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EFFECTS OF MULTIPLE TREATMENTS AND REINFECTIONS ON THE LEVELS OF SCHISTOSOME SPECIFIC ANTIBODY ISOTYPES IN OCCUPATIONALLY EXPOSED INDIVIDUALS IN WESTERN KENYA

BY

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ABSTRACT

Previous studies have demonstrated that treatment of schistosome infection in people from endemic areas results in significant alterations of their parasite specific humoral responses. However, these responses have not been fully characracterised. This longitudinal study was carried out to investigate the effects of multiple treatments and reinfections on the levels of schistosome specific antibody isotypes in chronically exposed sand harvesters and newly exposed car washers in Kisumu District in western Kenya. ELISA was used to detect specific levels of IgE, IgG1 IgG2, IgG3 IgG4 and IgM isotypes against *Schistosoma mansoni* soluble worm antigenic preparation (SWAP) before treatment, 6 months post treatments, and up to 3 years post treatments. Infection intensities were determined by modified Kato/Katz thick smear technique and expressed as egg per gram (EPG) of feaces. Results showed that IgG4 responses significantly positively correlated with the intensity of the infection in car washers ($r = 0.6066$, $P < 0.0001$) and sand harvesters ($r = 0.4557$, $P < 0.0001$) at baseline, and IgG1 responses significantly positively correlated with the intensity of infection in chronically exposed sand harvesters ($r = 0.3960$, $P < 0.0001$). At 6 months post treatments, IgE and IgG2 responses showed significant mean increase in sand harvesters. At 3 years post treatments, IgE, IgG1 and IgG4 responses against SWAP showed an increasing tendency with a significant increase in the levels of IgE responses from pre-treatment levels in both groups. IgG1 levels also increased significantly from pre-treatment levels in car washers at the same time. IgG3 responses against SWAP showed a declining trend in both groups with a significant decline in both groups from pre-treatment levels 3 years post multiple treatments. IgG2 and IgM responses also showed a declining tendency in both groups at last follow up 3 years post multiple treatments. From these results, it is clear that multiple episodes of treatments influenced the isotype responses towards protective immunity development with a significant elevation of potentially protective IgE responses in both groups at last follow up coupled with a significant increase in the levels of protective IgG1 in car washers and the response elevatation levels in sand harvesters. These results suggest that it is possible to define vaccine capabilities that have responses associated with resistance to schistosomiasis infection. However, further research into the precise roles of the blocking antibody isotypes in human schistosomiasis in relation to expression of immunity is also necessary.

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background Information

Schistosomiasis, a snail transmitted trematode infection, is a major parasitic disease that is reported to rank second only to malaria in terms of human suffering in the tropical and sub tropical world. It is estimated that approximately 200 million people are currently infected and a further 600 million are exposed to the risk of infection (Chitsulo *et al.*, 2007).

Five species of *schistosoma* are known to infect human beings: *Schistosoma mansoni, Schistosoma japonicum, Schistosoma mekongi, Schistosoma haematobium,* and *Schistosoma intercalatum*. Infections with *Schistosoma mansoni*, *Schistosoma japonicum* *Schistosoma mekongi,* and *Schistosoma intercalatum* are associated with chronic liver and intestinal fibrosis, whereas chronic *Schistosoma haematobium* infections lead to fibrosis, structuring and calcification of urinary tract. Based on prevalence, distribution and pathogenicity, the most important human schistosome species are *Schistosoma mansoni*, *Schistosoma japonicum* and *Schistosoma haematobium*.

Generally, schistosomiasis is characterized by a variety of symptoms including anemia, abdominal pain, hepatosplenomegaly, diarrhea or bloody urine. In severe cases, symmers peripotal fibrosis of the liver and its sequelae of portal hypertension and eosophageal varices may develop and finally death may occur due to haematemesis (King *et al.*, 2005). In affected populations, children carry the heaviest parasite burden (Fisher, 1934) and while most infected individuals develop subtle or moderate morbidity (King *et al.*, 2005), a few develop severe pathology in the second decade of life or beyond. This

form of severe pathology may cause 200,000 deaths every year in Sub Saharan Africa (Van der werf and de Vlas, 2002).

In Kenya, *Schistosoma mansoni* and *Schistosoma haematobium* occur in the eastern, western and coastal regions. According to unpublished reports of the Ministry of Health, Kenya, over 3.5 million people in Kenya are infected with the parasites. Currently, control of schistosomiasis is based on chemotherapy, snail control, sanitation and health education. Severe disease is largely controlled by treatment of infected people with praziquantel, a drug of choice due to its safety and efficacy (WHA, 2001).

Studies have demonstrated that treatment of schistosome infection in people from areas endemic for the infection results in significant alterations of their parasite specific humoral and cellular responses (Mutapi, 2001). However, these responses have not been fully characterized in order to understand how schistosome specific acquired immunity develops, and for accounting for the observed heterogeneities in the immune responses in endemic populations which have experienced at least a single chemotherapeutic intervention from those of populations with repeated chemotherapeutic interventions upon re-infections. This longitudinal cohort study therefore monitored for a period of 3 years, the kinetics of various specific antibody isotypes (IgE, IgM, IgG1, IgG2, IgG3 and IgG4) against *Schistosoma mansoni* soluble worm antigenic preparation (SWAP) in occupationally exposed adult individuals living in *Schistosoma mansoni* endemic area in western part of Kenya, who have been treated with praziquantel upon re-infection in a treatment re-infection-re-treatment study design. The effects of multiple treatments on the changes of various schistosome specific antibody isotypes are also reported.

1.2 Statement of the Problem

Human schistosomiasis is still a major public health problem in developing countries despite numerous intense efforts directed at controlling it. This is due to rapid reinfection as a result of continued exposure to contaminated water sources. Development of an effective vaccine would offer protection against reinfection, however, it is still not yet clear as to which specific antigens and immune responses are responsible for conferring protection. Chemotherapy induced changes in response to schistosomiasis infection have been reported on a number of occasions with schistosome-specific antibody responses being affected by multiple praziquantel treatments. Insights into treatments induced humoral immune response changes associated with resistance to re-infections with *Schistosoma mansoni* which can contribute towards development of a vaccine have yet to be fully understood.

1.3 Justification

Treatment-re-infection studies have shown tremendous variation in the changes in immune responses between and within host populations (Mutapi, 2001). Characterization of changes of humoral immune responses induced by repeated praziquantel treatments is useful in contributing to our understanding of the development of schistosome specific acquired immunity. In addition, it helps in differentiating between immuno-epidemiological changes that may occur between endemic populations which have experienced at least a single chemotherapeutic intervention from those of populations with repeated chemotherapeutic interventions upon re-infections. This study was therefore carried out to investigate the effects of multiple treatments and reinfection on

the levels of schistosome specific antibody isotypes in occupationally exposed individuals in Western Kenya. Information generated from this study will add to the understanding of the immune responses against schistosome infection and will support the development of an anti schistosomiasis vaccine.

1.4 Research Questions

a) How do schistosome specific antibody isotypes levels vary with the intensity of the infection before treatment?

b) What are the effects of chemotherapy with praziquantel on the levels of schistosome specific antibody responses after initial treatment?

c) What are the effects of repeated praziquantel chemotherapy on the levels of schistosome specific antibody responses following multiple re-infections and treatments?

1.5 Objectives of the Study

1.5.1 General objective

To demonstrate the dynamics of schistosome specific antibody isotypes against schistosome soluble worm antigenic preparation (SWAP) in relation to multiple treatments and re-infections.

1.5.2 Specific objectives

a) To compare the pre-treatment antibody isotypes levels against SWAP and the post treatment levels as related to intensity of the infection,

b) To investigate the effects of multiple treatments with praziquantel on human IgE, IgM and IgG subclasses levels during a treatment re-infection –re-treatment study period.

1.6 Null Hypotheses

a) There is no difference in the pre-treatment antibody isotypes levels against SWAP and the post treatment levels and also there is no relationship between them and the intensity of the infection.

b) There is no relationship between multiple treatments with praziquantel and the levels of schistosome specific antibody isotypes before and after treatments.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Life Cycle of Schistosomiasis

Schistosomiasis is a chronic infection of the circulatory system caused by trematodes that inflame mainly the intestines, bladder, and liver. There are five types that affect humans: *S. haematobium*, which migrates to the perivesical and periureteral vessels, *S. mansoni* to the inferior mesenteric, *S. japonicum* to the superior mesenteric and the two others, *S. intercalatum* and *S. mekongi* to both mesenteric vessels (Goldsmid *et al.*, 1989).

