), and alteration of microvasculatures are found mainly in the upper parts of dermis. Broad cuffs are formed around dermal appendages and vessels, which greatly thickens the dermis (Buck, 1974; MacKenzie, 1987). Papular eruption is as a result of microabscesses containing dying mf and varying numbers of eosinophils, monocytes, and lymphocytes. Initially, neutrophil polymorphonuclear leucocytes first infiltrate around the parasite and are quickly succeeded by eosinophils, lymphocytes, plasma cells, and fibroblasts. Histologic appearance of early lesions shows mf lying free in the connective tissue and lymphatics of the dermis, particularly numerous in the papillae which are oedematous and hyperaemic. Later there is loss of papillae, with atrophy and hyperkeratosis of epidermis (Roger, 1962; Edington and Gilles, 1981). An intense irritation due to onchodermatitis

may produce secondary lesions and infection from scratching may lead to regional lymphatic gland enlargement.

c. *LICHENIFIED ONCHODERMATITIS*: This is a regular feature of onchocerciasis in Yemen and Sudan, and is seen less frequently elsewhere (WHO, 1987). Teenagers and young adults are usually affected. There is high reactivity of antifilarial IgG and IgE and specific IgG3. Blood and tissue eosinophil counts are high with T- helper to T-suppresor cell ratio increased considerably (Gasparini, 1964; Anderson *et al.*, 1973; Buttner *et al.*, 1982; Buttner and Racz, 1984). There is abundant inflammation around vessels.

d. *LYMPHADENITIS:* Among the superficial lymph nodes, cervical, axillary, epitrochlear, inguinal, femoral, and popliteal may be affected. Also, another clinical outcome related to lymphadenitis include hanging groins (Buck, 1974). These nodes under the microscope show fibrosis of the capsule, trebaculae and medulla, and contain only few germinal centres which are reduced in size, or they may be absent. A collection of histiocytes are found in the sinosoids and large numbers of inflammatory cells, including plasma cells, eosinophils and mast cells are present throughout the substance of the node.

e. *ATROPHY*: This may appear following chronic infection and it implies there is loss of skin substances with the degeneration of one or more structural elements of the skin. It adapts the charateristic of ageing such as loss of elasticity and contours.

2.5.2. *OCULAR CHANGES*

a. *ANTERIOR SEGMENT LESIONS:* Acute microfilariae infection of cornea give rise to punctate keratitis due to leucocytic inflammatory infiltrate around dead or dying mf in the cornea

stroma (Garner, 1976). They appear as "fluffy" or "snow- flake" opacities. Since they resolve completely, are rarely seen in necropsy specimens. Clinically comparable lesions have been demonstrated experimentally in rabbit inoculated with mf into the limba conjuctiva (Duke and Anderson, 1972). Histology of the lesions revealed necrotic parasite in the cornea stroma surrounded by lymphocytes and eosinophils (Garner *et al.*, 1973). After prolonged cornea infection by mf sclerosing keratitis developed (Garner, 1977; Thylafors, 1978) from chronic inflammation with occasional eosinophils and neutrophils in the fibrovascular pannus of the stroma (Paul and Zimmerman, 1970). Scar tissue, fibrovascular in origin or pannus from limbus between the subtantia propia and covering the epithelium characterised sclerosing keratitis. Also, as a consequence of long standing infection fibrosis, necrosis, hyalinization, and pigmentary changes of the iris may occur.

Anterior synechiae may be present in addition to the chronic inflammatory cells infiltrate (Garner, 1977). The inflammatory cells are found to be predominantly T-lymphocytes ($CD3^+$) subset. A considerable increase in T suppresor/cytotoxic ($CD8^+$) subset has been reported in ocular onchocerciasis patients by Chan *et al.*, (1989). Moreover, non-lymphoid cells of the conjuctiva and iris such as the vascular endothelium, perycytes and fibroblasts were in an activated state as evidenced by increased expresion of class II major histocompatibility (MHC) antigens.

b. *POSTERIOR SEGMENT LESIONS*: Observed variability in posterior segment lesions from patient to patient and even within a single eye range from an unequivocal inflammatory process to an exclusively atrophic lesion (Bird *et al.*, 1976). Chronic

inflammation may give rise to focal aggregation of pigmented cells leading to pigmentary retinopathy. It is attributed to either migration and clumping of degenerate retinal pigment epithilium or focal hyperplasia. Microfilarial invasion of optic nerve, retina, choroid, and sclera has been documented (Donnelly *et al.*, 1985). Choroidoretinal changes present polymorphic appearance and it is common to observe lesions of different appearance in the same fundus (Anderson *et al.*, 1976). Findings from cross-sectional studies show atrophy of retinal pigment epithelium and choriocapillaries, ill-defined pale swelling at the level of the choroid and pigment epithelium, hyperpigmentation at the level of the pigment epithelium and posterior retinal oedema, and uveitis (Anderson *et al.*, 1976). There is subretinal fibrosis with wide spread atrophy of RPE. Most common site of fundus disease was temporal to the fovea while those lesions nasal to the optic disc were relatively less common. Longitudinal studies carried out by Semba *et al.*, (1990) using fundus photographs, flourescein angiograms with detail ocular examination has provided comprehensive information on fundus changes. High frequency of pathologic changes in the vitreous, retina, retinal pigment epithelium, and choroid were described. Among the retinal changes are brown or black intraretinal pigment (bone spicules), white intraretinal deposits, shiny intraretinal deposits, intraretinal haemorrages associated with a cotton-wool opacities, and retinal pigment epithelial (abnormalities) window defects.

Two forms of retinal atrophy have been defined (Anderson *et al.*, 1976; Semba *et al.*, 1990). One is diffuse and the other is geographic retinal pigment epithelial atrophy. In diffuse cases

there are areas with scattered loss of pigment, whereas in the geographic type retinal pigment epithelial loss is within well-defined borders. Geographic retinal pigment epithelial atrophy is usually surrounded by diffuse retina pigment epithelial atrophy. As the area of geographic type grow larger the diffuse type spread into areas of normal retina.

Changes in optic disc include retina nerve fibre loss which is indicated by defects in the superficial retinal white reflex, and barring of the retinal blood vessels was apparent (Hoyt *et al.*, 1973). Major retinal vessels on the optic disc are sheated, and extension of one to two disc diameter away from the disc may be pronounced. Optic atrophy may have associated choroidoretinal changes, but because fundus changes were mild it was unlikely that the atrophy was consecutive to retinal. The inflammatory process is essentially a choroiditis with lymphocytic plasma cells and eosinophil infiltration- as such is not strictly a granulomatous lesion despite the frequent clinical use of this designation- with secondary degeneration of the overlying retinal pigment epithlium and neurotina (Garner, 1976). It has been proposed by Donnelly *et al.* (1985) that eosinophil factors, e.g. major basic protein that are released during interactions with dying or degenerate mf may damage sensitive ocular tissues like the neurotina, a condition that may lead to generalized ocular disease.

2.5.3. *MICROFILARIAE KILLING:* Studies carried out by Mackenzie *et al.* (1980); Williams *et al.* 1987; Greene *et al.* (1981; 1985) revealed that antibody directed against surface antigens elicit antibody-dependent cell mediated cytotoxicity (ADCC) involving human eosinophils, neutrophils, macrophages, and platelets are

capable of killing microfilariae *in vitro*. Recent findings from experimentally infected chimpazees demonstrate that neither lymphocytes nor monocytes adhered to immobilized or killed mf (Johnson *et al.*, 1991). The densities of mf were about thousand fold higher in ivermectin treated patients and more than 90% of the mf in the lymph nodes showed distinct signs of degeneration (Darge *et al.* (1991). They observed that the disintegrating mf were always encircled by eosinophils or macrophages or both. Medina-de la Garza *et al.* (1992) had documented greater ability of eosinophils than neutrophils in cytoadherence to *O. volvulus* mf. Similarly, only infection sera exclusively mediated eosinophil adherence to mf, in the case of L3 adherence occurs in the presence of infection and normal sera from endemic and non-endemic controls. None of the sera mediated adherence to L4 (Brattig *et al.*, 1991). The rate of adherence and mf killing is more effective in the presence of homologous immune serum. A similar trend had been reported for schistosmes and the capacity of eosinophils to bind to schistosomula is mediated by IgG1 antibodies (Butterworth *et al.*, 1984; 1988; Khalife *et al.*, 1989), and not by IgG3, IgG4 and IgM which can interfere with and block the activity of IgG1. It will be of interest to identify the target antigens implicated in the adherence and killing of mf by leucocytes. To this end, a cocktail of anticuticular monoclonal antibodies to LMW ($\leq$ 27 KDa) antigens termed MOV GV produced adherence in 74% of the mf with 10 or more cells after 16-20 hrs incubation (Titanji *et al.*, 1992), and by 36-40 hrs up to 54% of the mf had been killed by the leucocytes in the presence of the cocktail mAbs.

2.6. *DEFINITION OF CLINICAL CHANGES*

2.6.1. *DERMAL CHANGES*: Skin changes have a number of different causes and onchocerciasis may be just one of them. One pattern may evolve into another such as chronic papular into lichenoid stage onchodermatitis. One pattern may coexist with another. An apt description and characterization for identifiction of the skin clinical changes by Murdoch *et al.* (1993) are thus: *Acute papular onchodermatitis* (APOD) are scattered pruritic papules which progress to vessicles and postules in more severe cases. Erythema and oedema of the skin may also be present affecting a single limb or area of the trunk or face. *Excoriation* are scratch marks with loss of epidermis. *Chronic papular onchodermatitis* (CPOD) are flat-topped papules which vary greatly in size (3-9mm in diameter) and the height is above the skin surface (some are almost macular). There could be post-inflammatory hyperpigmentation. *Lichenified onchodermatitis* (LOD) are pruritic hyperpigmented hyperkeratotoic plaques. The distribution is usually assymetric and may involve one limb. *Atrophy* is when skin appears smooth and shiny or else wrinkled. There may be loss of hair and reduced sweating in affected areas. It is only scored in individuals aged less than 50 years not to confuse it with senile atrophy. *Depigmentation* or *leopard skin* (Ls) refers to patches of complete pigment loss with islands or "spots" of normally pigmented skin centred around hair follicles. *Hanging groin* (HG) could be uni- or bilateral folds of skin present in the inguinal region. They may be inelastic and may contain enlarged lymph nodes. *Lymphadenopathy* (LA) is taken as lymph nodes greater than 1cm in diameter, non-tender or tender.

2.6.2. *OCULAR CHANGES:* Visual status was determined by World Health Organization (WHO) guidelines: *blindness* is visual acuity (VA) of less than 3/60 in the better eye, *visual impairment* is an acuity between 6/24 and 3/60 in the better eye, and *normal sight* is taken as an acuity of 6/18 or better in either eye. *Punctate keratitis* (PK) is presence of stromal opacities and *sclerosing keratitis* (SK) is when opacities are nasal or temporal periphery or both and semilunar or confuent. *Iritis* (Ir) is where there is anterior segment cells without posterior synechiae with or without anterior chamber cells. *Cataracts* (C) is an obvious lens opacity seen through undilated pupil reducing VA below 6/18. *Optic nerve disease* (OND) is presence of pallor, white or pale non-glaucomatous optic disc while *chorioretinitis* is retina epithelial atrophy with or without subretina fibrosis. *Vascular sheathing* (VS) is opacification of walls of major retinal vessels at or extending beyond the optic nerve head. *Glaucoma* is measured as an intra-ocular pressure of 25mm Hg or more in the presence of pathological cupping of the optic disc (with C/D ratio of 0.6 or more) or 40mm Hg having a C/D ratio 0.8 or more.

2.7. ***IMMUNOLOGY***

Like most tissue parasite with multi-antigenicity, *O. volvulus* elicit vigorous humoral response by IgM, IgA, IgE, and IgG class and subclass antibodies. These responses are polyclonal and parasite-induced (Greene *et al.*, 1985; Cabrera *et al.*, 1986). Autoantibodies to skin and ocular tissue components are enhanced (Vinghtain *et al.*, 1986; Petralanda *et al.*, 1988), but their role in the aetiology of clinical disease has not been elucidated. Eosinophilia is a common feature of helminthic parasite infection, including onchocerciasis (MacKenzie, 1987), where they

are suspected to be involved in depressing immune response and in immunopathology.

2.7.1. *HUMORAL IMMUNE RESPONSES:* All the known methods in clinical immunology have been tried to measure both quantitatively and qualitatively antibodies against the parasite antigens. They include immunodiffusion in gels (Neppert, 1974), haemagglutination tests (Ishii, 1974), skin reaction (Hashigushi *et al.,* 1979), immunoflourescent antibody technique, IFAT (Ambroise-Thomas, 1974), solid-phase radio-immunoassay (Hussain *et al.,* 1981, Weiss *et al.,* 1981), immunoblot or western blot technique (Cabrera *et al.,* 1986) and the now widely used enzyme-linked immunosorbent assay, ELISA (Bartlett *et al.,* 1975). Hitherto, assays were performed with crude parasite antigens.(Tada *et al.,* 1987). A major limiting problem to these assays is non-specificity due to cross-reactivity caused by shared homologies of common antigens that are also found in other parasites that are often co-endemic with onchocerciasis (Marcoullis and Grasbeck, 1978). A major cause of cross-reativity in filariasis is attributed to an ubiquitous molecule, identified as phosphorylcholine (Pery *et al.,* 1979; Lal *et al.,* 1987; Maizels *et al.,* 1987; Scott *et al.,* 1987; Wenger *et al.,* 1988). Purification of antigen through physicochemical and immunochemical (including monoclonal antibody based affinity) approach have been attempted with remarkable improvement (Marcoullis *et al.,* 1978; Philipp *et al.,* 1984; Lobos and Karam,1986; Nogami *et al.,* 1988; Weiss and Karam, 1989). A tremendous advancement in this regard has been facilitated by application of recombinant DNA tailored molecules that is now a dependable source of getting defined antigen, and even their supply in larger quantity is

guarranteed (Lucius *et al.*, 1988; Gallin *et al.*, 1989; Garate *et al.*, 1990; Chandrashekar *et al.*, 1991). A major problem is that potentially important antibody response against individual antigen may be hidden by a dominant response to another. This will preclude the detection of antigen(s) inducing responses with probable clinical trends (Lal and Ottesen,1988; Weil *et al.*, 1990; Engelbrecht *et al.*, 1992). The highly immunogenic epitope of PC molecule are detectable in essentially all individuals (Brile *et al.*, 1987). Another alternative approach is exploring of subclass restricton in immune response to carbonhydrate moiety reported by Natvig *et al.*, (1979). A test for specific antibody class and isotypes in onchocerciasis demonstrated difference in antibodies as IgE was found to be more parasite species-specific than IgG (Weiss *et al.*, 1982); much more IgG than IgE antibodies were removed by heterologous sorbents. Onchocerciasis is one of the very few disease conditions in which IgG4 predominate (Aalberse *et al.*, 1983; Lal and Ottesen, 1988; Weil *et al.*, 1990), a situation possibly linked to chronic stimulation.

Defining the immunogens involved in triggering any arm of immune response could be achieved employing serological assays which give better understanding of immunopathology and protective immunity, and could be fashionable tool for immunodiagnosis, drug screening and monitoring treatment efficacy. A unique IgG3 reactivity against low molecular weight antigens is exhibited by sowda patients differentiating it from the generalized disease (Cabrera *et al.*, 1988). High IgG4 levels is reported to be associated with increasing age, skin mf densities and severity of skin diseases (Ghalib *et al.*, 1987; Weiss and Lobos, 1989; Engelbrecht *et al.*, 1992; Egwang *et al.*, 1993). The

diversity in host immune response is attributable to variation in parasite strain (WHO, 1992), and inherent individual difference in ability to induce cytokine responsible for modulating or regulating immunoglobulin class and subclass production (Nutman and King, 1990). A role for interferon gamma (IFN- ¥), interleukine-5 (IL-5) and IL-6 influencing both IgE and IgG_4 production has been suspected. King and his colleagues have shown that IL-4 and IFN-¥ essentially mediate and down regulate IgE production. Simultaneous addition of anti-IL-4 completely inhibited antigen-stimulated IgE production while simultaneous addition of anti-IFN-¥ to the peripheral blood mononuclear cells (PBMC) culture significantly augmented filarial antigen-stimulated IgE production by between 33% to 1238% in the same patients (King *et al.*, 1989). Addition of 10-1,000 units per ml of recombinant human IFN-¥ completely inhibited parasite antigen-induced IgE production.

A similar role has been attributed to IFN-¥ with suppression of ongoing IgE production in patients with unusual hyper-immunoglobulin-E (HIE) syndrom. Indeed, elevated serum IgE and IgG4 levels in patients correlate with lymphatic filariasis generally and the high level of one correlate with high level of the other (Rivat-Peran *et al.*, 1981). It is thought that parallel regulating mechanisms exist for both of them as failure of pokeweed mitogen stimulated lymphocyte to produce neither of the two indicate they may be under similar regulating mechanisms (Nutman *et al.*, 1984; Hussain and Ottesen, 1985; 1986). Other factors are nutritional status, coexistent infection with other parasites, and hormonal influence could predispose individual to variability in immune response.