The life cycles of the common human schistosomes (*S. mansoni*, *S. japonicum* and *S. haematobium* is shown in figure 1) schistosomes. Parasite eggs are released into the environment from infected individuals and hatch on contact with fresh water to release the free-swimming miracidium. Miracidia infect specific fresh-water snails (*S .mansoni* infects biomphalaria species, *S. haematobium* infects bulinus species and *S. japonicum* infects comelania species) by penetrating the snail's foot. After infection, close to the site of penetration, the miracidium transforms into a primary (mother) sporocyst. Germ cells within the primary sporocyst will then begin dividing to produce secondary (daughter) sporocysts, which migrate to the snail's hepatopancrease. Once at the hepatopancreas, germ cells within the secondary sporocyst begin to divide again, this time producing thousands of new parasites, known as cercariae, which are the larvae capable of infecting mammals. Young cercariae are highly mobile, alternating between vigorous upward movements and sinking to maintain their position in the water. Cercarial

activity is particularly stimulated by water turbulence, by shadows and by chemicals found on human (Jordan *et al.,* 1993; Waine & McManus, 1997)

Infective schistosome cercariae gain entry to the mammalian host via a percutaneous route and use a number of proteolytic enzymes to digest a route through the skin prior to their exit via blood capillaries or lymphatic vessels (Pearce and MacDonald, 2002; Mountford and Trottein, 2004). Ordinarily, schistosomes traverse the skin of their primary host within days and as they penetrate the skin, they transform into migrating schistosomuli and the vast majority enters the circulation via lung to the liver and transform into young worm or schistosomulae. These mature in 4-6 weeks in the portal vein where they mate and migrate to the superior mesenteric veins (in the case of *S. mansoni*) the inferior mesenteric and hemorrhoidal veins (in the case of *S. japonicum*) or the vesical plexus and veins draining the ureters (in the case of *S .haematobium*). The precise amount of time larvae spend in the skin has recently been debated (Mountford and Trottein, 2004), although evidence from tracking studies of *S. mansoni*, using both rodent (Whitefield *et al.,* 2003) and primate (Mountford *et al.,* 1988) models, show that migration out of the skin can occur within 48–72 h, with the epidermal basement membrane providing the major obstacle for larvae to negotiate.

Egg production commences four to six weeks after infection and continues for 3-7 years which is usually the life of the worm (Fulford, 1995). Eggs pass from the lumen of the blood vessels into adjacent tissues, and many then pass through the intestinal or bladder mucosa and are shed in the feaces (In case of *S. mansoni* and *S. japonicum*) or urine (in case of *S. haematobium*).The life cycle is completed when the eggs hatch, releasing miracidia that in turn infect fresh water snails (*S. mansoni* infects biomphalaria

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species, *S. haematobium* infects bulinus species and *S. japonicum* infects comelania species). After two generations, primary and then daughter sporocysts within the snail, cercariae are released and the life cycle continues (Jordan *et al.,* 1993; Waine & McManus, 1997).

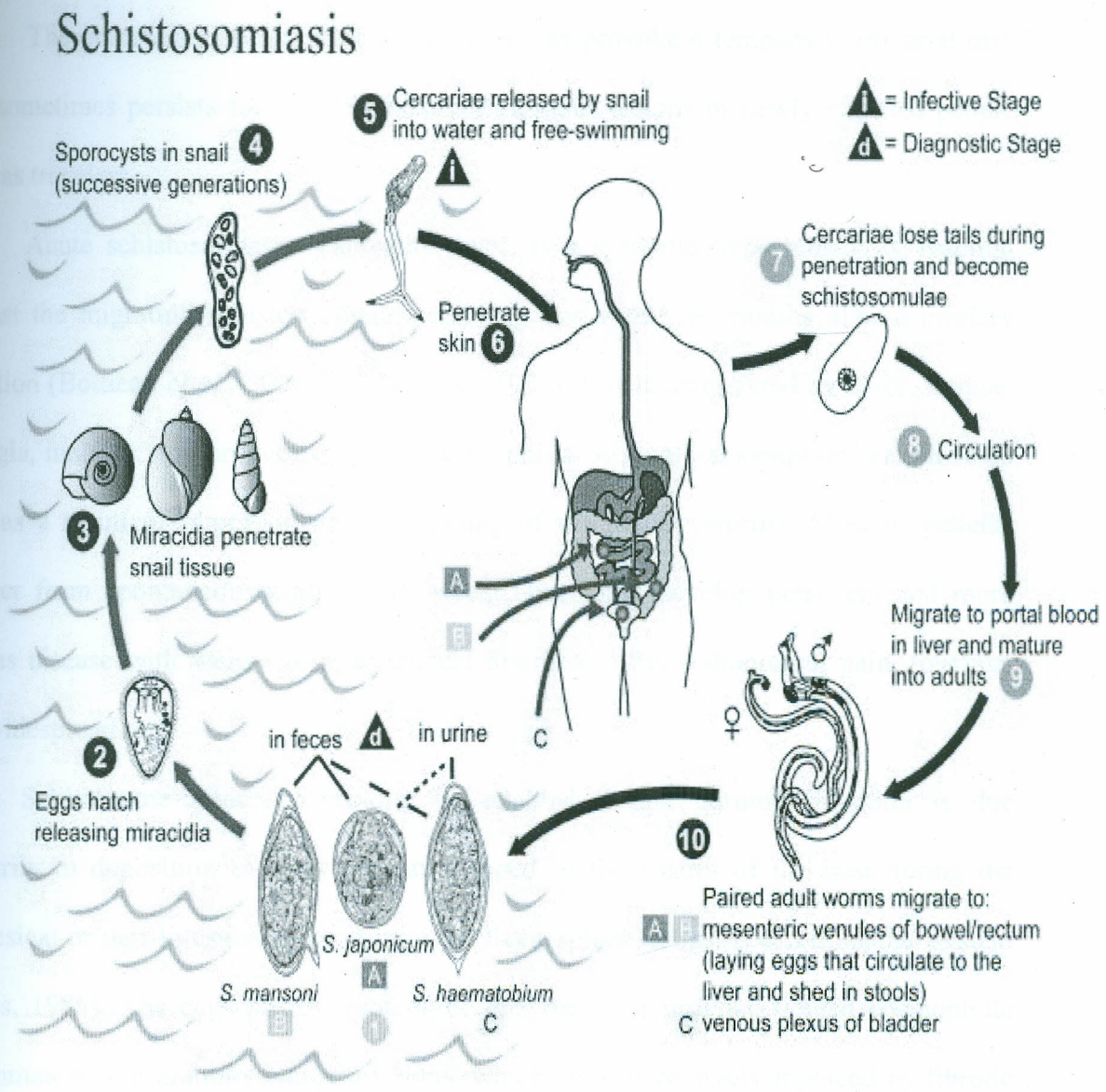

Figure 1. Schistosomiasis life cycle, from the DPDx website of CDC's Division of Parasitic Diseases: http://www.dpd.cdc.gov/dpdx

2.2 Pathogenesis of Schistosomiasis

The percutanous penetration of cercariae can provoke a temporary urticarial rash that sometimes persists for days as papulopruriginous lesions in newly exposed people such as travelers.

Acute schistosomiasis (katayama fever) is a systemic hypersensitivity reaction against the migrating schistosomulae, occurring few weeks to months after a primary infection (Bottieau *et al.,* 2006; Lambertucci, 1993). It is characterized by fever, fatigue, myalgia, malaise, non productive cough eosinophilia. Abdominal symptoms can develop later as a result of migration and positioning of the mature worms. Most of patients recover from spontaneously after 2-10 weeks, but some develop persistent and more serious disease with weight loss, dyspnoea, diarrhea, diffuse abdominal pain, toxemia, and widespread rash.

Schistosome-induced morbidity in established and chronic infection is due primarily to deposition of eggs that are trapped in the tissues of the host during the perivesical or peri-intestinal migration in the liver, spleen, lungs or cerebrospinal system (Boros, 1989). The eggs secrete proteolytic enzymes that provoke typical eosinophilic inflammatory and granulomatous reactions, which are progressively replaced by fibrotic deposits (Cheever *et al.,* 2000). In intestinal schistosomiasis, eggs stimulate development of intestinal polyps and ulcerations. Eggs lodged in the pre-sinusoidal capillary venules of the liver induce granulomatous inflammation and, with time, symmer' clay pipe-stem fibrosis (Boros, 1989). The consequences of chronic infection are development of hepatomegaly, portal hypertension, splenomegaly, ascites and esophageal varices. Rupture of varices leads to gastrointestinal bleeding with, occasionally, a fatal outcome

(King *et al.,* 2005). The severity of the symptoms is thus related to both intensity of infection and to individual immune responses.

2.3 Antibody Dependent Cell Mediated Immunity

There are a number of studies that relate to human immune responses and resistance to infection with *Schistosoma mansoni* and *Schistosoma haematobium,* which have describe possible roles played by antibodies in schistosomiasis infection (Butterworth *et al.,* 1985 and 1987). Accumulating evidence has shown that antibody dependent, cell mediated cytotoxicity (ADCC) plays a critical role in the mechanisms of defense against schistosomes (Capron *et al.,* 1982; Butterworth *et al.,* 1982). ADCC reactions on schistosomula were initially described experimentally with IgG antibody and neutrophils in rat (Dean *et al.,* 1974) and later, with eosinophils in baboon, rat and human beings (Butterworth *et al.,* 1977; Capron *et al.,* 1978). Eosinophils damage of schistosomula in the presence of both IgE and IgG antibodies has demonstrated its possible active role in protective immunity (Khalife *et al.,* 1989; Hagan *et al.,* 1991).

Evidence from earlier rat studies (Grzych *et al.,* 1984) which showed that IgM containing fractions failed to exhibit antiparasite killings in the presence of eosinophils led many investigators to recognize the possible roles of blocking antibodies in schistosomiasis infection. Investigators (Butterworth *et al.,* 1987; Butterworth *et al.,* 1988) also hypothesized that the presence of blocking antibodies elicited against carbohydrate epitopes expressed on the parasite egg surface could be reactive with epitopes present on the schistosomular surface, hence competing for the epitopes and thereby blocking the binding of protective antibodies. This has been a possible suggestion

to explain the continuous susceptibility of children as a group to reinfection even after treatment. Although children are capable of mounting both cellular and humoral responses against the parasite antigens, it is hypothesized that IgM and IgG2 antibodies elicited against carbohydrate epitopes cross react with epitopes present in schistosomula tegument thus competing for the epitopes and therefore blocking the binding of protective antibodies (Butterworth *et al.,* 1987; Dunne and Bickle, 1987). Khalife *et al.,* (1986) reported the blocking effects of IgM antibodies and indicated that susceptibility to reinfection after treatment in *S. mansoni* infection in man might be explained in part by the presence of schitosomulum specific IgM blocking antibodies. IgG4 interference with IgE mediated degranulation of mast cells has been reported with a suggestion that IgG4 responses block the effector IgE antibodies (Holfsetter *et al*., 1982; Hussain *et al*., 1992; Hagan *et al.,* 1991; Demeure *et al.,* 1993).

2.4 Heterogeneity in Anti-schistososme Isotype Responses Following Chemotherapy

A number of studies have shown that anti-helminth treatment of schistosome-infected people with praziquantel alters schistosome specific cellular (Colley et *al*., 1986; Ottensen *et al.,* 1978) and humoral immune responses (Grogan *et al.,* 1996; Naus *et al*., 1998; Mutapi *et al.,* 1998). This change is believed to be due to an increase in the amount of antigens to which the immune system is exposed by worm death (Mutapi, 2001) and a removal of immunosuppressive effects of adult worm (Ottensen *et al.,* 1978; Feldmeir *et al.,* 1988; Grogan *et al*., 1998). Some of these changes have been associated with resistance to infection/re-infection with schistosomiasis and protection against the development of severe forms of the disease (Correa-Oliveira *et al.,* 2000). Furthermore,

studies on *S. mansoni* infected/exposed adults have indicated that treatment can result in resistance to re-infection and that development of resistance to re-infection is independent of age (Karanja *et al.*, 2002).

Susceptibility to re-infection does not appear to be due to general failure to respond against the schistosomes, as all infected individuals are found to be hyper-responsive to parasite antigens (Butterworth *et al.*, 1988). However, the isotype composition of the antibody responses against the parasite does vary greatly between infected individuals. As different human antibody isotypes differ in their biological properties (Jefferis and Kumararatne, 1990), including their ability to mediate or block killings of schistosomes via a variety of immune effector mechanisms (Khalife *et al.*, 1986; Khalife *et al.*,. 1989; Dunne *et al.*, 1993), this heterogeneity in isotype responses may influence the outcome of infections in different individuals.

2.5 Humoral Immunity

A number of *in vitro* studies on the immunology of schistosomiasis have indicated the possible role played by antibodies as various effector or regulatory mechanisms (Butterworth *et al.*, 1977; Capron *et al.*, 1984; Khalife *et al.*, 1989). However, the underlying immuno- epidemiology of the humoral immune response against schistosomiasis is poorly understood. Within endemic areas, infected individuals of all ages have been shown to have high levels of circulating antibodies with anti-schistosome specificity and the class and sub-class composition of these antibodies have been shown to vary with age, sex and intensity of infection (Van Dam *et al.*, 1996; Mutapi *et al.*, 1997; Webster *et al.*, 1997; Naus *et al.*, 1999 and 2003). The class and sub-class

distribution of the antibody response in various individuals is considered critical because each isotype has specific biological functions to effect (Spiegelberg, 1989).

Many investigations have demonstrated associations between schistosome specific isotype responses and the infection intensity. For instance, potentially protective immunoglobulins E (IgE) and IgG1 antibodies responses have been associated with resistance against re-infection after chemotherapy (Hagan *et al.,* 1991; Dunne *et al.,* 1992; Zhang *et al.,* 1997; Satti *et al.,*1996; Khalife *et al.,*1989), whereas IgG2 (Butterworth *et al.,* 1988) and IgG4 (Hagan *et al.,* 1991) responses are thought to block the binding to the target antigens on schistosomula surface of protective responses, and possibly correlate with susceptibility to re-infection after chemotherapy. Moreover, protective immunity expression is thought to depend on favourable balance towards the production of IgE, rather than exclusively on the levels of IgE and IgG4, and high susceptibility especially in young individuals could be related to an IgE/IgG4 balance that favours IgG4 production (Hagan *et al.,* 1991; Dissein *et al.,* 1992; Demeure *et al.*, 1993). In addition, susceptibility to reinfection after chemotherapy has been associated with high levels of IgM antibodies (Dunne *et al.,* 1988; Khalife *et al.,* 1989). However, anti-schistosome IgG3 responses has been shown to be largely driven by chronic exposure to malaria, perhaps via cross-reactive antigens (Naus *et al.,* 2003) in areas endemic for both schistosomiasis and malaria.

2.6 Praziquantel Treatment

Adult worms are thought to possibly suppress schistosome specific immune responses so that the worm death following chemotherapy results in increased responsiveness to schistosome antigens (Ottensen *et al.,* 1978; Feldmeir *et al.,* 1998). In addition, drug treatment can damage the adult worm tegument as it kills the schistosomes (Andrews *et al.*, 1986), thereby possibly releasing previously un-exposed antigens and making them accessible to the immune systems (Fallon and Doenhoff, 1995). Further more, experimental work report in mice has shown that praziquantel chemotherapy results into tegumental damage and release of antigens including 28-kDa glutathione S-transferase (28GST), the leading vaccine candidate (Drupe *et al.,*1999), Sm23 antigens (antibody responses to Sm23 are associated with resistance to infection), tubercle glycoprotein, alkaline phosphatase and actin (Redman *et al.,*1996). Worm death, either naturally or due to drug treatment has been shown to induce changes in cellular and humoral immune responses against schistosome parasites, and previous studies have reported changes in the levels and types of antibody responses (Grogan *et al.,* 1996; Gryzch *et al.*, 1993) and cytokine responses following chemotherapy (Feldmeier *et al.,* 1988; Roberts *et al.,* 1993).

Praziquantel, an acylated quinoline-prazine that is active against all schistosome species, is now the most widely used drug for treating this infection, and forms a critical part in community-based schistosomiasis control programs. Since its discovery in mid 1970s (Thomas and Gonnert, 1977), its safety and efficacy has ensured its widespread use. It is mostly marketed as 600mg tablets with a recommended standard regimen of 40mg/kg body weight in a single dose (WHO, 2002). It is well absorbed but undergoes

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extensive first pass hepatic clearance. The drug acts within one hour of ingestion by paralyzing the worm and damaging the tegument hence, causing the worm to detach from the wall of the vain and die. Side effects are mild and occasionally include nausea, vomiting, malaise and abdominal pain. In heavy infections, acute colic with bloody diarrhea can occur shortly after treatment, probably provoked by massive worm shifts and antigen release (Stelma *et al.,* 1995). The drug has a low toxicity in animals and no important long term safety difficulties have been documented in people (Dayan, 2003). It is judged safe for treatment of young children and pregnant women (WHO, 2002).

Praziquantel drug however, has been shown to have little or no effect on eggs and immature worms. Tissue dwelling eggs can be excreted for several weeks after treatment and during the same period pre-patent or newly acquired infections can become productive because praziquantel is only effective against mature worm. The preferred timing of follow up is therefore 4-6 weeks after treatment (Rengmathan and Cioli, 1998). After a single dose of 40mg/kg, 70-100% of patients cease to excrete eggs. In most of those not cured, egg counts and antigen concentrations are nevertheless greatly reduced in number by 95% (Stelma *et al.*, 1995; Davis, 1993; Utzinger *et al.*, 2000). The efficacy of praziquantel has been shown to be unaltered in patients who are coinfected with HIV type 1 (Karanja *et al.,* 2002), and evidence has also shown that the S. *mansoni* infection treatment with praziquantel does not influence the viral load of HIV type 1 (Lawn *et al.,* 2000). The possibilities of resistance to praziquantel, the drug of choice for treating schistosomiasis continues to exist, but has not been reported, even in the face of heavy use for over 20 years.