2.7.2. *AUTOIMMUNE RESPONSE:* A role for ocular autoimmune disease has remained equivocal as animal model studies suggest that retinal autoantigenicity may play a potentially important role in ocular immune response and ocular immunopathology (Gery *et al.*,1986). Also, using indirect immunofluorescent staining of frozen sections of human retina, Chan *et al.*, (1987) demonstrated that anti-photoreceptor and outer plexiform autoantibodies were more in patients with retinal vasculitis than those with systemic vasculitic condition in which the eye was not involved. Early research undertaken in this direction by Vinghtain *et al.*, (1986) implicated type I hyper-sensitivity to filarial antigen and retina tissue antigen demonstrated by human basophil degranulation in onchocerciasis patients with chorioretinitis. Moreover, Chan *et al.* (1987) found that antibodies could not be absorbed by conventional techniques using either retinal surface (S-Ag) or the interphotoreceptor retinoid binding protein (IRBP), an observation suggesting that other ocular antigens are involved. Also, high frequency antibodies against autoantigen of inner retina (nerve fibre, ganglion cell and muller cell) and outer segment of the photoreceptor were noted more in onchocerciasis patients.

It has been observed that the level of parasite-induced antibodies in ocular fluid generally mirrored those of the sera (Chan *et al.*, 1987). But various studies by van der Lelij *et al.*, (1991;1992) demonstrated no difference in most ocular autoantigens in ocular and non-ocular onchocerciasis. A high intraocular levels of antibodies against the parasite suggest a direct involvement of host-parasite interaction in the pathogenesis of onchocercal chorioretinopathy. A cDNA clone (Ov39) isolated from lambda gt11 expression library of *O.*

volvulus show cross-reactivity with component of retina pigment epithelium (RPE). Polyclonal antibody raised against this recombinant molecule immuno-precipitated an M_r 22,000 Dalton from *O. volvulus* antigen and recognizes 44,000 M_r component of bovine RPE (Braun *et al.*, 1990). Antibody response to this cloned antigen is detected in all onchocerciasis cases with posterior segment pathology (McKennie *et al.*, 1993a;1993b). It is then proposed that immune response to the parasite by mimicking host tissue component may bring about autoreactivity that can cause tissue damage.

Humoral and cell-mediated responses to retina S-Ag and IRBP did not show any relationship between anti-retinal antibody level and occurrence of chorioretinitis (van der Lelij *et al.*, 1990a; 1990b). Both levels of antibodies were elevated in those with proven onchocerciasis. Also, variably reported circulating immune complexes could be a manifestation of an end stage immune response as it did not support any pathogenic role, but rather express defence mechanism against the infection agent or autoimmune process (Panganelli *et al.*, 1980; Steward *et al.*, 1982; Vinghtain *et al.*, 1986; Sisley *et al.*, 1987; Chandrashekar *et al.*, 1990). Local intraocular antiretinal IgG antibody production could not be demonstrated in onchocerciasis patients with or without ocular symptoms (van der Lelij *et al.*, 1991). Analysis of humoral immune response to purified connective tissue components (such as collagen, laminin, elastin and fibronectin) by Petralanda *et al.*, (1988) showed high level specific antibodies to some of the vertebrate basement membrane components (collagen IV and laminin).

2.7.3. *PROTECTIVE IMMUNITY.* The question of immunity in onchocerciasis stems from observation that some persons living under high risk of exposure to ongoing transmission are free of infection and disease (Ward *et al.*, 1988; Nutman, 1989). This category of people were termed as putatively immune (PI) or endemic normals (EN) or infection free. As there are no clinical definition, hence identification relies on criteria variously proposed to include residence in endemic area for over 10 years; negative annual evaluations for onchocercal nodules and skin microfilariae for the prior 3 years; no clinical evidence for onchocerciasis at evaluation (medical history, physical examination, ophthalmologic evaluation); no mf in four skin snips and no reaction to 50mg oral test dose of DEC (WHO, 1987; Freedman *et al.*, 1989). This group of people are found to be hyper-responsive to 20,000 dalton molecular weight glycoprotein, 45- to 50-KDa triplet and a 22-KDa antigen of *O. lienalis* infective (L3) stage. When L3 antigens of *Brugia malayi* were used, sera from putatively immune individuals identified a high-molecular-weight triplet/quadruplet plus several antigens of lower molecular weights that was recognized by sera from few (or none) of the infected patients. In contrast to hypo-responsiveness of infection-free group indicated by serum antibody levels to adult worm antigens did not extend to the T cell-mediated lymphocyte proliferative responses (Ward *et al.*,1988). The heightened T cell response to parasite antigen in the infection-free group was more apparent compared to those with infection when IL-2 production was assessed with OvAg, but purified protein derivative (PPD) equally stimulated lymphocytes from these same individuals to produce the IL-2. Elimination of

either IL-5 or IL-4 by treatment of Balb/cBYJ mice immunized with irradiated *O. vovulus* infective L3 significantly reduced the protective effects of vaccination against larval *O. volvulus* (Lange *et al.*, 1994). Also, proliferative responses to OvAg, mycobacterial PPD and Streptococcal SL-O were greater at 84 months post initial treatment in amicrofilaridermic than mf-positive onchocerciasis patients. In amicrofilaridermic patients the reactivity approached the magnitude observed in endemic normals (Soboslay *et al.*, 1994). Moreover, the peripheral blood mononuclear cells (PBMC) from patients and endemic normals produced equivalent amounts of IL-2, IL-4 and IFN-¥ in response to mitogenic stimulation with phytohaemagglutinin (PHA); in response to OvAg, however, significantly more IL-2 and IFN-¥ were produced by PBMC from subclinical amicrofilaridermic patients or endemic normals than by mf-positive patients. In another related study carried out by Steel and Nutman, (1993), immune individuals had significantly higher levels of both IL-2 and IL-5 in response to parasite antigen than did those individuals with active infection. Furthermore, there was a direct correlation between the production of IL-2 and IL-5. Up-regulation of IL-5 mRNA seen in all patient was specifically inhibited by neutralizing anti-IL-2 antibodies. These findings suggest that IL-2 production is required to induce IL-5 and further implicate IL-5 as a possible mediator of protection in onchocerciasis.

Another evidence supporting existence of immunity is hinged on mf rates (i.e., patent infection) and number of nodules in the dermis which often reach a plateau at 30-40 years age bracket, and remains constant or decline. This is the best evidence for

naturally occurring protective immunity in humans (WHO, 1987). It either connotes that there is mf clearance and or resistance against reinfection; concomitant immunity (Duke, 1968). Repopulation and reinfection by mf or parasites after treatment or nodulectomies have similar frequency in naive patients as in those with previous exposure and this is a strong indication that there is no active immunity. Reports of immunosupression especially cell-mediated immune response that is concommitant with infection is another possible explanation that protective immunity is lacking. Studies carried out by Killian and Nielsen, (1989a;b) have established suppression of CMI to tetanus and BCG vaccines in onchocerciasis patients.

2.7.4. *DIAGNOSTIC PATHOLOGY.* Parasitological detection based on skin snipping (Collins *et al.*, 1980; Taylor *et al.*, 1987;1989) and palpation for nodules are definitive diagnosis. In addition to the later, examination for clinical signs like leopard skin (Ls) has been adapted as RAM (Edungbola *et al.*, 1990). Although these methods are specific, they are less sensitive; many patients with low mf or those without overt clinical onchocerciasis are missed, and this could confound case detection when there is an operative and effective control method to break transmission cycle. Early infection or prepatent case espacially in children will invariably be missed by the conventional diagnostic methods.

Serodiagnosis is envisaged to complement skin snipping and RAM. Effort aimed at achieving this goal has resulted in identification, characterization and purification of parasite antigens found to be mostly low molecular weight (Philipp *et al.*, 1984; Lobos and Weiss, 1986; Nogami *et al.*, 1988; Weiss and Karam, 1989; Garate *et al.*, 1990; Engelbrecht *et al.*, 1991; Lucius

et al., 1993) with promising diagnostic potential as these antigens are more species-specific. Improved sensitivity upto 96% and specificity up to 98% efficiency has been attained using various separate recombinant antigen clones. Among them are 33-KDa and 22-KDa peptides described by Lucius *et al.*, (1988) and Lucius *et al.*, (1992) designated Ov33 and Ov22. Gallin *et al.*, (1989) and Maizels *et al.*, (1990) isolated cDNA cloned antigens that previously showed 88% sensitivity. The two recombinant antigens Oc3.6 (50 kDa) and Oc9.3 (40 kDa) obtained from *E. coli* plasmids constructed to produce fusion proteins with glutathione S-transferase (GST) of *Schistosoma japonicum* at the carboxyl terminal (Smith *et al.*, 1988; Chandrachekar *et al.*, (1991) attained 95% sensitivity. In all these assays best results were obtained by IgG_4 isotype assay which gives enhanced specificity without compromising sensitivity (Lal and Ottesen, 1988; Weil *et al.*, 1990; Ogunrinade *et al.*, 1990; 1991; 1993).

Another worthy aspect of this IgG4 assay is its potential reliability for the diagnosis of infection in children who are mostly mf negative or present with low mf rates without overt clinical signs that can easily be missed by the conventional detection methods (Ogunrinade *et al.*, 1992a; 1992b; Gbakina *et al.*, 1992). It is anticipated that a cocktail of some of these antigens (WHO, 1989) may provide even better results. This expectation may be realized in the not too distant future. However, the outcome of trial carried out by Bradley *et al.*, (1993) using a cocktail recombinant protein antigens showed an impressive 98% sensitivity and 100% specificity.

Inability to distinguish current or active infection from previous exposure or covalescent cases is one serious limitation

against serodiagnosis of parasitic diseases. Detection of parasite materials eliminate this problem and as well as it is indicative of infection intensity (Ouaissi *et al.*, 1981; Des Moutis *et al.*, 1983; Tittanji *et al.*, 1988; Schlie-Guzman and Rivas-Alcala, 1989, Mbacham *et al.*, 1992). A major hinderance in antigen detection assays is interference by host antibodies. Drop in the amount of detectable antigen is accompanied by rise in specific host antibody (More and Copeman, 1991a). They exist possibly as circulating immune complex (Paganelli *et al.*, 1980; Chandrashekar *et al.*, 1990). Attempt to dissociate immune complexes aimed at increasing sensitivity of assay first proposed by Hamilton *et al.*, (1984) have been approached through increased incubation times, pretreatment of serum with heat, and acid conditions (More and Copeman, 1991a). They were of the opinion that pretreatment of serum by boiling for 5 minutes in the presence of an equal volume of 0.1 M Na_2EDTA $_pH$ 4.0 and recovery of the supernatant fluid following centrifugation at 16000 g is the most effective method of restoring the sensitivity of each of these three assays. A novel approach likely to circumvent this drawback is application of DNA probes. A 150 base pair repeated DNA sequence family was found to detect upto 20pg DNA in fly, skin or blood sample (Erttmann *et al.*, 1987; Harnett *et al.*, 1989; Unnasch *et al.*, 1988; Meredith *et al.*, 1991a). With polymerase chain reaction (PCR) oligonucleotide sequences are amplified thereby improving characterization of parasite as subsequent hybridization of amplification products to the previously developed DNA probes increases the detection signals (WHO, 1989). Both strain and species specific members of the repeat family of oligonucleotide sequence are faithfully amplified

allowing characterization of a parasite on the basis of hybridization of the PCR amplification products to previously developed DNA probes. This method has been shown to be applicable to all diagnostically important forms of the parasites, including adults, infective larvae, and skin mf (Meredith *et al.*, 1991b). This technique can only be available for field use in endemic areas if the efforts geared towards replacing the radio-active label with the less harzadous enzyme labels is achieved and actualised. Digoxigenin labelled DNA probes coupled with an antibody-enzyme detection method (Marthin *et al.*, 1990, Kimpton *et al.*, 1989) has proved to be a useful means of detecting genes and gene expression by *in situ* hybridization. Apart from overcoming the risk associated with radio-active materials other advantages of immunogold detection include elimination of diffuse signals, its rapid detection with maximum sensitivity can be achieved within 20-30 min, insolubilty in common laboratory dehydrating and mounting agents and does not fade with time and is suitable for use with wide range of counterstains (Thomas and Chandler, 1991). Also, there is no interference from endogenous enzyme activity. DIG is a steroid which occurs naturally exclusively in digitalis plants, *Digitalis purpurea and D. lanata.* The antibody produced against it shows no cross-reaction with other steroids such as human oestrogen or androgen hormones (Digest, 1990). It does not exhibit any non-specific side-reactions in plant cells.

2.7.5. *CELLULAR IMMUNE RESPONSE.* Impairment of cell-mediated immunological (CMI) reactivity to both parasite and non-parasite antigens have been recorded from *in vitro* and *in vivo* studies using lymphocyte proliferation assays, blastogenesis and skin

test for delayed hypersensitivity reaction (Bartlett *et al.*, 1978; Gallin *et al.*, 1988; Ward *et al.*, 1988; Killian and Nielsen, 1989a; 1989b; Luty *et al.*, 1990). Lymphocyte proliferative responses to soluble *O. volvulus* antigen (sAg) were poor in infected persons while PPD responses were maintained in one group and depressed in another group from different locality in Sudan (Elkhalifa *et al.*, 1991). Proliferative response and INF-¥ were depressed in the presence of live mf of *O. volvulus* or secretion/excretion (S/E) from mf or from female but not male adult parasites. B-cell response in microfilaraemic patients is reduced as indicated by both their relatively low serum levels of parasite-specific antibodies (Ottesen *et al.*, 1982), and their impaired immunoglobulin production to parasite antigen *in vitro* (Nutman *et al.*, 1987). The mechanisms underlying this immunological hyporeactivity remained poorly defined, although a number of possibilities have been proposed (King and Nutman, 1991), including the development of immune tolerance (Nutman *et al.*, 1987), active suppression by monocytes or soluble adult and larva parasite products (Piessen *et al.*, 1980, Greene *et al.*, 1983; Ouassi *et al.*, 1983; Williams, 1987; Luty *et al.*, 1990) or suppressor T cells (Piessen *et al.*, 1982). The CD4/CD8 ratio of onchocerciasis patients is to some extent different from those of the controls (Soboslay *et al.*, 1992). However, they show increased numbers of circulating CD4+CD45RA+ lymphocytes and coexpression of activation marker HLA-DR was increased on CD4+ cells from infected individuals (Freedman *et al.*, 1991). On the basis of differences in individual ability to mount cellular response is likely to influence observed diversity in severity and presentation of clinical outcome.

Granulocyte reactivity is one arm of the immune system that is elicited, and eosinophilia occur frequently in parasitic diseases and is very prominent in onchocerciasis (McKenzie, 1987). An average of 21% eosinophilia has been reported and upto 70% (around 7,000/mm^3) are not uncommon. A temporal relationship between IL-5 and subsequent development of eosinophilia implicates IL-5 as an important mediator of eosinophilia (Limaye *et al.*, 1991). Neutrophils to a lesser extent and in most cases eosinophils are involved in microfilariae clearance through cytoadherence which is complement or antibody dependent, or both (Greene *et al.*, 1981; Williams *et al.*, 1987) as in the case of eosinophil. Since direct contact between host cells and parasites is required for killing of *O. volvulus* L3 in immunized hosts, the time influx of cells and cytokines into the infection site (L3 implanted in diffusion chambers) was compared with the time challenge infections were killed. The only cell type that was found to increase in diffusion chambers in immunized mice was eosinophils; maximal level were found to be coincident with the time of parasite killing; IL-5 was not found in diffusion chambers recovered from control mice (Lange *et al.*, 1994). Significant levels of IFN-¥ were absent in the diffusion chambers of both immunized and control groups.

2.8. *AN OVERVIEW OF INTERVENTION OPTIONS*

In the priorities for control of global parasitic diseases in developing countries enunciated by Walsh and Warren, (1979), onchocerciasis was classified under medium priority based on the following criteria: it is medium in prevalence, it has high mobidity and low motality, but difficult to control. Of recent, there has been increased inpetus in its surveilance as it is one of

the parasitic diseases included among the six tropical diseases recommended by the World Health Organization for control and eradication through an agency co-sponsored by United Nation Development Project; World Bank and World Health Organization (UNDP/WORLD BANK/WHO) Special Programme for Research and Training in Tropical Diseases (TDR). Consequently, a 11-member nation onchocerciasis control programme (OCP) whose headquarters is based at Upper Volta, was set up in West Africa in 1978 excluding Nigeria which later set up her own National Onchocerciasis Control Programme (NOCP) in 1982. There are other numerous non-governmental organizations (NGOs) involved in studies aimed at supporting the control of the disease. Effort geared towards control is directed at both vector and parasite.

2.8.1. *VECTOR CONTROL:* Basically it employs larviciding and spray of adult black flies in their ecological niches (river courses and bushes adjoining it) an approach that has been discontinued because of its attendant serious negative impact on the environment. Formerly, control campaigns carried out by OCP used organochlorines, in particular DDT that is now replaced by temephos (ABATE®). It is applied at a dosage of 0.05 mg (or 0.05ppm) for 10 minutes in wet season and at 0.1mg/litre in the dry season. It acts as a stomach poison and is available as slow release solid formulation and water dispersible forms (Davies and Crosskey, 1991). Other compounds are chlorphoxim which act as contact insecticide while permethrine (Pyrethroid) and carbosulfan (carbamate) are less selective as regard non-target invertebrate fauna. As emphasis is being placed on the use of biodegradable insecticides to kill blackfly larvae in their riverine breeding sites (WHO, 1990), more attention is being focussed on

insect growth regulators (i.e. that inhibit production of chitin), and biological vector control specific for Diptera e.g. by mixing the peanut butter-like paste residue of *Bacillus thuringiensis* serotype H-14 in water. A common limitation to these approaches include development of resistance, non-specific action and moreover environmental pollution.

2.8.2. *TREATMENT*: Available drugs only kills mf (microfilaricidal) except suramin which is effective against adult *O. volvulus* (i.e. macrofilaricidal) at high concentration. In addition, another drug, diethyl carbamazine citrate (DEC) present with high toxicity. Similarly, Levamisole require high and toxic doses to attain microfilaricidal and macrofilaricidal activities (Duke, 1975). Some important limitations to the mass application of these drugs are the dosage and route of administration. DEC is taken at a total dose of 2.4 g, given in a single daily oral doses of 200 mg for an adult while suramin is given intravenously at weekly intervals for six weeks. Others are the serious side effects notably the Mazzotti adverse reaction following DEC administration (Mazzotti and Herwitt, 1947 as cited by Bryceson *et al.*, 1977) and nephrotoxicity of suramin. Often, the onset of Mazzotti reaction occurs within 20-30 minutes after treatment. It is characterised by itching accompanied by anaphylatoid response and later by maculopapular eruption which can coalesce to produce plaque-like areas of induration, vessiculation (intra-epithelial), exfoliative dermatitis which is more common with suramin, headache, vertigo, arthralgia, myalgia, eye soreness, and watery eyes (MacKenzie, 1987). Depending on the parasitic load of the patient, the severity could be serious, and in some cases could be fatal (Oomen, 1969). Moreso, the drugs are known to

exacerbate the clinical presentation of individual host ranging from inflammation of skin and eye.

Suramin is reported to cause pathological changes even in patient not infected with onchocerciasis resulting in kidney damage through degeneration of the tubules (Gibson *et al.*, 1977). There is petechial hemorrhages in the intestines, ulcerative colitis, degeneration of epithelium of renal tubules, and splenic atrophy. Other idiosynchratic reactions documented for suramin are vomiting, shock, hypertensive collapse, tenderness of soles and palms, renal signs, fibrile reactions, and severe stomatitis, and even death. Elevation in IL-6 but not tissue necrosis factor (TNF) correlated with the occurrence and severity of clinical symptoms after treatment (Turner, *et al.*, 1994), and IL-6 levels directly reflected pretreatment intensity of infection. All the foregoing make the use of these drugs discouraging.

2.8.3. *CURRENT THERAPY.* Ivermectin was initially patented for veterinary use against a wide range of parasites (Campbell *et al.*, 1983), and was found to act by distrupting the invertebrate neurotrasmission mediated by binding to ¥- amino butyric acid (GABA) receptors. A very successful trial in human against *O. volvulus* was first reported by Aziz and his colleagues (Aziz *et al.*, 1982a; b). Subsequently, it was widely reported to have relatively transient and mild side effects (Awadzi *et al.*, 1985; Lariviere *et al.*, 1985; Greene *et al.*, 1985; Whitworth *et al.*, 1991). WHO, (1989) adjudged the drug to be relatively safe, and without known pharmaccological side-effects in humans. The drug is excluded for certain categories of people (pregnant and lactating mothers, children less than 5 years, and those with central nervous system disorders e.g. meningitis, epilepsis and

trypanosomiasis, and those with concurrent illness). Based on results of more than 70,000 people treated, with a total of 120,000 treatment some with more than one dose as at January, 1989, no death attributable to ivermectin had been reported. A mild Mazzotti type reaction occurs approximately in 10-30% of person usually within the first 2-3 days, and in most cases it resolves spontaneously. Few cases (less than 1 in 1000) show severe side-effect. These include orthostatic hypotension and ashma attack (in known asthmatics) both occurring within 36 hours after ingestion of ivermectin.

The drug is a microfilaricide with the capability of temporarily impairing adult worm fertility, thereby delaying the repopulation of the skin by new microfilariae. This observed slow repopulation of the skin may indicate reduced turnover of mf production and release, or an increased and sustained mf elimination on the part of the host (Duke *et al.*, 1991a;b) suggesting partial drug-facilitated immunity (Schulz-key *et al.*, 1992). It has been observed that intrauterine stages of precursor to mature mf continue to develop normally even after ivermectin treatment (Awadzi *et al.*, 1986; Albiez *et al.*, 1988, Duke *et al.*, 1991a; b). It has been further observed that a small number of female worms have normal series of intrauterine stages and viable mf 3-4 months after single ivermectin treatment, a clear indication of an early phase of their reproductive cycle. Intrauterine mf accummulation and their subsequent degeneration ensures several weeks later, allowing another normal reproductive cycle to be initiated, beginning with reinsemination and terminate once again in sequestration and resorption of mf (Schulz-key *et al.*, 1992). They proposed that since recurrence

gradually reverses by the first year after treatment, a role for direct embryotoxic and embryostatic drug effects could not be advanced, rather it strongly favours the hypothesis that sustained alterations of biological and immunologocal processes interfere with delicate dynamic parasite-host equillibrium and parasite reproduction.

Convincing ample indications exist from studies on viability of adult worms that after a single dose of ivermectin the number of female worms producing live mf decreased. Subsequent to four doses there were more moribound/dead female worms and live uninseminated females compared to controls (Duke *et al.*, 1991a). Live adult worms were seen in only nine of the 21 nodules examined by Edgeworth *et al.*, (1993). They claimed three regions were decernible within the nodules: an outer fibrovascular capsule, an inner adult worm bundle with surrounding hyaline extracellular matrix interspersed with solitary cells, and a dense cellular infiltrate surrounding and in contact with a variable proportion of the worms. All zones contain predominantly macrophages. Eosinophils were seen in only six nodules and the macrophages in the inner zones were positive for a calcium-binding protein, MRP8/MRP14. The whole infiltrate was surrounded with extracellular MRP8/MRP14 with greatest concentration seen adjacent to the worm. The authors are of the opinion that there is an association of this protein with adult worm death.

2.8.4. *CLINICAL BENEFIT:* Apart from reducing mf load of the skin and the anterior chamber (MFAC) of the eye to less than 5% of pretreatment value for upto 12 months (Greene *et al.*, 1985; Lariviere *et al.*, 1985; Awadzi *et al.*, 1985; Remme *et al.*, 1989),

there is no direct clinical benefits from ivermectin therapy except reduction in severity, but not prevalence of clinical signs and symptoms (Whitworth *et al.*, 1991). A reduction in eosinophil counts was seen after therapy (Whitworth *et al.*, 1991; Steel *et al.*, 1991) which did not correlate with decrease in mf levels. Ivermectin was found to reduce the incidence of anterior segment diseases with significant lower prevalence of mf in anterior chamber and cornea, and punctate keratitis documented for treated group in relation to those receiving placebo (Whitworth *et al.*, 1991). Observation of progression of anterior segment lesions over a 12 month period showed regression. There was no difference between the groups in prevalence of sclerosing keratitis, optic atrophy and chorioretinitis. Recently, Abiose *et al.* (1993) showed that ivermectin treatment was capable of reducing the incidence of optic nerve disease particularly in subjects with microfilarial load greater than 10 mf per mg skin.

2.8.5. *EFFECT OF THERAPY ON ANERGY:* Various independent findings show there is up-regulation of immunosuppression consequent to treatment (Lee *et al.*, 1990; Steel *et al.*, 1991; Luty *et al.*, 1992). Steel and her research collaborators observed an increase in the stimulation index (SI) of the low responder, and previous nonresponders demonstrated proliferation to *O. volvulus* antigen (OvAg) 6 months after first ivermectin dose. The SI returned to preivermectin levels from 12 months assessment point. Mitogen response did not differ from normals and it remained so throughout the 2 year period of assessment. Change in elevated IgG antibody levels did not occur until day 7, and there was no change in parasite-specific IgE antibody. On the contrary, Steel *et al.*, (1991) reported an increase in IgE (137% of

pretreatment) levels at the 6 month point. Of the IgG isotypes only IgG_4 values did not decrease until the 1-year sampling. Patients responded with selective increases in their antibody production to certain previously recognized antigens or they developed "new" antibody responses to antigens not previously recognized (or not definable by immunoblotting techniques). The frequency of changes in antibody levels after DEC treatment was almost 50% for both IgG and IgE (Lee *et al.*, 1990) which showed intensification of binding in qualitative analyses and additional one-third of the patients showed the development of an antibody to newly recognized antigens. Freedman *et al.*, (1991) reported improvement in percentage CD4+CD45RA- cells and mitogen induced lymphokine production (IL-2 and IL-4). While the parasite-specific IL-2 and IL-4 remained undetected even before and after semiannual doses with ivermectin by 2-years follow-up, increase in delayed cutaneous hypersensitivity (DCH) to seven recall antigens occured 6 and 14 months after treatment.

Also, *in vitro* cellular reactivity to OvAg was reportedly reduced in untreated patients as compared to controls (Soboslay *et al.*, 1992), and the lymphocyte blastogenic responses to OvAg and streptolysin-O clearly improved upto 14 months after treatment. Repeated drug treament over two year period had no effect on either mitogenic or non-parasite antigen like the purified protein derivative of *Mycobacterium tuberculosis* (PPD) responses in majority of individual (Luty *et al.*, 1992). There is absolute increase in T-helper-inducer cells (CD+CD45RO+), and the CD4/CD8 ratio is temporally improved. Freedman *et al.*, (1991) opined that observed defects in the T-cell activation pathway in *Onchocerca volvulus*-infected individuals may thus

exist at several independent points; is an indication that a state of antigen-specific tolerance appears to remain even after relative reversal of other generalized immunoregulatory defects.

2.8.6. *AMOCARZINE: A PROSPECTIVE: FUTURE DRUG:* A novel compound amocarzine or Thiocarbamazin (CGP 6140) is now undergoing multicentre clinical trial (WHO, 1988). So far, the results obtained were very attractive as it has proved effective as both macrofilaricide and microfilaricide (Poltera *et al.*, 1991; Guderian *et al.*, 1991; Zea-Flores *et al.*, 1991). Majority of adult worms, 65% were dead, 20% were moribund or necrobiotic, and 15% were alive four months posttherapy. The microfilaricidal effect was reflected by reduced microfilaridermia to about 10% of the initial value within first week and 20% level was sustained for up to one year after start of therapy. Butterworth *et al.*, (1991) has described distinct signs of damage to mf at 20-48 hrs after treatment to include disintegration of the cytoplasm of the afibrillar portion of the muscle cells. Some mf showed disintegration of the mitochondria in the muscle cells. Furthermore, they reported progressive separation of the cuticle from the hypodermis, increase of intracellular vacuoles and clefts and in some mf there was condensation of cytoplasm. Various amocarzine regimens have produced a prolonged reduction in microfilaridermia at the end of the second year following initial therapy (Poltera *et al.*, 1991b), the best levels were about 7-17% of the initial parasite load in the skin for some three days amocarzine regimens. Variability in plasma concentration and urinary recovery of the N-oxide metabolite was greater in fasted patients (Lecaillon *et al.*, 1990). In effect, a 3mg/Kg amocarzine postprandially twice daily for three consecutive days (Zak *et al.*,

1991) reduced skin mf density by 45% on day 4 and 95% on day 8. This regimen is adjudged the most effective (Poltera *et al.*, 1991) killing 73% of adult female worms at nodulectomy 4 months after treatment, and was the best tolerated. A microfilaridermia of 7-9% of initial value was achieved 2 years posttherapy.

CHAPTER THREE

MATERIALS AND METHODS

3.1. *STUDY AREA*

Six villages in Kachia Local Government Area of Kaduna State have been identified as onchocerciasis endemic area. Kachia LGA is located between 9° 20'-10° 10' N and 7° 15'-8° E. The study area is within 9° 37'-9° 45' N and 7° 44'-7°48' E as shown on the map (Figure 2.). The population based on census figures compiled for ivermectin dosing was estimated at 1400 (adult 800 and chilren 600). The people are mostly indigenous farmers and their only cash crop is ginger. Most probably this informed the establishment of Kachia Ginger Refinery, at Kachia, the administrative headquarters of the LGA. Also, the area is noted for growing groundnut, corn, millet, and soya bean (which is fast becoming an attractive source of additional income as a cash crop). In a pre-survey earlier undertaken, rapid assessment method (WHO,1992) was used to establish the endemicity of the disease in these areas. During the pre-survey visit, discussions were held with the LGA Health officials and Village Heads soliciting for their cooperation, and the purpose and method of the proposed study to obtain baseline data prior to commencement of ivermectin dosing of the people was made known to them.

3.2. *PATIENTS*

The epidemiological studies included only adults (age ≥ 15 years). Participating individuals were duely informed of the purpose and method of the study through a member of the survey

team and the two LGA Primary Health workers who speak the local dialect. Their verbal consent for skin and eye clinical examinations, skin snipping and bleeding were freely obtained.

3.3. *CLINICAL EXAMINATION*

All adults presenting themselves for screening were subjected to clinical examination for the presence of onchocerciasis induceable skin and eye diseases. Palpation for nodule were carried out with the patient been asked to locate any before thoroughly palpating every part of the body to the bearest limit decency permits. At the same time skin clinical examination and snipping were performed by an Assistant Chief Rural Health Superintendent, Mr. M. S. Mohammed from N. I. T. R. The eye examinations were performed by a Senior Ophthalmic Nurse with the National Eye Centre, Mr. P. Damboyi. Both staff have been on secondment (since inception in 1988) with WHO Onchocerciasis Ivermectin Project based at Ahmadu Bello University Teaching Hospital (ABUTH), Kaduna and have been trained specifically for their respective roles. Individual was examined for palpable nodule (N), acute or chronic papular onchodermatitis (APOD or CPOD), and hypopigmentation or leopard skin (Ls). Other changes that were examined for were skin atrophy (ATR) and lichenoid changes; hanging groin (MacKenzie, 1987). Each eye was examined for visual acuity (VA) using the Snellen illitrate E-chart. Anterior segment ocular lesions were classified as punctate keratitis (PK), sclerosing keratitis (SK), iritis (Ir) and cataract (Cat.). Posterior segment lesions were classified as chorioretinitis (CR), optic nerve disease (OND) and glaucoma (Dadzie *et al*.,1990). A torch loop and a lens, and direct ophthalmoscopy were used to

examinine the anterior segment and posterior segment lesions, respectively.

3.4 *PARASITOLOGY*

Two skin snips were taken from skin overlying both iliac crests using a 2mm corneoscleral biopsy punch (Roboz Surgical Instrument, Washington, DC.). Each person's skin snips were placed in two separate wells of flat-bottomed microtitre plate containing 100µl normal saline. Plates were incubated overnight at ambient temperature sealed with platesealer. The skin snips were removed with a hypodermic needle (cleaned with a cotton wool in between paired well) and 10% formol saline was added at 50µl per well, and then sealed. Thereafter, on getting to the laboratory at the Nigerian Institute for Trypanosomiasis Research, N. I. T. R. microplates were examined under inverted microscope for emerged microfilariae (Weil *et al.*, 1990). Results of mf count were entered into form designed by the Ivermectin Project, ABUTH, Kaduna. The total count for both snips were obtained for every individual.



Figure 2. Map of Kachia Local Government Area. (The six villages surveyed are indicated with closed circle (●))

3.5. *BLOOD AND SERUM SAMPLING*

About 7ml blood was collected through venepuncture from 90 persons with one or more clinical signs and from 30 persons without any sign using sterile disposable syringes and needles. Each blood sample was shared ~2ml into tube containing ethylene diamine tetracetic acid (EDTA) anticoangulant and the other portion into clean vacutainer tube with cap. Serum was extracted after allowing the blood to clot under ambient temperature for 2 hours before centrifuging. Serum was neatly withdrawn with 1ml adjustable pipette into clean screw-capped cryotubes. Sera were stored and transported frozen in liquid nitrogen in and from the field to the laboratory, and thereafter stored at -20^{o} C in deep freezer before use.