2.7 Vaccination Against Schistosomiasis

Due to the possibility of drug resistance, there is clear need for a safe, affordable and effective schistosomiasis vaccine. This is because it is hoped that morbidity due to schistosomiasis might be suspended permanently even with continued transmission if chemotherapy is followed with vaccination (Berquist, 1995), should an effective vaccine be developed. Therefore, with a view of developing a vaccine against schistosomiasis, considerable effort has been devoted to the identification of relevant schistosome antigens that may be involved in inducing protective immune responses. There is a possibility for developing a recombinant protein, synthetic peptide, or DNA vaccine (McManus, 1999) with the advancements in recombinant technology. There is evidence of protection against *S .mansoni* infection attainment in mice and a similar high level of level of protection against *S. japonicum* infection in mice, pigs and buffaloes after immunizing these animals with irradiated cercariae (McManus, 1999). There is evidence that both type 1 and type 2 helper-T-cell responses may contribute to protection (McManus, 1999). Several antigens have also been judged to be potential vaccine candidates and have been tested in animals with varying results (Berquist and Colley, 1998; Hewitson *et al.*, 2005).

The recombinant rShGST-28 (Bilhvax; Eurogentec, Herstal, Belgium) has already undergone phase I and II clinical trials involving human volunteers and has been judged safe and demonstrated excellent immunogenicity (Capron *et al.*, 2005). Other promising vaccine candidate antigens under various stages of experimental trials include:

a.) Triose Phosphate Isomerase (TPI, MAP-4) a 28 kDa antigen from all stages of the parasite,

b.) Paramyosin (Sm97), a myofibrillar muscle protein expressed in schistosomula and adult worms,

c.) Fatty acid binding protein (Sm14),

d.) Internal membrane protein (Sm23),

e.) Irradiated cercarial antigen and irradiation-associated vaccine antigen like IrV-5, which is similar to vertebrate muscle protein (myosin heavy chain) expressed in all stages of the schistosome life cycle.

Descriptions of how each of these antigens was first identified have been reviewed (Dunne and Mountford, 2001). Recently, studies in water buffaloes have shown that the protection accorded by the *S. japonicum* antigens paramyosin (Sj-97) and GST-26 (Sj-GST26) have yielded good results, and it may be feasible to develop a vaccine that block transmission for use in reservoir hosts (Ross *et al.,* 2001). There is a considerable optimism about possible future vaccine development (Hagan *et al.,* 2000) given the breadth of the multinational efforts to generate antischistosome vaccines.

2.8 Immunity to Schistosomiasis

A vital component of vaccine development is understanding wich immune responses are responsible for conferring protective immunity to reinfection. Debates in terms of whether protective mechanisms observed in experimental hosts are comparable to those observed in human populations with a view to define the mechanisms of protective immunity to schistosomiasis has been on for long (Gryseels, 2000; Druille *et al.,* 2002; James and Colley, 2001). Epidemiological and clinical evidence show that people living in endemic areas acquire some form of immune resistance after years of

exposure (Butterworth, 1993). In terms of population dynamics, host related factors such as innate or acquired immunity are likely to have an important role in truncating the enormous reproduction potential of schistosomes to the endemic equilibrium of one (Gryseels,1996). Previous studies indicated that the intensity of infection peaks among older children (adolescents) and declines to lower levels in adults (King, 2001), even in situations when adults have greater exposure to infection than the children in endemic areas (Butterworth *et al.*, 1999). This pattern has been used to classify older individuals as being partially resistant and the changes that occur in the immune response as adolescents become older are considered to be important in immunity to schistosomiasis (Hagan *et al.,* 1991; Grogan *et al.,* 1996). However, the decrease in infection rates after adolescence can also be explained by reduced water contact, and this makes it difficult to prove the acquisition of effective

Nonetheless, the changes which occur in the immune response of adolescents as they become older are considered very important in immunity to schistosomiasis (Hagan *et al.*, 1991; Grogan *et al.,* 1996). A previous study has shown that the transition from a child like to an adult like antibody repertoire occurs at younger age in populations subjected to higher levels of transmission (Mutapi *et al.,* 1997). Studies have indicated that populations with recent exposure to transmission have a strikingly similar age related infection pattern to those in long standing endemic conditions. Since slowly acquired immunity cannot be invoked in such circumstances, it is postulated that some form of age related innate resistance could also play an important part in epidemiology of schistosomiasis (Gryseels, 1994; Naus *et al.,* 1998; Kabatereine *et al.,* 1999). Findings in both human and animals suggest that acquired immunity is mediated by IgE against

larvae and adult worms' antigens which trigger eosinophils release of cytokines such as IL-5 and IL-4 (Th2 cytokines) against schistosomulae (Butterworth, 1993).

The slow development of acquired immunity is thought to be due to potential blockage of the IgE receptors by excess antischistosome IgG4 and possibly other blocking isotypes in the first year of infection, with some evidences indicating that protective immunity to schistosome infection is associated with a skewed Th-2 immune response with high levels of worm specific IgE and eosinophilia (Dunne *et al.,* 1992; Hagan *et al.,* 1987; Hagan *et al.,* 1991; Hagan *et al.,* 1985; Demeure *et al.,* 1993; Rihert *et al.,* 1991).

Cellular immune responses induced by the parasite eggs in the host tissues have been shown to result in schistosomiasis related pathology (Cheever, 2000). Evidence show that granulomatous reactions around the eggs are orchestrated by CD4-positive T cells and involves eosinophils, monocytes and lymphocytes (Cheever, 2000). In mice, a predominantly T-helper-1 reaction has been shown to occur in early stages of infection but shifts to an egg induced T-helper 2-biased profile later in the progression of the infection and evidence has it that an imbalance between these responses lead to severe lesions (Pearce, 2005), and it is thought that these similar mechanisms could be the basis of fibrotic pathology in human beings (Abath *et al.,* 2006).

3.4 Inclusion and Exclusion Criteria

Study participants were included in the study if: a) they were 18 and above years of age and willing to participate in the study, b) they were residents of the study area, and c) they were occupational car-washers or sand harvesters with documented exposure to Lake Victoria waters.

Participants were excluded from the study if: a) they were unwilling to participate in the study and under 18 years of age, b) they were not residents of the study area, and c) they were neither car-washers nor sand harvesters.

3.5 Sample Collection

About 30mls of peripheral venous blood was obtained by venipuncture into heparin-coated vacutainer tubes (Becton Dickinson), by a qualified KEMRI phlebotomist. The blood samples were then put in a cooler box with ice packs and immediately transported to the laboratory within 2-3 hours of collection for processing. Three consecutive stool samples and a blood sample were taken upon enrollment and *Schistosoma mansoni* positive participants were treated using praziquantel. There was a routine collection of three consecutive stool samples from the participants after every four weeks (every month) for detection of infection/re-infection of *Schistosoma mansoni.* Four to six weeks after treatment was a preferred timing of stool follow up because praziquantel had been reported to have little or no effect on eggs and immature worms. It takes 4-6 weeks for immature worms to start producing eggs. Blood samples were collected after every six months for the whole 3 years period of the study. Treatments were given upon detection of *S. mansoni* eggs

and other helminthes ova in the stool every month, for the whole of the 3 years period of the study.

3.6 Parasitological Screening and Chemotherapy

Quantitative evaluation of *Schistosoma mansoni* eggs was determined by the modified Kato/Katz feacal thick smear technique on duplicate slides of each of the 3 feacal specimen collected on consecutive days from each participant, and the result expressed as mean eggs per gram of feaces (EPG) (Katz *et al.*, 1972). Using a wooden applicator stick, a small amount of the feacal material in a stool cup was placed on the newspaper and a piece of nylon screen (80 mesh) pressed on top so that some of the feacal material sieved through. The sieved feaces was collected by scrapping the flat-sided spatula across the upper surface then transferred to a template with a hole (pre-measured to hold 41.7) placed on the center of the microscope slide until the hole completely got filled. The template was then passed over using the side of the spatula to remove excess feaces from the edge of the hole then carefully removed to leave a cylinder of feaces on the slide.

The feacal material was then coved with cellophane strip pre-soaked with 3% malachite green, 50% glycerol and 50% water, and the microscope slide inverted so that the feacal material pressed firmly against the hydrophilic cellophane strip on a smooth hard surface to allow the feacal material to spread evenly between the slide and the strip. The slide was then carefully pushed sideways to avoid separating the cellophane strip and the slide with the feaces placed on the bench facing upwards for water to evaporate and glycerol to clear. The smear was then read immediately for

hookworm eggs as the egg clears first, then examined in a systematic way for *S. mansoni* infection and the scored number of eggs reported multiplied by 24 to obtain the total number of eggs per gram (EPG) which is an estimation of the worm burden (WHO,1993) (see Appendix III).

From the same stool examinations, other soil transmitted helminthes present were also noted. Schistosomiasis infected participants were offered treatment with the recommended dose of praziquantel (40mg/kg of body weight) and participants coinfected with other helminthes were treated with albendazole as required. In case malaria parasites were detected in the blood samples, the participants were treated with coatem™.

3.7 Laboratory Procedures

3.7.1 Plasma Separation

All blood processing procedures were carried out in a sterile Biological safety cabinet (NuAire, Inc.Minnesota, U.S.A.). Room temperature Ficoll/Hypague (F/H) solution (Pharmacia, Uppsala, Sweden) was placed into 50ml centrifuge tubes (Becton Dickenson and Co., U.S.A). Ten ml pipettes were used to draw heparanised blood from vacutainer which was then gently layed over the F/H for isolation by density gradient centrifugation (Bayum, 1968).