3.6. *BLOOD COUNT*

3.6.1. *WHITE BLOOD CELL DIFFERENTIAL COUNT:* Thin blood films were prepared, air dried and stored in a slide box. The films were fixed with undiluted Leishman stain for one minute and staining by diluting (1:3) with distill water, and washed in PBS pH 7.2 after 10 minutes.. Slides were allowed to dry in slanting position on a wooden rack. Films were stored in a slide box until when examined under the light microscope (x100 objective) with oil immersion. Leucocyte or white blood cell count was done using differential counter to enumerate 200 cells.

3.6.2. *TOTAL LEUCOCYTE COUNT:* A 1:20 (20µl/380µl) dilution of anticoagulant blood in WBC diluent (3ml of glacial acetic acid and 2ml methylene blue was made up to 100ml with distill water). Standard method of preparing an improved Neubeur haemocytometer chamber was used. A glass cover slip was pressed on to the chamber and observing the formation of

Newton's rings at the edges. The chamber was charged with diluted blood avoiding air bubble, kept for 3-5 min. in improvised moist chamber (petridishes with water soaked tissue paper), and leucocyte count was done under x40 objective using tally counter to enumerate.

3.7. ***ANTIGENS***

3.7.1. *CRUDE PARASITE ANTIGENS:* The adult worms were recovered from nodules incubated for periods up to 24 hrs at ambient temperature in 0.5% (w/v) collegenase Type vii (Sigma C-0773) in RPMI 1640 medium supplemented with 10% foetal calf serum. Worms were extensively washed in PBS prior to further processing. The worms were stored frozen in deep freezer. Worms were ground to powder under LN_2 then resuspended in PBS containing 1 mM phenylmethylsulphonyl fluoride and 50mg/ml N-tosyl-phenylalanine chloromethyl ketone. The suspension was centrifuged at 1300 g for 5 min and the supernatant fluid designated PBS-extract.

The 'PBS-pellet' was then resuspended in PBS containing 1% SDS for 10min at 28°C. The suspension was cenrifuged at 1300g for 5 min and the supernatant fluid designated 'SDS-extract'.

3.7.2. *RECOMBINANT ANTIGEN:* A recombinant antigen coded Ov1.9 was a protein derived from the *in vitro* translation of mRNA from adult worm of *O. volvulus* was prepared following the procedure described by Connor *et al*, (1988). Adult parasites of *O. volvulus* were recovered from excised nodules by digestion with collagenase (Engelbrecht and Schulz-Key, 1984). Nodules were incubated in 0.3% (w/v) collagenase in RPMI 1640 medium as already described above. For the isolation of RNA for the molecular cloning of *O. volvulus*, the hot phenol/SDS procedure

described by Taylor *et al.*, (1984) and extraction in guanidine isothiocyanate according to the protocol of Chirgivin *et al.*, (1979) were used. This antigen was prepared and supplied to the Immunology Research Laboratory, at the Pathology, Epidemiology and Statistics Division, N.I.T.R., Kaduna, by Dr. C.G. Braun (Cambridge University, London).

3.7.3. *MONOCLONAL ANTIBODIES:* Monoclonal antibodies (mAb) designated Cam8, Cam22 and Cam28 directed against adult worm antigenic material were produced by Engelbrecht *et al.*, (1991) following the standard procedures described by Fazekas de St. Groth and Scheidegger, (1980). Both Cam8 and Cam22 were selected based on their reactivity with the antigen from among the monoclonal antibodies provided by Dr Fred Engelbrecht (a visiting scientist) to the IRL, N.I.T.R., Kaduna. The role of these mAb in protection against the development of pathology was investigated to verify findings earlier reported by Engelbrecht *et al.*, (1992) for Cam28.

3.8. *ELISA*

3.8.1 *INDIRECT SANDWICH ELISA*: Serum IgA, IgM, IgG class and IgG1, IgG3, and IgG4 isotypes antibodies were measured using a modified standard protocol (Engvall *et al.*, 1975; Bartlett *et al.* 1978; Engelbrecht *et al.*, 1990) at the I.R.L., N.I.T.R., Kaduna. Procedure and concentration of reagents and buffers (see appendices 1-3) are as follows: microtitre plates were sensitized with antigens diluted in carbonate/bicarbonate buffer (pH 9.6) at optimum concentration, 150µl per well and incubated overnight at 4°C. All other steps were performed at room temperature(RT^{o}) with a minimum of 5 washings in between. The unspecific sites were "blocked" with 1-2% bovine serum albumine (BSA) at 200µl

per well for 1hr before serum was added at 100µl per well for 2hrs. Anti-human IgG horseradish peroxidase conjugate was added for IgG assay at 150µl per well for 1hr 30min. For isotype assays, monoclonal antibody specific for each isotype was added 110µl per well after the serum step followed by anti-mouse horseradish peroxidase conjugate at 150µl per well for 1hr 30min. Antigen and antibody reactions were detected by addition of subtrate solution made up of orthophenylene diamine (OPD) with hydrogen peroxide (0.02M) in citric acid (0.1M); Na_2HPO_4 buffer at 150µl per well and allowed to stay for 15 minutes. The reaction was terminated with 2M H_2SO_4 at 30µl per well and rock gently for 5 min. Colometric analysis based on optical density (OD) of microtitre plates were measured in a Dynatech ELISA reader (model MR4000) at 490nm test filter and 650nm reference filter.

3.8.2. *INHIBITION (COMPETITIVE) ELISA:* After the serum step the mAb was added at 110µl per well and incubated for 1hr 30min. All subsequent steps were the same for isotype assay.

3.8.3. *ASSAY CONTROL:* Each assay included internal controls for negative and positive standards (each in 5 wells) and they had been selected from among the serum samples in pretest experiments. In addition, 4 wells were left blank (without serum) per replicate plate. Optimum concentration of antigens, serum, monoclonal antibodies, and conjugates were determined in a series of pretitration experiments. The final assays were performed in duplicate.

3.9. *DATA STORAGE AND ANALYSES*

The survey population were enumerated and registered with identification number, name, age, occupation, and years of

domicile. Enlisted patients' parasitological and clinical data were recorded in seperate record notebook for ophthalmic and dermatologic examinations and were later entered into computer. The OD matrix of assay results were fed directly into the computer. All data were stored in both floppy and hard disc using compatible computer, Macintosh Classic. Data are presented in tabular and graphic format; frequency tables, histograms and line graphs were prepared with existing computer programme, Cricket graph.

Positive and negative reactions were determined from cut off point of mean OD values ± standard deviation (SD) of non-infected normal controls. Correlation between detection of antibodies and parasitaemia or clinical signs (those with acute or chronic skin and optic nerve diseases e.t.c.) were sought. The sensitivity and specifity of assay were calculated based on percentage positives in confirmed onchocerciasis patients and in controls.

CHAPTER FOUR

RESULTS

4.1. *PARASITOLOGY*

Among the 531 people screened (aged ≥ 15 yrs), 201 (37.9%) and 77 (14.5%) were at least one mf skin snip and palpable nodule positive respectively. The mean mf count per iliac crest skin snip for the whole population was computed at 6.7±17.6 and geometric mean or community microfilarial load (CMFL) of 1.5 was calculated as sum of $e^{\Sigma \log(count+1)/n}$. The male (n=237) and female (n=294) cohorts accounted for 99 (41.8%) and 102 (34.7%) mf skin snip positives, and 33 (13.9%) and 44 (15%) cases of palpable nodules. The skin MFD was slightly higher in male than female group (as shown in Table 1) with a mean±standard deviation (SD) mf per skin snip of 7.4±15.9 versus 6.1±.18.8 with a slight difference in geometric mean mf count 1.5±1.8 and 1.4±1.7.

Prevalence of infections among the settlements show inter-village variation with Bomjock (n=50) having the highest, 36 (72%) and 11 (22%) skin snip microfilarial and palpable nodule positive rates followed by Sabon Gantan (n=95) with corresponding 50 (52.1%) and 26 (27.1%) prevalence rates. The former was hyperendemic (≥70%) while the later was mesoendemic (40-60%) by World Health Organisation (WHO, 1987) classification of community endemic status based on mf prevalence. Other villages were hypoendemic (≥10-39%) with mf and nodule positive rates ranging between 22-37% and 7-14.6% respectively as shown in Figure 3. There was a significant correlation between mf prevalence and nodule carrier rates

(r=0.633) on one hand and between these two variates and MFD (r=0.869 and r=0.763) on the other. Prevalence rates and infection intensity based on skin MFD increased with increasing age (Table 2). Also, a gradual decrease in frequency occurred with increased infection intensity graded as low, 0.5-4.5mf/skin snip (37.8%); medium, 5-19.5mf/skin snip (33.3%) and high, ≥20mf/skin snip (28.9%).

From Figure 4, nodule, skin and ocular diseases were more in those with medium and high MFD. Among the mf positive cases, 27% were positive in a skin snip. One or more palpable nodules were found in 8.8% of mf-ve (0mf/snip, n=329) and in 20.9% of mf positive (n=201). The highest detected number of palpable nodules per person was 8 and a total of 98 nodules were palpated in 77 persons. About 60% of them were located at the pelvic region. There was no strong positive association between prevalence rates and ocular symptoms on one hand and MFD on the other.

4.2.1. *CLINICAL MANIFESTATIONS*: Complaint of itching was a commonplace among the villagers but was not scored. 55 (10.4%) of those examined presented with one or more skin clinical signs. Prevalence of clinical manifestations (Table 1) was more in the female (8.8%) than male group (4.6%) in those manifesting POD; 6 (2%) and 4 (1.7%) cases of skin atrophy (ATR), and 5 (1.7%) and a case (0.4%) of herniae, respectively. Table 2 and Figures 5 and 6 show that there were increases in skin microfilaria prevalence and intensity, skin and ocular manifestations with increasing age. Nodule prevalence appears two- to threefold less sensitive in those aged ≥20 years but tenfold less than skin snip in those ≤20 years. When data were analysed for those aged ≥20years, the nodule prevalence rates for the villages was >10%. Observed skin

clinical changes were POD 37 (7%); Ls 8 (1.5%); skin atrophy 12 (2.3%), and 6 (1.1%) cases of herniae. The incidence of onchocercal induceable eye lesions were recorded in 31 (5.8%) persons. The cases of visual impairment and blindness in one eye were 4 (0.8%) and 5 (0.9%), while those affecting both eyes were 1 (0.2%) and 2 (0.4%) as shown in Table 3. Optic nerve disease (OND) was the predominant ocular lesion, 24 (4.5%) with male accounting for 5.1% and female 4.1%.

The following cases of ocular lesions were also found: glaucoma, 3; OSK, 3; cataract, 4; a case of punctate keratitis in a 16 year old boy, and chorioretinitis with OND was the cause of blindness in one eye. Blindness in one/both eyes was attributed to glaucoma 1/1; OND 4/1, and a case of non-onchocercal blindness due to trachoma which manifested as cornea opacity with pannus, 0/1. In addition, a case each of poor refractive media, cornea opacity due to ulcer or measles, unilocular or bilateral pterygium, cornea or macular scars, 2 cases of cornea oedema due to trachoma, and leucoma due to measle were the non-onchocercal lesions detected in 10 persons some of whom were mf positive. An association between skin MFL and clinical manifestions could not be established as there were mf negative with observable symptoms and cases of mf positive without overt clinical disease.

Table 1. Gender distribution of parasitologic and clinical manifestations

Description	Male	Female	Total
Study population	237	294	531
Mean age±SD	32±15	37±17	35±16
Mean±SD mf count per snip	7.4 ±15.9	6.1 ±18.8	6.7 ±17.6
*Community mf load (CMFL)	1.5±1.8	1.4±1.7	1.5±1.8
Skin mf positive cases (%)	99(41.8)	102(34.8)	201(37.9)
Nodule positive cases (%)	33(13.9)	44(15)	77(14.5)
POD cases (%)	11(4.6)	2.6(8.8)	37(6.9)
Leopard skin (%)	1(0.4)	8(1.5)	9(1.7)
Skin atrophy (%)	4(1.7)	11(13.7)	15(2.8)
Lichenified skin	0	1 (0.3)	1 (0.2)
Oedema	1 (0.4)	0	1 (0.2)
Hernia (%)	1 (0.4)	5 (1.7)	6 (1.1)

*Community microfilarial load or geometric mean mf count was calculated as $e^{\Sigma \log (count+1)/n}$. POD =papular onchodermatitis

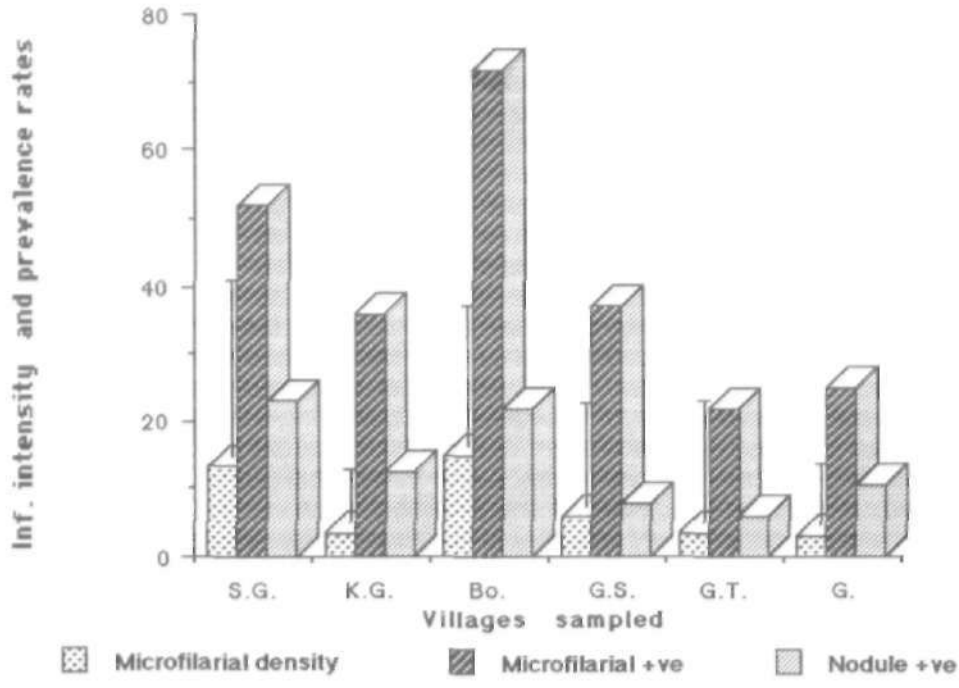


Figure 3: Parasitologic profile of Bomjock (Bo.), Gantan (Ga.), Gidan Shaho (G.S.), Gidan Tama (G.T.), Kurmi Gwaza (K.G.) and Sabon Gantan (S.G.) villages. Bomjock and S. Gantan were hyperendemic and mesoendemic while the others were hypoendemic by WHO, (1987) standard. +ve= positive. Infection intensity is mean$\pm$SD number of emerged microfilariae per skin snip.

Table 2. Age distribution of population sampled and microfilarial rates

Age group	Number Examined	Microfilarial prevalence (%)	*Goemetric.mean ±SD mf load
15-19year	90	11.11	1.29±2.34
20-29year	151	37.09	2.69±4.57
30-39year	108	49.37	3.80±5.37
40-49year	67	49.25	4.47±5.62
50-59year	52	32.69	2.63±4.90
≥60year	63	57.14	5.37±6.03

*Geometric mean mf.load or community mf load (CMFL) was calculated as $e^{\Sigma \log(count+1)/n}$. Both rates increases with increasing age.

Table 3: Visual acuity and ocular impairment or blindness

Visual acuity	Left eye	Right eye	Both eyes
6/9	4	1	17
6/18	4	1	2
6/36	1	1	0
6/60	1	1	1
NPL	0	2	2
PL	1	1	
HM	0	1	0
Blind	1	4	2
Not evaluated			1
Myopia	0	0	1

Note: A person with visual acuity above 6/60 in one or both eyes was regarded to be blind. NPL=no perception of light, PL=perception of light and HM= can not see hand movement.