Tubes containing the F/H-blood were centrifuged at 1700 rpm for 35 minutes at room temperature without using brakes. Using a 5ml pipette the plasma layer from the top of the F/H gradient was collected and put in a well labeled 15ml centrifuge tubes (Becton Dickenson and Co., U.S.A). This was aliquoted into a 1.5ml cryotubes (Nunc A/S, Kamstrup, and Roskilde, Denmark) and stored in the freezer at -20°C to be used later for Antibody ELISA assays (see Appendix IV).

3.7.2 Antibody enzyme-linked immunosorbent assay (ELISA)

Enzyme Linked Immunosorbent Assay (ELISA) was performed using SWAP (soluble worm antigenic preparation) with 25ug/ml as antigens used to coat the surface of microtitre plate wells. For each antigenic preparation, anti-human isotype-specific antibodies conjugated to horse radish peroxidase was used to quantitate the levels of IgG1, IgG2, IgG3, IgG4, IgM and IgE antibodies present in the plasma of each individual that bound to the immobilized SWAP. The method used with some modifications, have been described previously (Lunde *et al.*, 1979). Briefly, Immunol II microtitre plates

(Dynatech Laboratories, Inc., St Louis, MO, USA) were coated with SWAP (100ul/well at 25ug/ml) in 0.5 M carbonate-bicarbonate buffer pH 9.6. The plates were then incubated at 4°C overnight, blocked for 60min (1hr) at 37°C with a non-fat dry milk (100mls 1PBS, add 5g non-fat dry milk and 300ul Tween 20, stirred until milk dissolved) in PBS containing 0.05% Tween 20 (PBS-Tween) and washed three times with PBS-Tween. Plasma at a dilution of 1:20 for IgE and IgG2, 1:100 for IgG1 and IgG3, 1:800 for IgG4 and IgM, was then added to each well at 100ul per well for each sample in duplicate. These dilutions were selected based on standard curves made from a plasma pool from schistosomiasis patients which was also used as the positive standard on each plate alongside a normal human serum pool. After an additional incubation overnight at 4°C, the plates were again washed three times with PBS-Tween and then 100ul/well of 1:400 of anti- IgE, 1:1000 of anti-IgG1, 1:500 of anti-IgG2, 1:500 of anti-IgG3, 1:1000 of anti-IgG4 and 1:1000 of anti-IgM dilution of biotin conjugated mouse anti-human conjugated monoclonal antibodies to human IgE, IgG1, IgG2, IgG3, IgG4 and IgM (Southern Biotechnology Associates, Inc., Birmingham, USA) was added to each respective wells followed by incubation for an additional 1 hour. Plates were again washed in PBS-Tween 20 and the substrate 2, 2'azino-bis-3-ethlyl-benzythiazolinesulphonicacid (ABTS) (Kikergaad and Perry Laboratories, Gaithersburg, MA, USA) added. After appropriate colour development, the reaction was stopped by adding 50ul/well of H_2O_4 and the O.Ds measured at 450nm using an automated ELISA reader (see Appendix V).

3.8 Ethical Considerations

Ethical approval for the study was obtained from Ethical Review Committee at Kenya Medical Research Institute. This study was part of a bigger study which was looking at the "Determinants of resistance in human schitosomiasis: longitudinal studies on the development of resistance" whose principal investigator was Dr.Diana Karanja who was also one my research supervisors. Informed consent was obtained from the participants by signing the consent forms (see Appendix I). All participants received adequate explanation of the study and their participation requested in the language they understood best which was either in Kiswahili, English or Dholuo. Recruitment was purely voluntary and participants were allowed to ask questions about their rights and the study as a whole before they were enrolled after providing written consent. The process of obtaining blood samples by venipucture exposes participants to a minimal risk of discomfort and a slight chance of bruise at the site of venipucture. To minimize risk of infection, qualified KEMRI trained phlebotomists carried out the process in a sterile manner.

Participants diagnosed for schistosomiasis and soil transmitted helminthes were treated using Praziquantel and Albendazole, respectively. Blood was also screened for malaria parasites and participants found to be parasitaemic were treated with Coartem™. Participants also benefited by access to frequent examination by the collaborating physician and prescription of drugs for other ailments other than those mentioned above. All the samples collected from the study participants were allocated unique study identification codes for purposes of sample tracking and identification for treatment. All information and medical records were confidential.

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3.9 Data Analysis

All data were analysed with GraphPad Prism 4.0 software (Graphpad Software, Inc., San Diego, A). The correlation between antibody levels and eggs per gram of feaces (EPG) was analysed using non parametric Spearman's correlation coefficient. Wilcoxon matched pair signed rank test was used for comparison of individual antibody isotypes responses against SWAP between pretreatment and first follow up (6 months post treatments) and pretreatment and last follow up bleed (3 years post treatments) in car washers and sand harvesters. The effects of treatments on antibody levels were determined by 1 way ANOVA on the antibody levels before treatment, first follow up (6 months post treatment and last follow up (3 years post treatments). Differences and correlations were considered significant at $P < 0.05$.

4.0 RESULTS

4.1 Demographics

Baseline characteristics of car washers and sand harvesters were shown in Table 1 below. Sand harvesters were older (mean age of 28.62 years) as compared to car washers (mean age of 19.6 years). Sand harvesters also reported working in the lake more years (mean number of years worked in the lake =11years) prior to study entry than car washers (mean number of years worked in the lake =2.4 years) prior to being enrolled in the study. Essentially all, (96%) sand harvesters reported being born in Usoma, the lakeside village where they harvest sand. Conversely, the car washers were mostly from the city of Kisumu or emigrants from other areas of Kenya, and only 24% reported being born in a village near Lake Victoria (see Appendix II).

Table 1. Baseline demographic characteristics of car washers and sand harvesters

	Car washers (CW) n=37	Sand harvesters (SH) n=58
Age in years [mean (range)]	19.6 (18.0-26.0)	28.62 (18.0-63.8)
Born in the lakeside village [n (%)]	9 (24)	56 (96)
Years worked in the lake [mean (range)]	2.4 (0-17)	11 (0.8-40)

4.2 Mean Egg Counts at Baseline, 6 Months and 3 Years Post Treatments.

The intensities of infections expressed in eggs per gram (EPG) and minimum- maximum egg counts for sand harvesters and car washers at baseline, first follow up (6 months post treatments) and last follow up (3 years post treatments) time periods were shown in Table 2 below. At pre-treatment, chronically exposed sand harvesters had a higher level of infection with a mean egg count of 856 eggs/g of feaces and a range of 0-7454 eggs/g and car washers had a mean egg count of 761 eggs/g and a range of 0-4786 eggs/g.

Table 2. Mean egg count (eggs/g)

Study group	Time points		
	Baseline (mean egg counts-EPG and minimum-maximum)	1st follow up (mean egg counts-EPG and minimum-maximum)	Last follow up (mean egg counts-EPG and minimum-maximum)
Sand harvesters (SH) (n=58)	856 (0-7455)	73 (0-1458)	34 (0-528)
Car washers (CW) (n=37)	761 (0-4786)	56 (0-1524)	9 (0-92)

4.3 Correlation of Specific Antibody Levels with Intensity of Infection at Baseline and 6 Months Post Treatments

Pre-treatment IgG4 levels against SWAP were positively significantly correlated with the intensity of infection (Spearman correlation test: $P<0.0001$) in both car washers (Figure 3) and sand harvesters (Figure 4). However, an inverse correlation between the intensity of infection and IgG2 response against SWAP was also observed at baseline in car washers (Spearman correlation test: $P=0.0138$) with an increase in the intensity of infection and a decrease in IgG2 response against SWAP (Figure 3). No significant correlations between other specific antibody responses against SWAP and the intensity of the infection were observed at first follow up, 6 months post treatments in car washers group (Table 3). A positive significant correlation between the intensity of infection and IgG1 response against SWAP was also observed in sand harvesters (Spearman correlation test: $P<0.0001$) at baseline (Figure 4). At first follow up bleed, 6 months later, a significant negative correlation was observed between IgG3 response against SWAP and the intensity of the infection in sand harvesters (Table3).