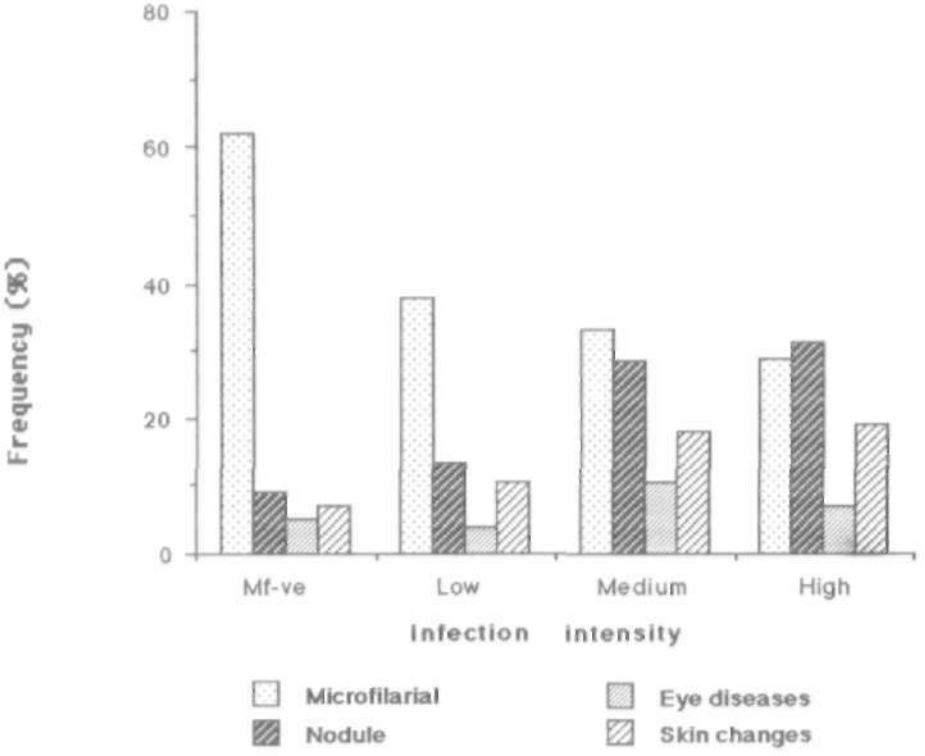


Figure 4: Impact of infection intensity on disease frquency. For purpose of analysis patients were subjectively graded into Mf-ve, microfilarial negative (0mf/snip), low (0.5-4.5mf/snip), medium (5-19.5mf/snip), and high (≥20mf/snip) infection intensity.

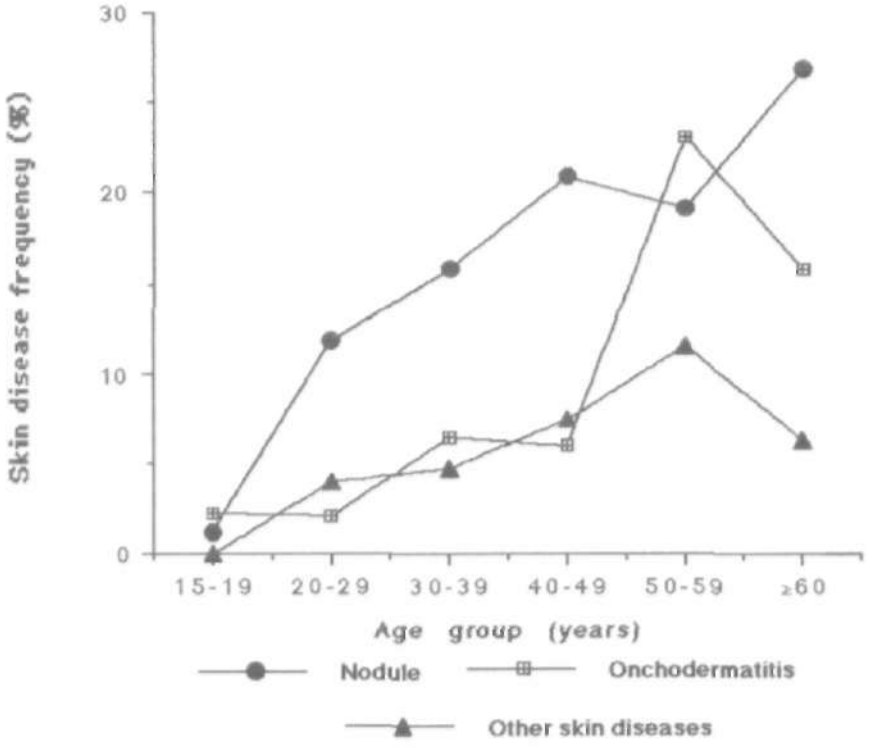


Figure 5. Age distribution of oncho-related skin diseases. The onchodermatitis include acute papular and chronic papular (APOD and CPOD) cases while the other skin diseases was made up of atrophy, lichenoid onchodermatitis, Leopard skin, and hernia. Percentage frequency (%) was calculated over the age group sample size (see Table 2). Hernia was not scored in patients ≥50 years.

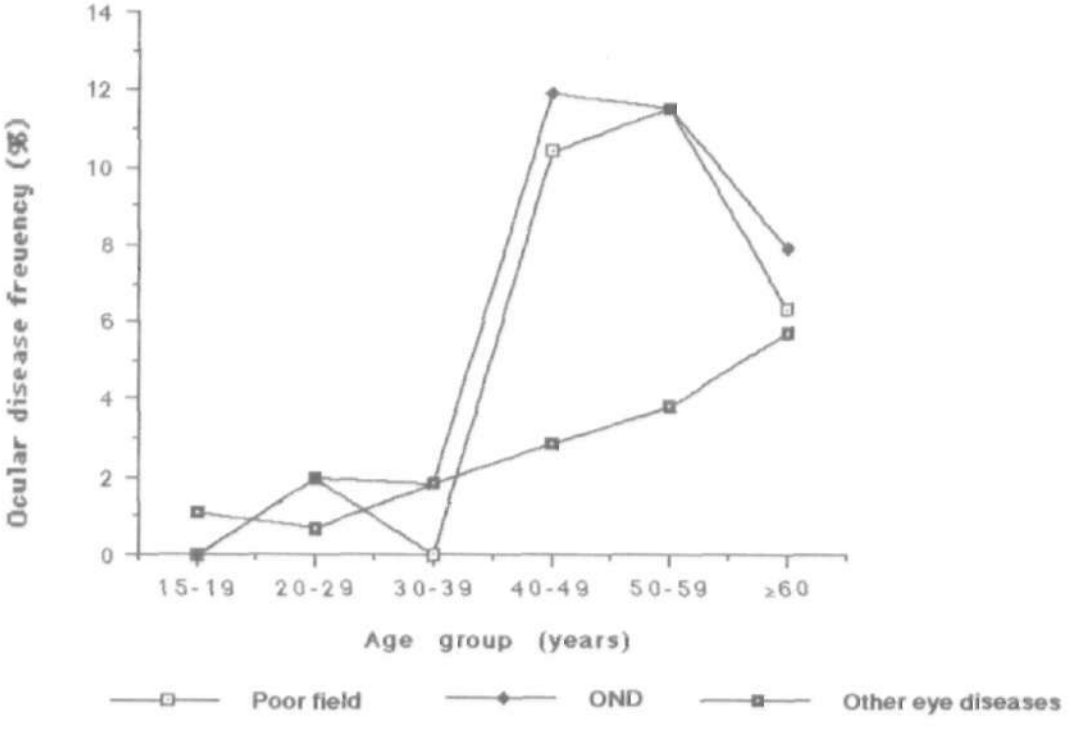


Figure 6: Age distribution of oncho-related eye diseases. Poor field of view is when a patient can not see at a wider angle. Pale disc or pallor of the optic nerve were taken as optic nerve disease (OND). Other eye dieases comprise glaucoma, cataract, punctate and sclerosing keratitis. The percentage frequency was

4.2. *EOSINOPHILIA*

It was observed to be generally moderate in 95 cases with a mean±SD percentage eosinophil count of 10.6±6.9 and absolute eosinophil count of 604±472. Apparently, nodule negative and mf positive subsamples had the highest with 12.9±7.4 and 760±541, and both nodule and mf negative cohort had the least percentage eosinophil count of 7.80±6.8 while nodule positive and mf negative had least absolute eosinophil count of 400±343 as shown in Table 4. Eosinophilia was generally higher in mf positive (Table 5), but decreases from 14.4±7.9 and 896±590 to 10.6±6.1 and 610±455 in low (0.5-4.5mf/skin snip) and high (≥20mf/skin snip) MFD compared to mf negative with 8.4±6.5 and 470±444. But patients with evidence of OND (Table 6.) had more eosinophil count, 11.9±7.04 and 767±558 while those presenting with CSD (8.9±7.4) and POD (520±335).had the least. Patients with no clinical manifestation of the disease had count approaching those recorded for OND.

Generally very low level eosinophil degranulation and vacuolation were observed in patients with eosinophilia during the process of differential WBC count. Hence, these parameters did not attract further detail evaluation. Ocassionally, *in situ* eosinophil lysis with resultant liberation of intact granules on thin films were noted.

4.3. *SEROLOGY*

4.3.1. *BASELINE DATA:* Level of antibody to SDS extract were significantly more in onchocerciasis patients showing overt clinical and/or with proven parasitologic evidence of infection. The endemic normals before (n=8) and after (n=11) treatment had raised antibody than the non-endemic group (n=7) as shown in

Figures 7-8. IgG4 response to SDS extract in patients and control groups (0.43 ± 0.12 and 0.08 ± 0.13) seem more specific than IgG (0.58 ± 00.15 and 0.31 ± 0.21) and other antibodies, IgG1, IgG3, IgM and IgA. Difference in antibody titre was recorded relative to the infection status as shown in Figures 7 & 8 with the nodule and mf positive cases (n=32) having the highest mean OD value for IgG, IgG1 and IgG4 while nodule and mf negatives (n=15) had the least.

Antibody reactivity varies among the clinical groups. There was a sharp difference between those with skin diseases and those having optic nerve disease. Patients with chronic skin diseases (n=20) had more antibody response (0.65 ± 0.14 for IgG and 0.39 ± 0.08 for IgG3 to SDS extract followed by those patients manifesting POD having 0.59 ± 0.13 and 0.33 ± 0.10 which did not differ much from those without clinical changes (n=33) in comparison with the OND group (n=12) with corresponding 0.50 ± 0.13 and 0.28 ± 0.10 OD values (Figures 9 & 10). The differences between OND and CSD groups in IgG and IgG3 antibody levels were statistically significant ($P<0.02$) by T test.

Sera from 35 (36.8%), 71 (74.7%) and 82 (86.3%) for Cam8, Cam22 and Cam28 respectively showed the capacity to competitively block the binding to epitope of *Onchocerca volvulus* antigen defined by these monoclonal antibodies in cases but non was recognised by the non-endemic controls. Cut-off points were based on mean+2SD of 16 control blank wells (without serum).

The percentage serum inhibition of binding of Cam22 and Cam28 mAbs (Figures 11 & 12) followed the pattern of antibody responses depending on infection status and clinical manifestation. Except that there was no remarkable difference in the inhibition of Cam22 by those without clinical symptoms and

those with chronic skin disease. Observed difference between OND and CSD groups in mean percentage blocking ability of Cam22 and Cam28 were statistically significant ($P<0.02$) by T-test.

For IgG antibody response to Ov1.9 a similar trend to what obtains in IgG response to SDS-extract was obtained, but the differences were very small. Antibody level was higher in cases than control, and slightly varies with infection status (Figure 13). It is higher in mf positive than negative cases. Individuals with chronic skin diseases (n=20) had more antibody levels in contrast to those manifesting OND (n=12) with corresponding mean plus or minus OD values of 0.64 ± 0.22 vs 0.48 ± 0.25 Figure 14 shows the differences in antibody profile among the clinical groups.

Table 4: Analysis of blood eosinophil percentage and absolute counts in cases: by gender and infection status

Description	Age group (years)	Eosinophil count	
		Percentage (%)	Absolute
mean(cases)	40.9	10.6	604.3
±SD(n=95)	14.2	6.9	472.4
mean(EN1)	32.9	13.6	878.9
±SD (n=8)	11.4	10.3	653.7
mean(NEN)	39.4	2.1	ND
±SD (n=7)	11.1	3.8	"
mean(male)	40.4	10.9	628.7
±SD (n=42)	16.7	7.5	507.2
mean(female)	41.5	11.6	678.9
±SD (n=53)	12.2	7	472.1
Nodule/mf			
mean(N-mf-)	39.6	7.8	544
±SD (n=15)	16.3	6.8	555.5
mean:N-mf+)	40.1	12.9	760.2
±SD (n=30)	14	7.4	541.3
mean:N+mf-)	42.6	8.9	400.1
±SD (n=18)	13.3	6.6	342.7
mean:N+mf+)	41.3	10.7	606.1
±SD (n=32)	14.4	6.	390.2

Note: Percentage eosinophil was based on differential white blood cell (WBC) count of stained thin blood film while absolute count was multiplication of this count (%) by total haemocytometer WBC count. EN=endemic control, mf=microfilaria, n=sample size, N= nodule, ND=not done, NEN=non-endemic control, SD=standard deviation, -=negative and +=positive

Table 5: Analysis of blood eosinophil percentage and absolute counts by microfilarial load and age

Description	Age group (years)	Eosinophil Percentage (%)	count Absolute
Mf load			
mean(0mf)	41.2	8.4	465.5
±SD (n=33)	14.6	6.6	450.2
mean(0.5-4.5mf)	40.7	14.4	895.8
±SD (n=18)	15.6	7.9	590.1
mean:(5-19.5mf)	40.6	10.9	581.7
±SD (n=22)	13.2	6.1	327.1
mean(≥20mf)	40.8	10.6	609.8
±SD (n=22)	14.5	6.1	455.4
Age(yrs)			
mean:15-29)	23.2	10	603.7
±SD (n=24)	4.2	7.8	590.9
mean:30-49)	39.3	11.5	661
±SD (n=41)	5.96	6.47	427.63
mean(≥50yr)	57.2	9.9	529.1
±SD (n=30)	7.3	6.8	427.1

Note: Percentage eosinophil was based on differential white blood cell (WBC) count of stained thin blood film while absolute count was multiplication of this count (%) by total haemocytometer WBC count. mf=microfilaria, n=sample size.

Table 6: Analysis of blood eosinophil percentage and absolute counts by clinical manifestations

Description	Age (years)	Eosinophil Percentage (%)	count Absolute
Clinical signs			
mean(Cs-)	38.1	11.1	604.8
±SD (n=39)	13.7	6.9	441.6
mean(OND)	46.1	11.9	766.9
±SD (n=12)	15.9	7	557.6
mean(POD)	41.4	9.5	519.9
±SD (n=18)	13.3	5.8	335.3
mean(CSD)	42.6	8.8	524.5
±SD (n=20)	13.2	7.4	501

Note: Percentage eosinophil was based on differential white blood cell (WBC) count of stained thin blood film while absolute count was multiplication of this count (%) by total hemocytometer WBC count. Cs-=Clinical sign negative, POD=papular onchodermatitis, CSD=chronic skin diseases (leopard skin, atrophy, hernia, and lichenified onchodermatis) and n=sample size.

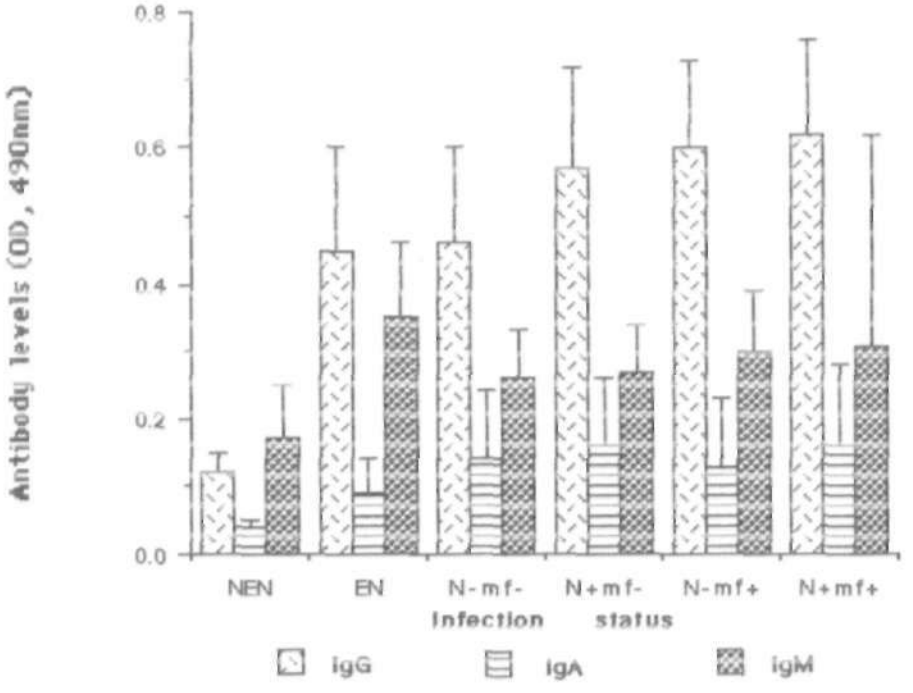


Figure 7: IgG, IgA and IgM responses in patients depending on infection status. Antibodies to *O. volvulus* adult worm extract in non-endemic controls (NEN) and endemic normals (EN), and in patients without nodules or microfilariae (N-mf-), with nodules and without mf (N+mf-), without nodules, but with mf (N-mf+) and with nodules and with mf (N+mf+). Antibody levels are given as mean optical density (OD) at 490nm.

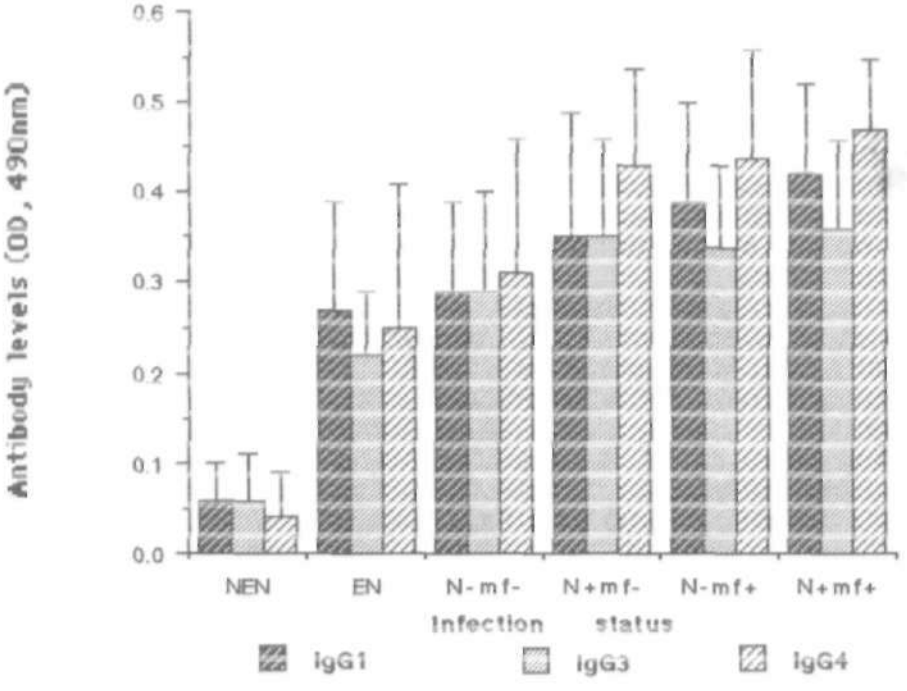


Figure 8: IgG1, IgG3 and IgG4 responses in patients depending on infection status. Antibodies to *O. volvulus* adult worm extract in non-endemic controls (NEN) and endemic normals (EN), and in patients without nodules or microfilariae (N-mf-), with nodules and without mf (N+mf-), without nodules, but with mf (N-mf+) and with nodules and with mf (N+mf+). Antibody levels are given as mean optical density (OD) at 490nm.

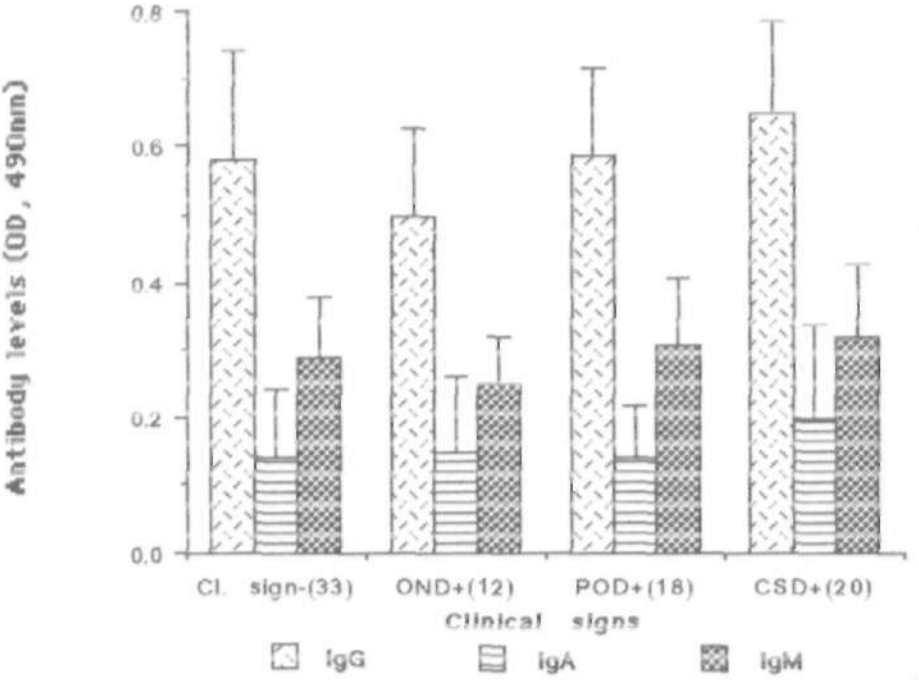


Figure 9: IgG, IgA and IgM responses in clinically defined groups of patients. Antibody levels in patients with no visible clinical signs (Cl. sign-), with optic nerve disease (OND), papular onchodermatitis (POD), and chronic skin diseases (CSD) which comprise leopard skin, hernia, atrophy, and lichinoid onchodermatitis. Figure in parenthesis is the sample size. Antibody levels are given as mean optical density (OD) at 490nm.

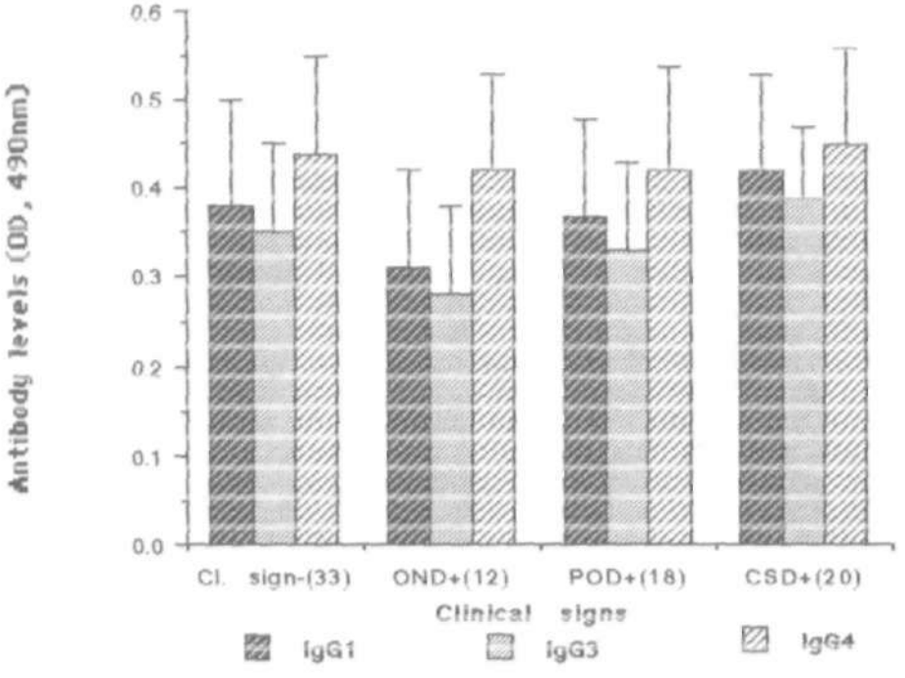


Figure 10: IgG1, IgG3 and IgG4 responses in clinically defined groups of patients. Antibody levels in groups of individuals with no visible clinical signs (Cl. sign-), with optic nerve disease (OND), papular onchodermatitis (POD), and chronic skin diseases (CSD) which comprise leopard skin, hernia, atrophy, and lichinoid onchodermatitis. Figure in parenthesis is the sample size. Antibody levels are given as mean optical density (OD) at 490nm.

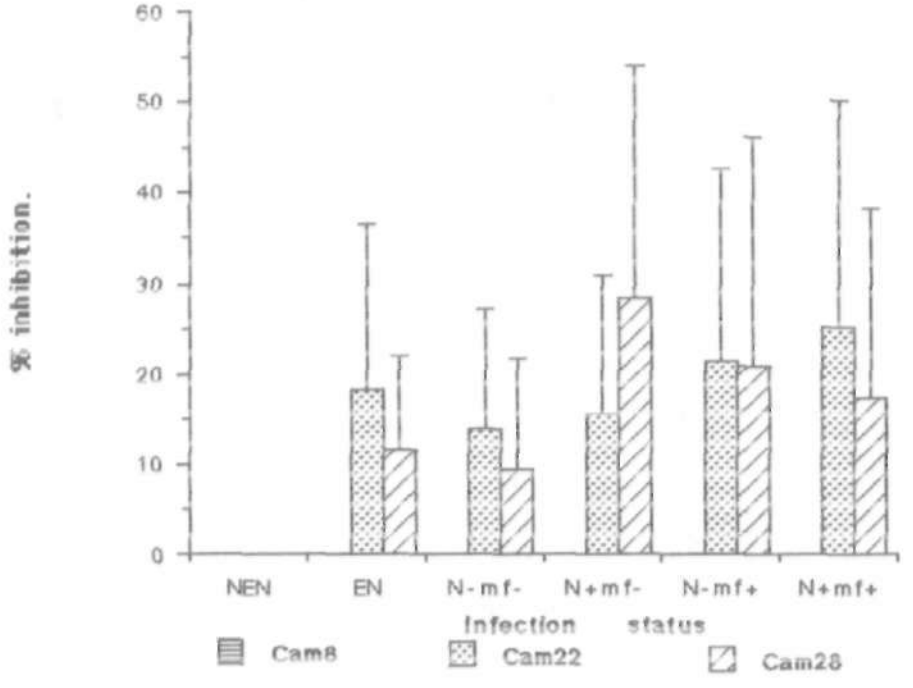


Figure 11: Serum inhibition of binding of Cam8, Cam22 and Cam28. monoclonal antibodies to antigenic epitopes. Percentage (%) inhibition by non-endemic controls (NEN) and in groups of patients with different infection status, endemic normals (EN), and in patients without nodules or microfilariae (N-mf-), with nodules and without mf (N+mf-), without nodules, but with mf (N-mf+) and with nodules and with mf (N+mf+). Inhibition was taken as the difference of individual mean OD value over the cut-off value (mean minus 2SD of 16 internal control blank wells, without serum)

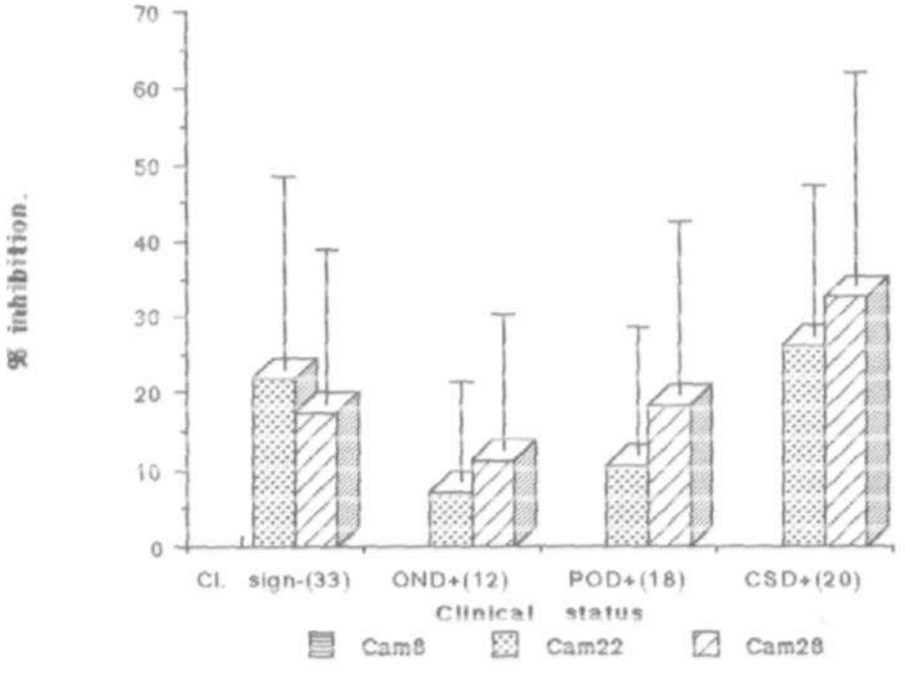


Figure 12: Serum inhibition of binding of Cam8, Cam22 and Cam28 mAb to antigen depending on clinical manifestations. The percentage (%) reciprocal effects of serum in patients with no clinical signs (Cl. signs-), optic nerve disease (OND+), with papular onchodermatitis (POD), and chronic skin diseases (CSD+) which comprise those with leopard skin, hernia, lichenoid onchodermatis, and skin atrophy. The number in parenthesis is the sample size (n).

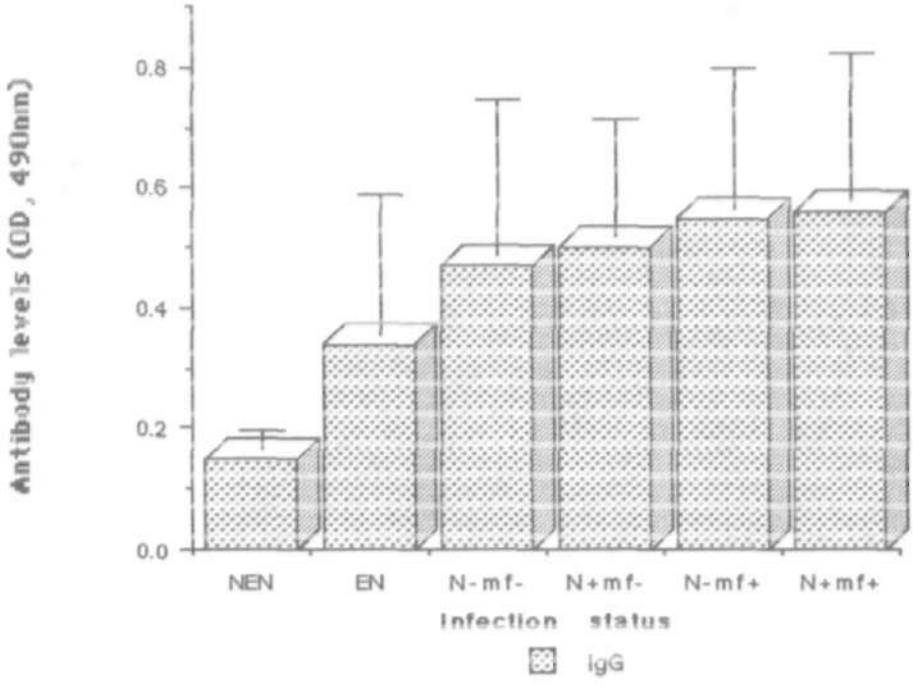


Figure 13: IgG reactivity in patients depending on infection status. Antibodies to *O. volvulus* recombinant Ov1.9 antigen in non-endemic controls (NEN) and endemic normals (EN), and in patients without nodules or microfilariae (N-mf-), with nodules and without mf (N+mf-), without nodules, but with mf (N-mf+) and with nodules and with mf (N+mf+). Antibody levels are given as mean optical density (OD) at 490nm.

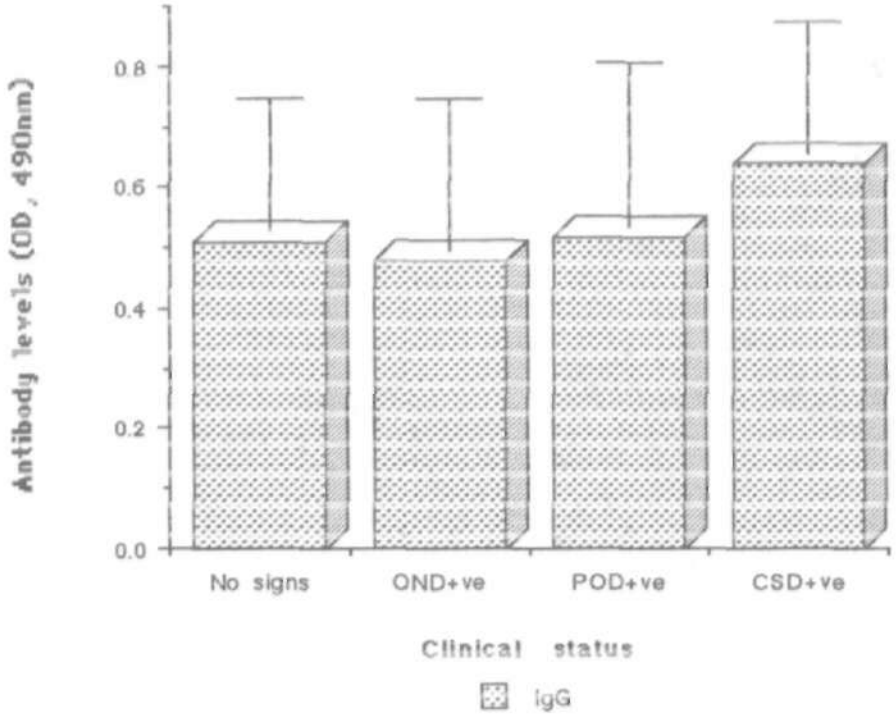


Figure 14: IgG antibody responses to *O. volvulus* recombinant antigen in clinically defined groups of patients. Antibody levels in patients with no visible clinical signs (no signs), with optic nerve disease (OND), papular onchodermatitis (POD), and chronic skin diseases (CSD) which comprise leopard skin, hernia, atrophy, and lichinoid onchodermatitis. Figure in parenthesis is the sample size. The ELISA optic density (OD) value were measured at 490nm.