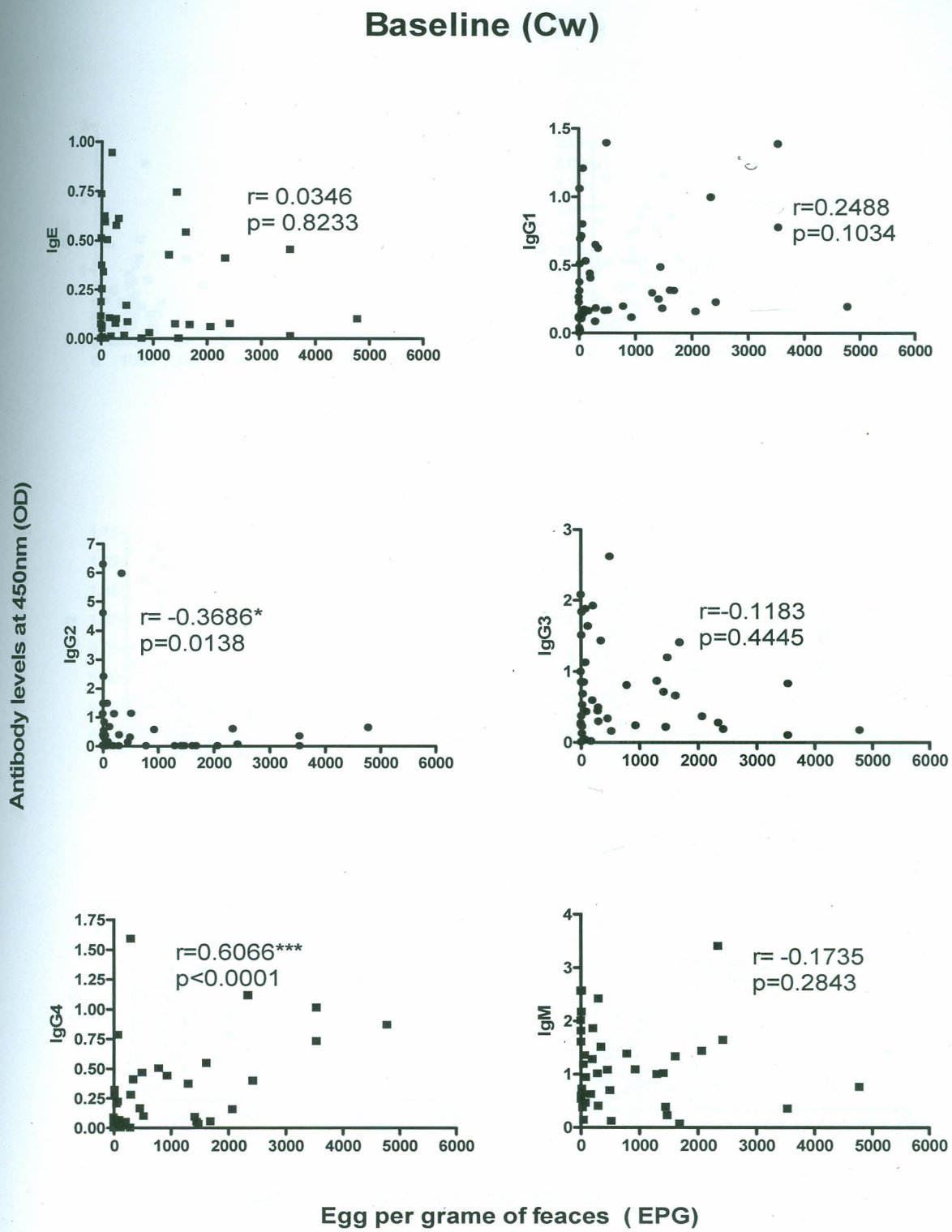

Figure 3. Correlations of specific antibody levels with the intensity of infection at baseline in car washers.

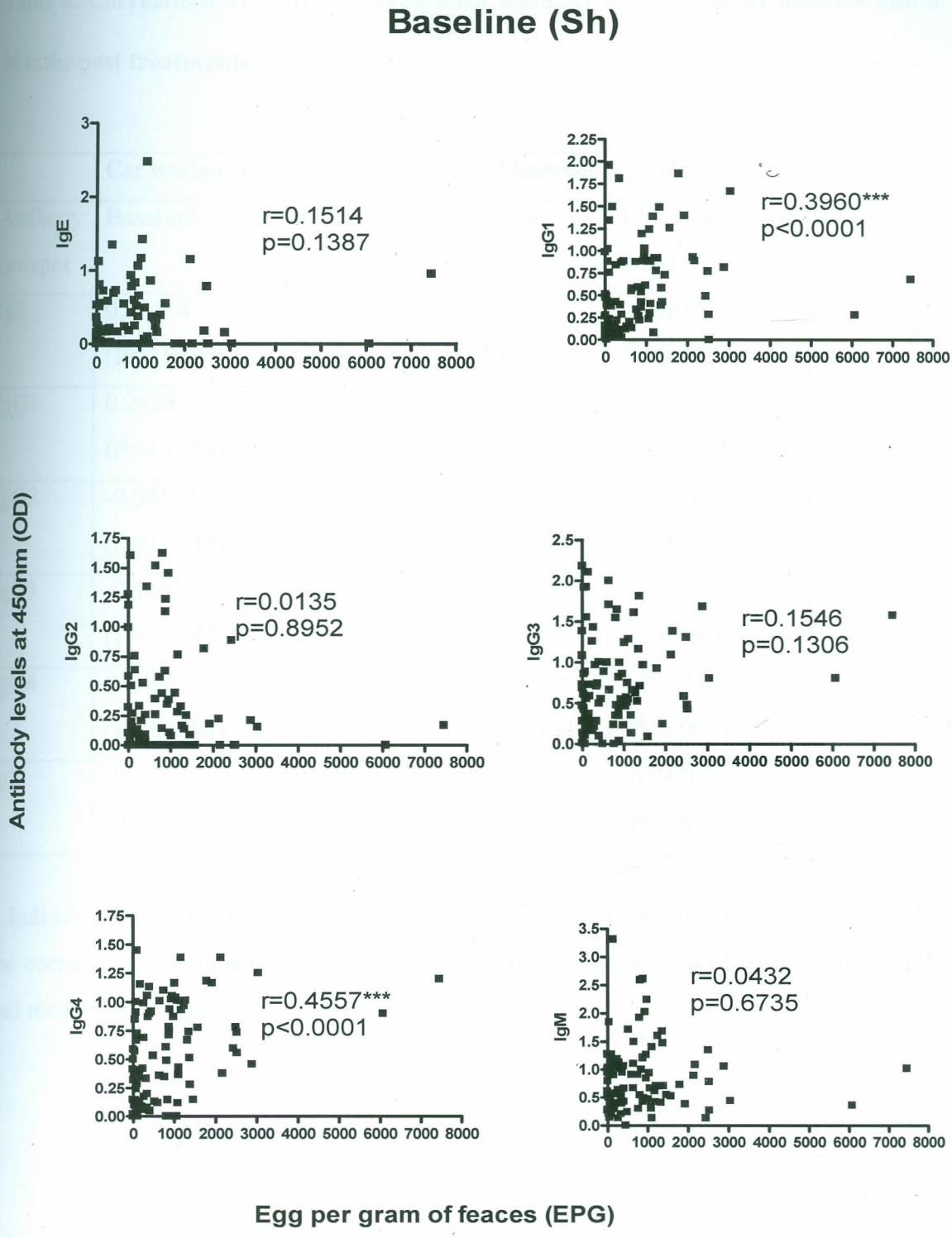

Figure 4. Correlations of specific antibody levels with the intensity of infection at baseline in sand harvesters.

Table 3. Correlation of antibody levels with intensity of infection at baseline and 6 Months post treatments.

	Car washers (CW)		Sand harvesters (SH)	
Antibody isotypes	Baseline	1st follow up bleed	Baseline	1st follow up bleed
IgE	0.03464 (P=0.8233)	0.08159 (P=0.6413)	0.1514 (P=0.1387)	0.03636 (P=0.8571)
IgG1	0.2488 (P=0.1034)	0.06964 (P=0.6910)	0.3960*** (P<0.0001)	-0.03162 (P=0.8756)
IgG2	-0.3686* (P=0.0138)	0.3307 (P=0.0523)	0.01354 (P=0.8952)	-0.1658 (P=0.4086)
IgG3	-0.1183 (P=0.4445)	-0.3225 (P=0.4445)	0.1546 (P=0.1306)	-0.4161* (P=0.0309)
IgG4	0.6066*** (P<0.0001)	0.1571 (P=0.3675)	0.4557*** (P<0.0001)	0.02341 (P=0.9077)
IgM	-0.1735 (P=0.2843)	0.04690 (P=0.7923)	0.04332 (P=0.6735)	-0.04207 (P=0.8350)

* Indicate probability values P< 0.05 and *** indicate probability values P<0.0001 for the correlation coefficient values (from Spearman correlation test) between time points and mean egg counts.

4.4 Comparisons of Pre- and Post-treatments of Specific Antibody levels

Figure 5 represents the differences in specific antibody responses against SWAP in car washers between baseline versus first follow up bleed 6 months post treatments, and Figure 6 represents baseline versus last follow up bleed 3 years post treatments following chemotherapy in the same group. In Figure 5, at 6 months post treatments in car washers IgG1 and IgG4 responses showed an increasing trend (Wilcoxon signed rank test: P=0.0435 and P=0.4560 respectively). However, in the same figure 5, IgE, IgG2, and IgG3 responses showed a decreasing trend (Wilcoxon signed rank test: P=0.7231, P=0.4458 and P= 0.0728 respectively). IgM response showed no significant change (Wilcoxon signed rank test: P= 0.8593). None of these responses showed a significant difference from pre treatment levels with the exception of IgG1 response in car washers (Wilcoxon signed rank test: P=0.0435). However, in Figure 6, pretreatment levels of IgE and IgG1 specific antibody responses against SWAP in comparison with the responses at last follow up in car washers showed a significant increase (Wilcoxon signed rank test: P=0.0101 and P=0.0480, respectively), but IgG3 and IgM responses showed a significant decrease (Wilcoxon signed rank test: P= 0.0091 and P= 0.0056, respectively).