4.3.2. *EFEECT OF IVERMECTIN.* Quantitative changes in antibody levels were observed 2 months after initial ivermectin dosing with percentage increase in OD value over the baseline sample taken just before treatment. The change in IgG antibody (14.9%±24.6) to SDS-extract was more than that of Ov1.9 (4.9%±19.1). From Tables 7-11, analyses of IgG, IgG1, IgG3, IgG4 and Cam22 changes by age, sex, presence or absence of nodule and skin mf are given. Skin disease cohort (n=18) had remakable increase with mean±SD percentage change of 20.5%±29.6 (pretreatment and post-treatment values of 0.59±0.15 vs 0.68±0.13 for IgG reactivity to SDS-extract) was statistically significant by the T-test for paired data ($P<0.01$), but the case was not the same for Ov1.9. The nodule positive and low MFD (≤19.5mf per skin snip) recorded appreciable increase in percentage inhibition than nodule negative and high MFD (≥20mf per skin snip) respectively.

Table 7 showed that serum IgG antibody reactivity to SDS-extract seem to appreciate 2 months after innitial ivermectin treatment. The increase was apparently expressed more in the older (≥41 year) and in female groups than the younger (≤40 yr) and male conterparts. Microfilarial load and absence or presence of palpable nodule had no remarkable effect on the antibody changes.

Table 7. Changes in IgG antibody levels two months after initial ivermectin treatment

Description	Predose SDS IgG¶	Absolute change	Percentage change (%)
mean (total)	0.60	0.07	15.1
±SD(32)	0.15	0.10	24.9
Age (years)			
mean(≤40)	0.62	0.04	10.2*
±SD (16)	0.16	0.07	15.3
mean(≥41)	0.58	0.10	19.6*
±SD (16)	0.14	0.12	31.1
sex			
mean(male)	0.67	0.04	9.4*
±SD (13)	0.13	0.07	15.1
mean(female)	0.56	0.09	18.6*
±SD (19)	0.15	0.12	29.2
Nodule load			
mean(N-ve)	0.59	0.06	13.8
±SD (16)	0.17	0.13	32.3
mean(N+ve)	0.62	0.08	16
±SD (16)	0.12	0.07	14.1
Mf load			
mean(<30)	0.57	0.06	15.1
±SD (18)	0.18	0.11	29.9
mean(>31)	0.64	0.09	14.7
±SD (14)	0.09	0.09	16.4

Note: The change in IgG level was expressed as % over the predose (baseline) indirect ELISA optical density (OD) (¶) values measured at 490 nanometer (nm) wavelenght. *The difference was significant ($P<0.05$) by T-test of unpaired data. N-ve and N+ve= palpable nodule negative and positive respectively.

Table 8. Changes in IgG1 antibody levels two months after initial ivermectin treatment

Description	Predose SDS IgG1¶	Absolute change	percentage change (%)
mean(total)	0.42	0.03	11.1
±SD (32)	0.11	0.06	22.5
Age (years)			
mean(≤40yr)	0.43	0.01	5.7*
±SD (16)	0.12	0.06	17.3
mean(≥41yr)	0.41	0.05	16.2*
±SD (16)	0.11	0.06	25.7
sex			
mean(male)	0.47	-0.01	-0.3*
±SD (13)	0.10	0.04	8.4
mean(female)	0.39	0.05	18.6*
±SD (19)	0.11	0.07	25.5
Nodule load			
mean(N-ve)	0.41	0.02	10.3
±SD (16)	0.13	0.07	28
mean(N+ve)	0.43	0.04	11.5
±SD (16)	0.10	0.06	15.2
Mf load			
mean(<30mf	0.40	0.02	9.7
±SD (18)	0.13	0.06	25.5
mean(>31mf	0.44	0.05	12.6
±SD (14)	0.08	0.07	17.7

Note: The change IgG1 level was calculated over the predose (baseline) indirect ELISA optical density (OD) (¶) values. *Indicate a remarkable difference between the subgroups were significant ($p<0.01$) by T-test.of unpaired data.

Table 9. Changes in IgG3 antibody levels two months after initial ivermectin treatment

Description	Predose SDS IgG3¶	Absolute change	Percentage change%)
mean	0.36	0.00	2.3
±SD (32)	0.09	0.06	18.3
Age (years)			
mean(≤40yr)	0.34	-0.01	-0.7
±SD (16)	0.11	0.05	17.9
mean(≥41yr)	0.38	0.02	5.9
±SD (16)	0.06	0.06	18.2
sex			
mean(male)	0.40	-0.01	-1.8
±SD (13)	0.09	0.04	15.3
mean(female	0.33	0.02	5.6
±SD (19)	0.08	0.06	19.5
Nodule			
mean(N-ve)	0.36	-0.01	-3.9
±SD (16)	0.10	0.06	17.6
mean(N+ve)	0.36	0.02	9.
±SD (16)	0.09	0.05	16.6
Mf load			
mean(<30mf)	0.35	0.00	2
±SD (18)	0.11	0.06	18.4
mean(>31mf)	0.37	0.01	3.4
±SD (14)	0.05	0.06	18.3

Note: The % change in IgG3 reponse to SDS-extract was expressed as percentage over predose (baseline) indirect ELISA optical density OD (¶) values. No remarkable change could be seen.

Table 10. Changes in IgG4 antibody levels two months after initial ivermectin treatment

	Predose SDS IgG4¶	Absolute change	Percentage change (%)
mean	0.46	0.04	13
±SD (32)	0.09	0.06	25.4
Age (year)			
mean(≤40yr)	0.45	0.04	14.8
±SD (16)	0.11	0.07	34
mean(≥41yr)	0.46	0.05	10.7
±SD (16)	0.07	0.04	11.3
sex			
mean(male)	0.50	0.02	4.3*
±SD (13)	0.05	0.03	6.8
mean(female)	0.43	0.06	18.5*
±SD (19)	0.10	0.07	31
Nodule			
mean(N-ve)	0.46	0.03	7.3*
±SD (16)	0.09	0.05	12.4
mean(N+ve)	0.46	0.06	18.1*
±SD (16)	0.09	0.07	32.8
Mf load			
mean(<15.5mf)	0.46	0.03	5.6*
±SD (18)	0.08	0.03	6.1
mean(≥16mf)	0.46	0.07	21.9*
±SD (14)	0.11	0.08	35.8

Note: The change in IgG4 level was expressed as % over predose (baseline) indirect-ELISA optical density OD (¶) values. *Indicate differences between the subgroups were significant ($P<0.02$) by T-test of unpaired data. Samples were arbitrarily divided into low (≤15.5mf) or high (≥16mf) per skin snip. N+ or N- = presence or absence of palpable nodule.

There was no remarkable change in antibody to recombinant parasite antigen designated Ov1.9 with percentage mean±SD change of 4.8%±19.4. For IgA and IgM response to SDS-extract the situation was the same with -7.1%±23.6% and 3.0%±23.6% respectively. Nonetheless, presence of nodule had effect on IgM antibody increase, 10.4%±19% compared to -1.0%±29.7% in those without palpable nodule. Level of antibody changes were not dependent on MFD and there was very low correlation with baseline antibody titre as indicated on the scattered graph (not shown) analysis using Cricket Graph Programme. The calculated regression coefficient, r=0.183. Since r<0.6, on transformation the correlation was not significant (P>0.05).

Patients that showed with antibody increase ≥10% were 16/32 for IgG, 9/32 for IgM and 4/32 for IgA response to SDS extract. In contrast, 16/32 had IgA decrease ≥10% followed by IgM (8/32) and IgG3 (7/32). Also, enhanced levels in 8/32 for Ov1.9 IgG; 13/32 for IgG1 and IgG4; and 8/32 for IgG3 responses to SDS-extract were recorded. On the other hand, increase inhibition ≥10% occured in 15/32 of Cam22, 13/32 of Cam8 and in 8/32 of Cam28. From Table 11 an appreciable increase in the capacity to inhibit Cam22 monoclonal antibody following ivermectin treatment was demonstrated. There was slight disparity between the age groups below and above 40 years on one hand, and between nodule positive and negative on the other. The differences were not statistically significant (p>0.05).

Comparatively, IgG trailed by IgG1 and both IgG3 and IgG4 was by far more sensitive than IgA and IgM antibodies to SDS-extract, and also to IgG antibody to Ov1.9. Table 12 shows that IgG detected 100% of the cases , while IgG1, IgG3 and IgG4 detected

98%, 97% and 96%. Only a case each of non-endemic normals (n=7) was weakly positive by IgG3 and IgG4 assay (with 0.02 and 0.01 above the cut off point respectively). The sensitivity among the endemic normals (n=19) follows the same pattern with IgG being the strongest (90%) and IgM was the lowest (32%). Across the board, sensitivity increase with infection status, and was more in mf and nodule positive group (n=32) and least in the mf and nodule negative group (n=15).

Table 11. Changes in serum antibody inhibition of Cam22 two months after initial ivermectin treatment

Description	Predose Cam22¶	Absolute change	Percentage change (%)
mean	0.39	-0.05	-14.7
±SD (32)	0.12	0.08	21.8
Age (years)			
mean(≤40yr	0.39	-0.03	-10.1*
±SD (16)	0.13	0.06	19.8
mean(≥41yr	0.39	-0.07	-19.3*
±SD (16)	0.11	0.09	22.6
sex			
mean(male)	0.36	-0.05	-17.6
±SD (13)	0.13	0.04	14.46
mean(female)	0.41	-0.05	-12.7
±SD (19)	0.10	0.10	25.3
Nodule load			
mean(N-ve)	0.39	-0.04	-10.1*
±SD (16)	0.11	0.10	23.7
mean(N+ve)	0.40	-0.06	-19.3*
±SD (16)	0.12	0.05	18.5
Mf load			
mean(<30mf	0.42	-0.05	-14.3
±SD (18)	0.11	0.09	24.7
mean(>31mf	0.36	-0.05	-15.3
±SD (14)	0.13	0.05	17.1

Note: The change in serum blocking capacity was calculated as % over the predose (baseline) competitive ELISA mean OD (¶) values. A negative result represent decrease or reciprocal effect of serum antibody which prevent binding of Cam22 to antigen. *These differences were not statistically significant (P>0.05).

Table 12: Sensitivity of serum antibodies for the detection of onchocerciasis

Infection status	IgG	IgA	IgM	IgG1	IgG3	IgG4	Ov1.9 IgG
NEN (n=7)	0	0	0	0	1	1	0
sero±ve(%)	0	0	0	0	14.3	14.3	0
EN (n=19)	17	9	6	15	15	11	12
sero±ve(%)	89.5	47.4	31.6	79	79	57.9	63.2
cases (n=95)	95	73	29	93	92	91	83
sero±ve(%)	100	76.8	30.5	97.9	96.8	95.8	87.4
N mf-(n=15)	15	13	2	14	13	12	11
sero±ve(%)	100	86.7	13.3	93.3	86.7	80.0	73.3
N±mf-(n=18)	18	15	3	17	18	17	17
sero±ve(%)	100	83.3	16.7	94.4	100	94.4	94.4
N mf±(n=30)	30	18	11	30	29	29	28
sero±ve(%)	100	60.0	36.7	100.	96.7	96.7	93.3
N±mf±(n=32)	32	26	13	32	32	32	27
sero±ve(%)	100	81.3	40.6	100	100	100.	84.4

Note: Those with mean optical density OD value greater than the cut-off point (mean±2SD of 7 non-endemic controls) were regarded as been seropositive (sero±ve). -=negative; ±=positive; %= percentage positive; mf= microfilaria; n= sample size; N=nodule; NEN=non-endemic controls; EN=assumed endemic normals, SDS=sodium dodecyl sulphate crude antigen extract; Ov1.9=*Onchocerca volvulus* recombinant antigen; and IgG=immunoglobulin gamma antibody.

CHAPTER FIVE

DISCUSSION

From the available parasitologic and clinical profile, onchocerciasis is hypoendemic with the generalized type in these agrarian communities using the WHO creteria (WHO, 1987). In the interpretation of this data we should take cognisance of the limit of skin snip detection which is known to improve with the number of snips (Taylor *et al.*, 1989). An explanation for the hyperendemicity and mesoendemicity of two villages, Bomjock and S. Gantan, respectively, is attributable possibly to their proximity to breeding site and are both relatively small settlements. It is very likely that these people are subjected to more vector contact. Age and occupation are two other variables influencing the community microfilariae load (CMFL) in the face of ongoing transmission. Prevalence rate, skin MFD and clinical signs increased with age irrespective of sex (Table 2 and Figures 5 and 6.). Sociocultural disposition of the people does not impose limitation on female involvement in farming and related activities putting them at par in risk of exposure to infection. But the significant difference in infection intensity in male than the female could be due to difference in severity of exposure rate, and this is not uncommon (Sesay and Trypis, 1992). Prevalence of onchocercomata and mf which correlated with infection intensity could be interpreted to indicate that mature reproductive worms are more common. The poor correlation of these variates have been documented in different epidemiological setting by Sesay and Trpis, (1992); Gbakina and Barbe, (1992), and Akogun *et al.*, (1994). Some possibilities likely to prevent palpation of a nodule

in a mf+ could be due to location particularly if it is deep seated and small in size (Albiez *et al.*, 1988), or due to observer error. A low prevalence of physical symptoms have similarly been reported by Akogun *et al.*, (1992) from elsewhere within the same savannah ecozone of the country. This coupled with the absence of chronic obstructive lymphatic manifestations like hanging groin and elephantiasis of limb or scrotum, and the low individual nodule carrier rate (1.3 per person) reinforces the nascent nature of the disease in these study communities. Severity and prevalence of lesions is a direct function of the endemicity status (WHO, 1987), and is more in communities with high endemicity and less in those with low endemicity. The parasitologic and clinical data from these villages which are graded as hypoendemic is vindicated when compared to the data collected elsewhere by Somo *et al.*, (1993) describing hyperendemic situation. Certain clinical manifestations of onchcerciasis suggested by Akogun *et al.*, (1992) may serve as a reliable and more rapid alternative to parasitological approach in the assessment of prevalence and severity of the disease in cummunity with similar socioeconomic charactristics. Large number of skin mf were not associated with severe dermal or ocular signs as hitherto reported by Withworth *et al.*, (1991) and Akogun, (1991a;1992). Expectedly therefore, the frequency of clinical cases which does not increase with increasing MFD could be taken to mean that the subjects mounting more vigorous immune response are invariably those that have the capacity to clear mf and cause tissue damage,as suggested by McKenzie, 1985) is not far from being correct. Moreso, physical symptoms were also seen among the mf positive individuals. Similar reports

have been made where filarial infections including onchocerciasis had cases of clinical symptoms with amicrofilaremia and microfilaremia without clinical sypmtoms (Akogun, 1991). The prevalence or intensity of infection does not correlate with ocular impairment or blindness (Burnham, 1991). No clear relationship is necessarily found between the number of microfilariae in the skin and extent or degree of the lesions (Reber and Hoeppli, 1964). Ghalib *et al.*, (1987) observed correlation of intensity of subjectively graded clinical manifestations with lower rather than high mf skin densities in Eastern Sudan. All these run contrary to other reports (WHO, 1976; Marshall *et al.*, 1986 and Akogun *et al.*, 1992).

Those with OND are already at risk of irreversible blindness from permanent damage to optic nerve (Anderson *et al.*, 1976; Dadzie *et al.*, 1990; Abiose *et al.* (1993) support early intervention to prevent the increase in incidence of blindness. OND is the predominant cause of onchocercal blindness in savannah ecozone (Abiose *et al.*, 1993). Hence, those with OND having >10 mf per skin snip will be prevented from going blind. This can only be achieved with the timely enlistment of these communities for participation in the network of cummunity based ivermectin distributon in Nigeria, which was a component of this study. WHO (1992) report of informal consultation had identified the need to collect data in lower and medium endemicity zones to improve the usefulness of clinical indicators for community selection for ivermectin dosing. Subsequent to this recommendation the evaluation of palpable nodule was found to be about two to three times less effective than skin snip examination for emerged mf in the hypoendemic and mesoendemic

situations, yet, nodule palpation remains a more viable option compared to leopard skin which is less sensitive for community selection for participation in ivermectin distribution. This view no doubt has been widely acknowledged (Taylor *et al.*, 1993). Based on the available data from this study, these villages are qualified for inclussion in the community-based ivermectin treatment as they have >10% threshold prevalence rate recommended by these authors (see Figure 3). Added to the capacity to clear mf in skin and anterior chamber and also prevent anterior chamber disease (Withworth *et al.*, 1991; Collins *et al.*,1992), and as those with >10 mf/skin may have the risk of developing OND reduced (Abiose *et al.*, 1993) in these cummunities, a long term longitudinal studies through annual follow-up is been articulated to monitor the impact of ivermectin treatment on the disease dynamic in these areas, viz, prevalence rates, MFD, clinical and antibody changes.