Figure 7 shows the differences in specific antibody responses against SWAP in sand harvesters between baseline versus first follow up and figure 8 shows the difference between baseline versus last follow up following chemotherapy. At 6 months post treatments, IgG2 responses against SWAP increased significantly with a significant decrease in responses of IgG3 from pre-treatments levels (Wilcoxon signed rank test: P=0.0141 and P=0.0003, respectively). At the same time, no significant change was observed in other antibody responses levels from pre-treatment levels in sand harvesters

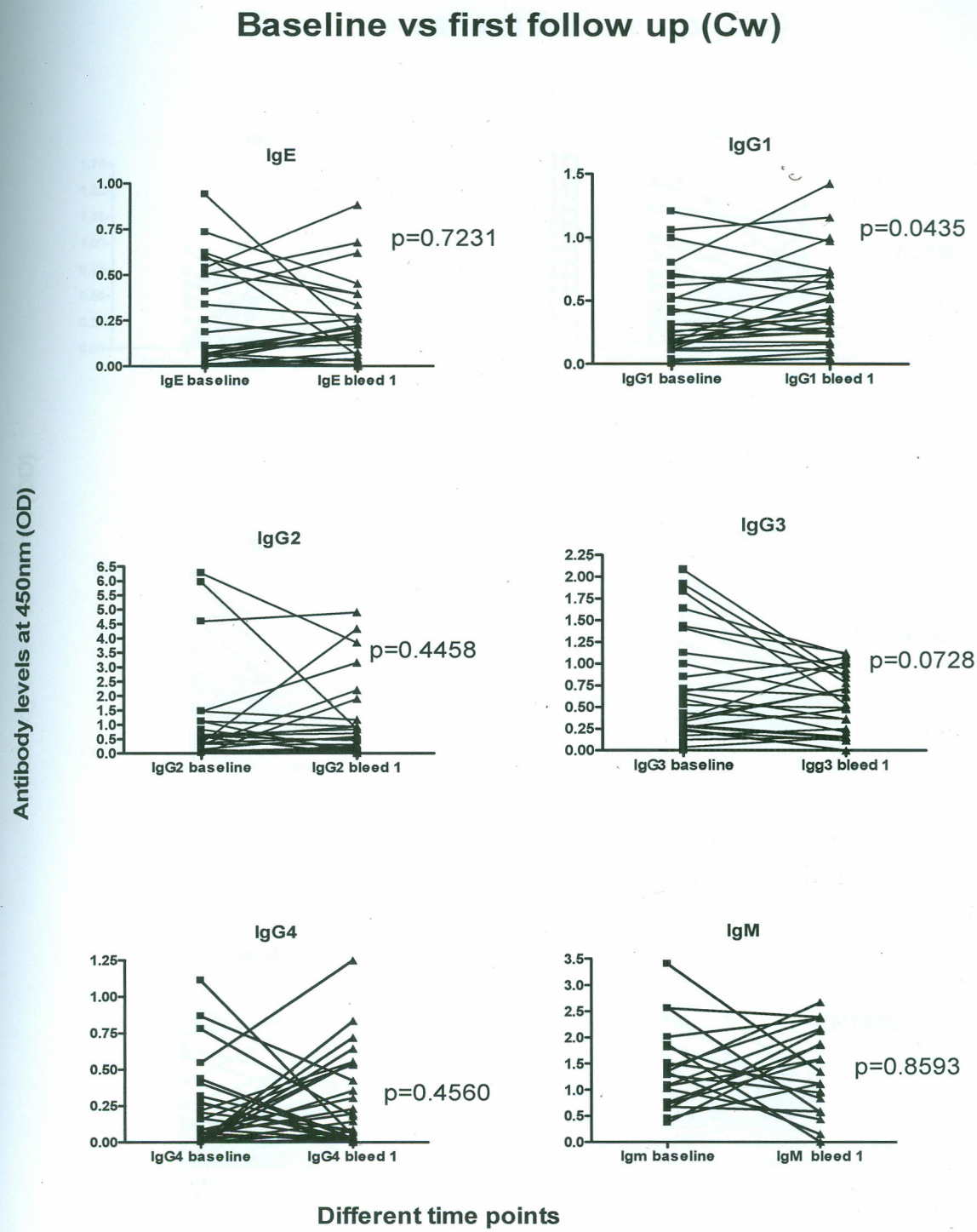

Figure 5. Comparisons of pre and first follow up post-treatments of specific antibody levels in car washers.

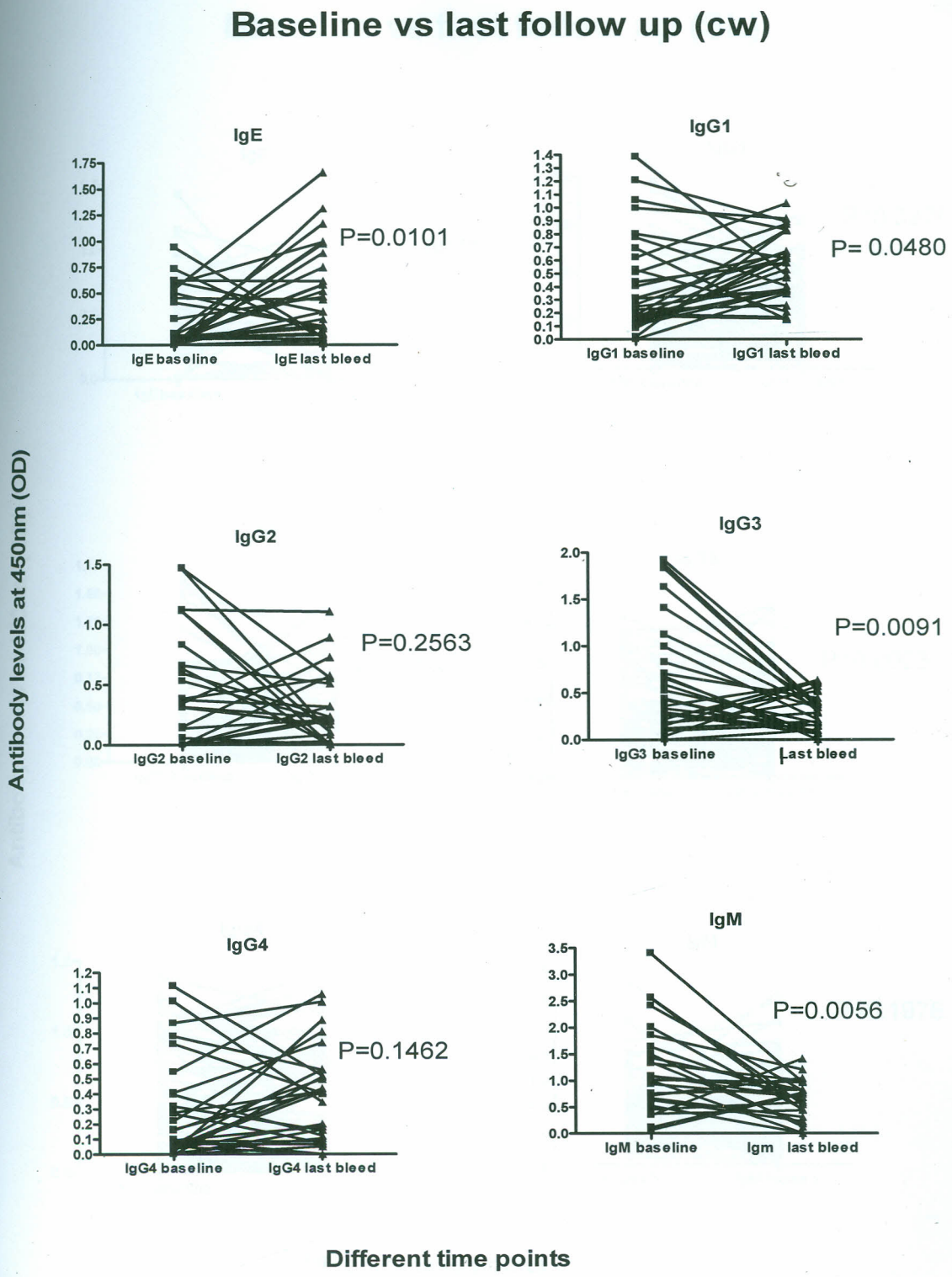

Figure 6. Comparisons of pre and last follow up post-treatments of specific antibody levels in car washers.