Eosinophilia which is a common feature in parasitic helminth diseases appeared to be of moderate levels commensurate to the prevailing parasitologic and clinical manifestations. Eosinophilia is no doubt reported to be a function of helminth loads certainly of *O. volvulus* and probably of other parasites including *Ascaris* (Withworth *et al.*, 1988). A difference between this and the hypereosinophilia reported from hyperendemic areas by Whitworth *et al.*, (1991) could be understood in the foregoing analogy. Eosinophil degranulation clearly occurs and toxic secretions circulate, but the reasons why these cells are called to play remains unclear (WHO, 1988). This scepticism proved unequivocal, in so far, the mean eosinophil level was obviously more heightened in patients with OND followed by those with no

untoward clinical signs, as against the low level recorded for those with chronic skin diseases. An insignificant degranulation and vacuolation seems to characterise eosinophilia due to onchocerciasis which is consistent with the early report by Newton and Ebeigbe, (1991) in helminthic infection including onchocerciasis. An inference to be drawn from this finding is that it implies the eosinophilia due to onchocerciasis neither cause nor is it associated with target organ diseases like Leoffler's endomyocardial fibrosis, nervous system disease, cutaneous disease, and other target organ diseases (Spry and Tai, 1976; Olsen, 1983; Moore *et al.*, 1985; Tai *et al.*, 1987). Eosinophil degranulation and vacuolation is strongly implicated in the aetiology of these diseases. In accord with the suggestion by Newsome and Ebeigbe, (1991) that finding of large numbers, e.g. 15% or more, of vacuolated and especially degranulated eosinophils in patients with helminth related eosinophilia should not be attributed to the helminth infection alone, but should raise the suspicion of eosinophil mediated host tissue damage. What then is the fate of these cells and how is the body able to cope with their clearance without having any deliterious effect on the tissues or organs. Unlike the case of lymphatic filarial infection i.e. with *Wuchereira bancrofti* it causes tropical pulmonary eosinophilia. The role of eosinophil in protection and pathology is well documented (Weller and Groetzl, 1980). Major basic protein an important constituent of eosinophil granules, is seen to be present in the serum at levels inverse to the circulating eosinophil levels (Kephart *et al.*, 1984). In addition, it is most likely that cationic protein and eosinophil-derived neurotoxin (Ackerman *et al.*, 1983) are liberated along with the MBP into the

serum. It helps to advance a hypothesis as a potential cause of tissue damage affecting the optic disc and is possibly responsible for the postneuritic optic nerve disease seen in onchocerciasis patients.

Several authors have evaluated both polyclonal and parasite-specific antibodies in onchocerciasis patients. Immune responses in infected patients were suspected to contribute to the pathogenesis seen in onchocerciasis (Engelbrecht *et al*, 1992). This study was aimed at establishing if there is an association between parasite antigens-specific antibody responses and clinical manifestations. Also, it is intended to investigate the impact the course of infection has on antibody changes following ivermectin treatment.

Serum antibody reactivity to adult worm SDS-extracted antigens reveals a diverse response pattern of IgG class and subclasses in patients with infection and overt disease. A similar pattern was obtained with IgG recognition of recombinant Ov1.9 and the antigenic epitopes defined by three monoclonal antibodies designated Cam8, Cam22 and Cam28 In contrast to IgG class and subclass responses, there was no appreciable difference between control and infection sera for IgM (except that it was slightly more in the endemic normals) and IgA antibodies. This result could be attributed to problem of cross-reactivity which is not unexpected as similar results have been documented by Cabrera *et al*, (1986). The choice of SDS-extract used in this study had earlier been found to contain fewer high molecular weight antigens than the PBS-extract (Cabrera *et al*, 1986; Engelbrecht *et al*, 1990). A very noticeable increase in antibody levels concormitant to infection status may explain the role adult worm

and mf status play in development of humoral immune response. Irrespective of the presence or absence of the parasite in cases, IgG response to the SDS extract were very much the same. IgG4 antibody reactivity depends on infection status as both palpable nodule and microfilariae positive were the important variables (Figure 8) But Egwang *et al,* (1993) study of a population of mixed filarial infection reported that in IgG4 antifilarial response, only mf status was the important variable. The question of mf being a major source of antigens that stimulate the bulk of antibody responses during infection is rather controvertible. Caution should be exercised when interpreting results relying on subjective classification into various infection groups as the criteria used was identified with limitations. One, mf detection efficiency based on skin snips is known to depend on the number of snips taken (Taylor *et al,* 1992). Secondly, apart from inter-observer variations in nodule palpation some nodule are deep seated and small to be detected, and may not be onchocercomata (Albiese *et al,* 1988). Again, mf load tend to heighten the IgG4 antibody as figure 8 supported this assertion: antibody levels were reduced in nodule and mf negative than in those positive for one or both.

Among the monoclonal antibodies, the binding of Cam28 was inhibited most by sera from patients with palpable nodule and mf negative (28.5%) and the least (17.3%) in those with palpable nodule and microfilaria positive. A finding which agrees with that earlier reported by Engelbrecht *et al,* (1992). On the other hand, the reverse was the case for Cam22 with 12.7% and 26% respectively. Anology to be drawn from this finding is that previously put forward by Engelbrecht *et al,* (1992) that Cam28

could be a potential candidate antibody which may be involved in regulating skin microfilarae. An attractive mechanism of action through interreaction with granulocytes particularly eosinophils in cytoadherence is very likely. This phenomenom has been demonstrated in vitro using human serum (Brattig *et al*, 1990; Medina De La Garza *et al*, 1990), but the *in vivo* correlate is yet to be observed. A failure of human infection sera to recognise a particular epitope or antigen also could be the result of specific suppression (tolerance) of the immune response (Engelbrecht *et al*, 1992). This is most likely the case with antigenic epitope defined by Cam8 as response was restored in some individuals after treatment.

As the total parasite specific IgG and IgG1 antibodies seem to be more elevated in those CSD group of patients atest to the widely held view that humoral immune response underline the outcome of pathology of onchocerciasis. Furthermore, the response to two monoclonal antibodies, Cam28 and Cam22 defined antigenic epitopes vividly illustrated pattern of clinical trend. Antibody build up ensues following infection. It can be speculated from these results that those manifesting skin diseases witnessed elevated total IgG and IgG1 response as the disease becomes chronic with time. In contrast, OND patients exhibit suppressed antibody response (possibly a state of anergy.). In lymphatic filariasis a prepondorant IgG4 antibody levels had been reported in microfilaremic patients than those manifesting chronic pathology (Hussain *et al*, 1987). Observed increased IgG antibody levels against bands of 100K in individuals with chronic disease had previously been documented (Green *et al*, 1985) Additional information emanating from this study is that total

IgG and IgG1 prominently recognized by those with chronic skin diseases. This is in agreement with reports by the other authors. Some other notable findings include high titres of IgG3 reactivity were very prominent in hyper reactive (sowda) cases (Lucius *et al*, 1986; Parkhouse *et al*, 1987). Subsequently, Cabrera *et al*, (1988) found two major antigens recognized by IgG3 that was unique to sowda patients distinguishing from the generalised onchocerciasis. A cDNA clone (Ov39) isolated from lambda gt11 expression library of *O. volvulus* cross-react with component of retina pigment epithelium (RPE). The polyclonal antibody raised against this recombinant molecule immuno precipitated an M_r 22,000 Dalton *O. volvulus* antigen and recognizes 44,000 M_r component of bovine RPE (Braun *et al.*, 1990). Recently, antibody response to this cloned antigen is detected in all onchocerciasis cases with posterior segment pathology (McKennie *et al.*, 1993a; 1993b).

Both onchocerciasis and lymphatic filariasis are extremely complex in their manifestations, and remarkably little is established about why they exhibit such a wide spectrum or how clinical progression or regression takes place. (WHO, 1988). In so far, further studies will be needed to throw more light on antibody response to the parasite antigens in groups of patients with well defined pathology of onchocerciasis. Cummunities not under any intervention measures, which can provide diverse active cases of disease manifestations will be suitable for such study. Mass distribution of ivermectin has made this proposition very difficult to achieve as the local government area of study has been well covered.

Generalised onchocerciasis are generally microfilaridermic, have little skin disease, and usually hyporesponsive to parasite antigens. In their assays of cellular immune reactivity (Ward *et al*, 1988; Gallin *et al*, 1988; Greene *et al*, 1983). Steel *et al* (1991) have emphasized the importance of characterizing the effects of treatment on the arm of immune response as immunologic responsiveness has been shown to be related to onchodermatitis. The slight increase in antibody in those with higher microfilarial load, palpable nodule and in female consequent to ivermectin is most probable in response to antigens released due to the drug. Qualitative immunoblot analyses had shown that IgG antibody to previously recognised and unrecognised antigens were remarkably improved in 50% and one third of the patients (Lee *et al*, 1990). A similar trend was observed which allows for speculation that about 50% of onchocerciasis patients may present with antigen-specific immune unresponsiveness due to B-cell tolerance. Wether this involves the T-lymphocyte as well remain a conjecture. T-cell tolerance has been reported in patients with elephantiasis, the most common clinical manifestations of bancroftian filariasis (King *el al*., 1987). It could be infered that the detected antibody changes was sustained up to 2 month period of evaluation. Clinical implication of IgG, IgG1 and Ig4, Cam8 and Cam22 antibody changes should attract further research attention.

An equal sensitivity of IgG3 and IgG4 responses to SDS-extract in serodiagnosis of onchocerciasis is a new development. Previous effort at defining a serodiagnostic assay has improved over the years since IgG4 was reported to be more species specific by Ottesen *et al*., (1985). This engineered the greater

attention that was devoted to exploring the antigenic distinction that permit serologic differentiation and the functional differences among the IgG subclasses that are well known. (Shakib and Stanworth, 1980; Ottesen *et al*, 1985). Later on, other investigators recorded appreciable improvement in specificity without compromising sensitivity (Lal and Ottesen, 1988; Weiss and Karam, 1989; Weil *et al*, 1990; Lal and Ottesen 1988; Ogunrinade *et al* 1990). It appears that this appealing result is an added advantage of SDS over PBS-extract used by the other authors. The latter contain more high molecular weight antigens than the former as reported by Cabrera *et al*, (1986). It may not be a different case with the SDS-extract used in this study because in pretitration of monoclonal antibodies with antigens, there was one that recognises an epitope found in PBS-extract which was not detected in SDS-extract (data not shown). Evaluating the specificity of IgG class and IgG1, IgG3 and IgG4 subclass reactivity to SDS-extract in other related and unrelated parasitic and bacteria infections will go a long way to determine their serodiagnostic value. Superiority of serodiagnosis over parasitological methods (skin snipping and nodule palpation is well documented (Engelbrecht *et al*, 1990; Ogunrinade *et al*, 1992; 1993; Lucius *et al*, 1993). Using more defined recombinant antigens up to 96% specificity has been reported. The number of patients tested by other authors were relatively fewer. One advantage of this study is that the sensitivity of the assays were demonstrated in patients with different infection status and SDS-extract was identified as a potential reliable serodiagnostic antigen preparation. Sensitivity of total IgG and IgG isotypes

compared very well with those reported previously by other investigators already cited.

Observed combination of highest eosinophils and reduced antibody levels which characterised those with OND and the other way round for those with chronic skin diseases (Tables 4-6) is an area requiring detail investigation. Update of scientific knowledge in this that regard will help to validate if this trend has clinical relevance and is not just mere epiphenomenal.

CONCLUSIONS

1. Onchocerciasis hypo endemicity in these agrarian cummunities has been established. Though the most socioeconomically important clinical aspect of the disease (blindness and visual impairment) seems to be very low in frequency, the potential for becoming a serious public health problem in not too distant future is not in doubt. Some people are already at risk of irreversible blindness as a result of optic nerve disorders.

2. Extension of the National Mectizan Mass Distribution Programme to the study areas was justified as highly desirable. The villages have been dosed as part of the study plan of this project.

3. Follow up study after two or more annual treatment regimens is suggested: this will facilitate adequate emperical assessment of the impact on transmission potential and clinical benefits that can be derived from ivermectin treatment.

4. Nodule palpation proved to be a better index than other physical manifestations as rapid assessment of disease endemicity of communities with similar ecology and prevailing socio epidemiological setting; the savannah type of onchocerciasis and hypoendemicity level.

5. A moderate level of blood eosinophil count reflects equally moderate frequency and severity of skin clinical manifestations in the study population.

6. Expectedly individuals presented with diverse antibody profile. Therefore, the hightened IgG and IgG1 reponses in patients with chronic skin diseases had clearly lent weight to the widely held view that immune response may underly the disease clinical outlook.

7. Furthermore, interaction of eosinophils and antibodies (IgG, IgG1 and IgG3) is implied as they have inverse and direct relationship with chronic skin diseases, and vice versa with optic nerve disease.

8. Individual immune status of onchocerciasis patients varies as not all had the capacity to mount antibody changes sequel to first dose ivermectin treatment. This infers a state of anergy or tolerance which could be general (against wide range of antigens) rather than selective (restricted to single antigen).

9. Adopting serodiagnosis in the management of onchocerciais will become increasingly relevant when there is an effective operational control method. Detecting more cases elusive to the standard diagnostic methods is a benefit which far outweighs its limitations, as serodiagnosis can detect early prepatent infections but can not differentiate current from past infections.

10. This project can be aptly described as an explorative research, so a correlative study should be undertaken to verify the findings reported hereof.

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APPENDIX 1

CONCENTRATION OF BIOLOGICAL REAGENTS

1(a) Antibody class responses to Oncho SDS extract (21/8/91) assays:

Assay	Antigen	Peroxidase	Serum
IgA	1:1000	1:500	1:80
IgG	1:1000	1:1000	1:200
IgM	1:1000	1:400	1:160

1(b) IgG antibody class response to Ov1.9 assay:

Assay	Antigen	Peroxidase	Serum
IgG	1:100	1:1000	1:20

2. IgG isotype responses to SDS extract(21/8/91) assays:

Assay	Antigen	IgG mAb	Serum
IgG1	1:2000	1:2000	1:500
IgG3	1:2000	1:8000	1:100
IgG4	1:2000	1:8000	1:200

Note: Anti mouse IgG peroxidase at 1:1000

3. Inhibition ELISA:

Monoclonal	Antigen	Serum
Cam8	1:1000	1:50
Cam22	1:1000	1:25
Cam28	1:1000	1:25

Note: Anti mouse IgG peroxidase at 1:1000

APPENDIX 2.

SERUM DILUTION STEPS FOR FINAL ASSAYS:

Indirect ELISA:

Plate	Master	Intermediate	Assay
1:500	1:05	1:05	1:20
Dilution	30µl+120µl	20µl+80µl	5µl+95µl
1:200	1:05	1:05	1:08
Dilution			12.5µl+87.5µl
1:100	1:05		1:20
Dilution			5µl+95µl
1:50	1:05		1:10
Dilution			10µl+90µl

Inhibition ELISA:

Plate	Master	Intermediate	Assay
1:50	1:05		1:10
Dilution			11µl+99µl
1:25	1:05		1:05
Dilution			22µl+88µl

APPENDIX 3.

CONCENTRATION OF BUFFERS AND WORKING SOLUTIONS

Coating buffer pH9.6 (50 mM carbonate)

1.59g Na_2CO_3

2.93g $NaHCO_3$

make up to 1litre with dist. water, add 5ml of each of the dye solutions and store in the fridge.

Phosphate buffered saline (PBS) pH7.6

42.5g NaCl

6.4g Na_2HPO_4

0.78g $NaH_2PO_4 . 2H_2O$

make up to 5litre dist. water

Incubation buffer:

PBS pH7.6 with 0.05% Tween 20

Make 1 l PBS and add 0.5 ml Tween 20 and 5 ml of each of the two dye solutions

Blocking buffer:

1 2% Bovine serum albumin (BSA)

dissolved in incubation buffer

Washing buffer:

45 g NaCl

1.5 ml Tween 20 (0.03%)

make up to 5 ml with dist. water

Subtrate solution:

10 ml H_2O dist.

4.96 ml 0.1 M citric acid (2.1 g in 100 ml H_2O)

5.04 ml 0.2 M Na_2HPO_4 (2.84 g in 100 ml H_2O)

0.2 ml OPD stock solution

20µl H_2O_2

OPD stock solution:

make always fresh (toxic! Wear gloves!)

100 mg orthophenylene diamine (OPD)

dissolved in 10 ml methanol.

Or: Dissolve 30 mg tablet in 3 ml dist. water.

Acid Inhibitors:

2 M H_2SO_4,

26.65 ml of conc. acid mixed with 250 ml water.

Dye solutions:

Amido Black 60 mg/100ml

Phenol Red 40 mg/100ml