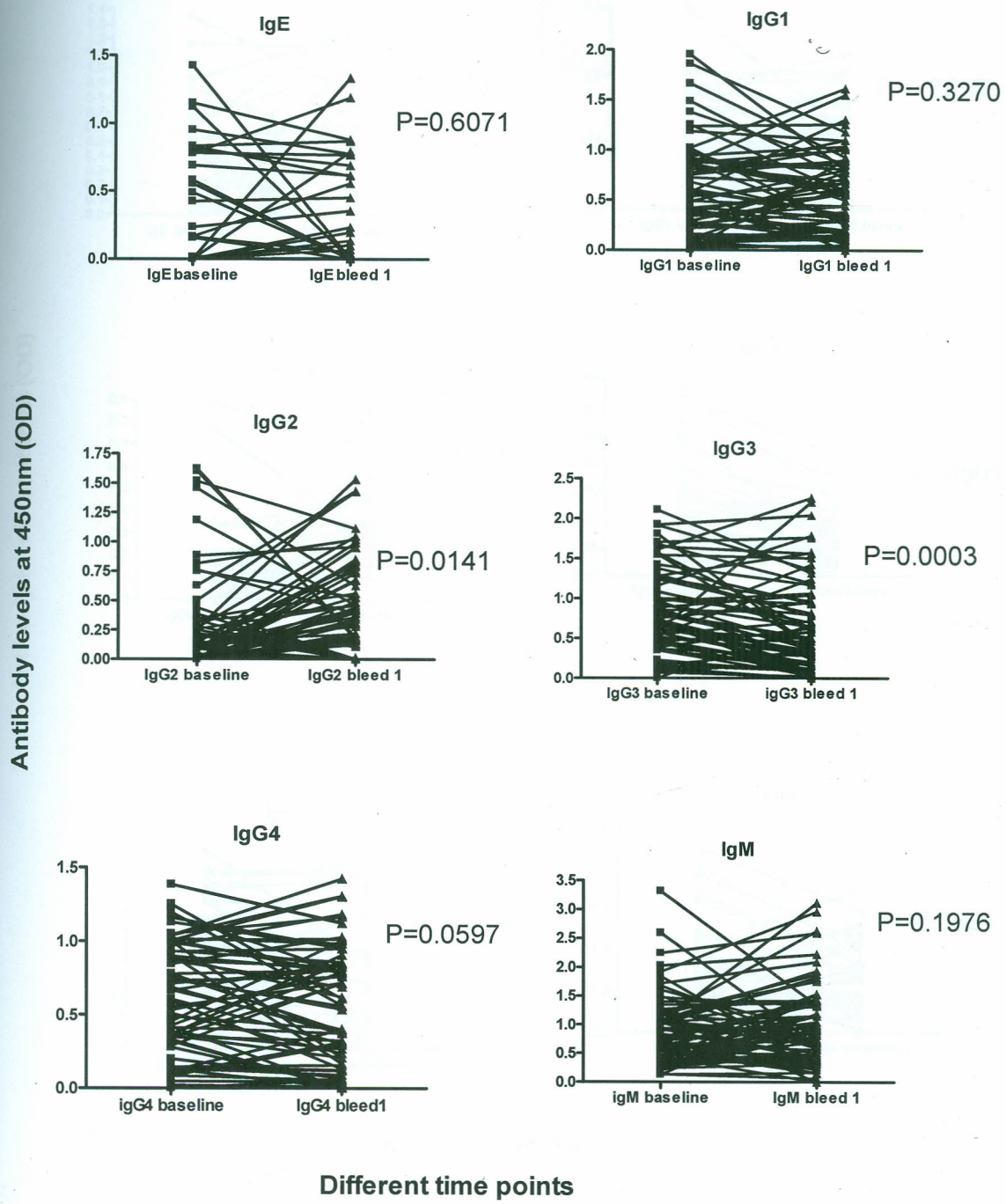

Figure 7. Comparisons of pre and first follow up post-treatments of specific antibody levels in sand harvesters.

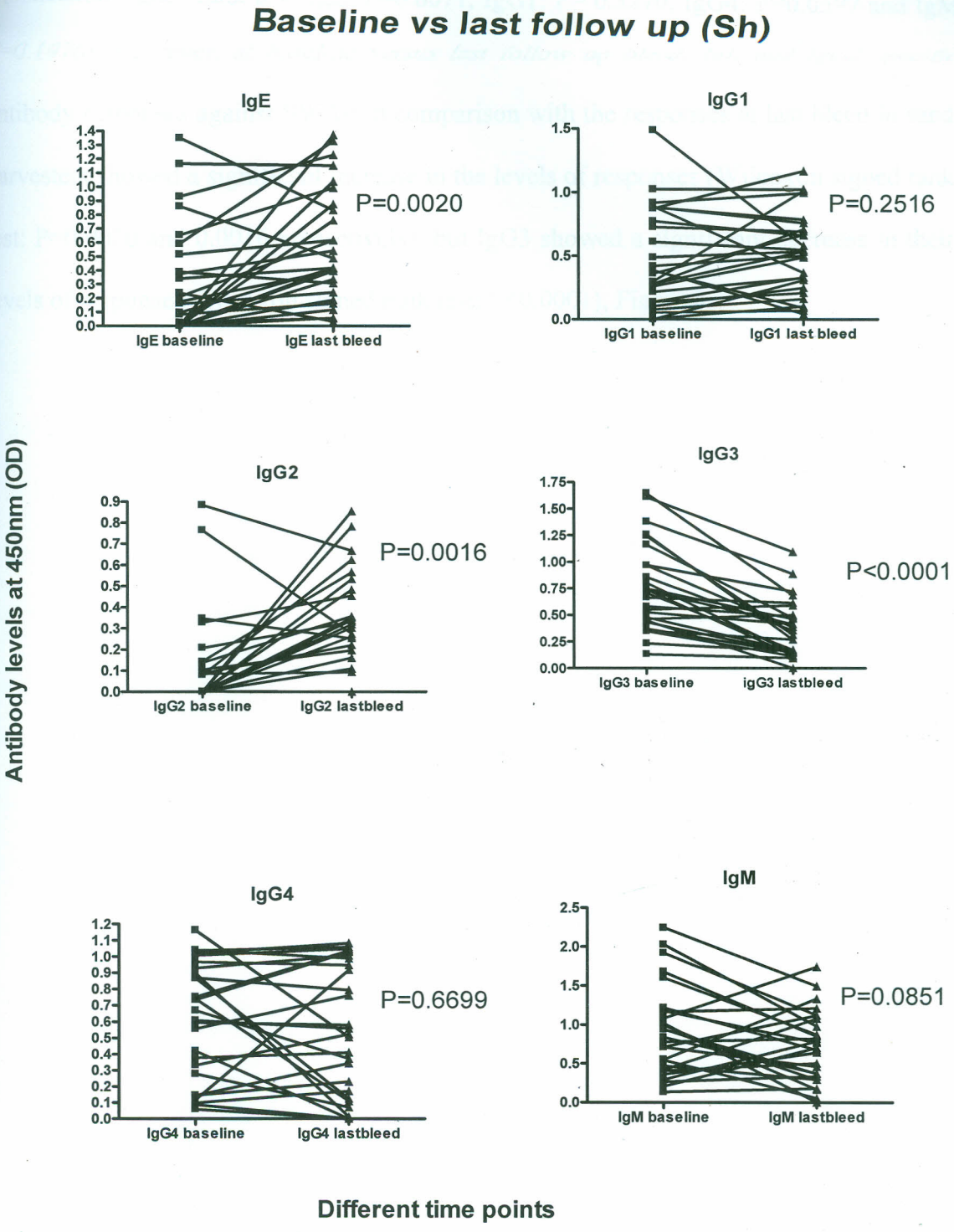

Figure 8. Comparisons of Pre- and post-treatments of Specific Antibody levels in sand harvesters (Sh).

(Wilcoxon signed rank test: IgE: P=0.6071, IgG1: P= 0.3270, IgG4: P=0.0597 and IgM: P=0.1976). However, at baseline versus last follow up bleed, IgE and IgG2 specific antibody responses against SWAP in comparison with the responses at last bleed in sand harvesters showed a significant increase in the levels of responses (Wilcoxon signed rank test: P=0.0020 and 0.0016 respectively), but IgG3 showed a significant decrease in their levels of response (Wilcoxon signed rank test: P<0.0001), Figure 8.

4.5 Effects of Treatments on Antibody Production

The effects of treatments on antibody levels were determined by one-way Analysis of Variance on the antibody levels before treatments, first follow up (6 months post treatments) and last follow up (3 years post treatments). At 6 months post treatments, there was an increasing trend, though not significant, in the mean antibody responses of IgE, IgG1, IgG2, IgG4 and IgM against SWAP with a non significant decrease in the levels of IgG3 in car washers as shown in Figure 9. At the same time, mean IgG1 and IgG4 levels showed no change but a significant mean increase in levels of IgE ($P<0.05$) and IgG2 ($P<0.05$) responses against SWAP in sand harvesters as shown in Figure 10. Mean antibody levels against SWAP 3 years post-treatments showed a varied responses pattern with IgE ($P<0.01$), IgG1 ($P<0.05$) and IgG4 ($P<0.05$) responses showing a significant increase while IgG3 ($P<0.01$) and IgM ($P<0.01$) levels remaining significantly lower than the pre treatment levels in car washers (Figure 9). In sand harvesters 3 years post-treatments. IgE and IgG2 responses showed significant mean increase ($P<0.01$) and $P<0.05$ respectively while IgG3 ($P<0.01$) levels declined significantly in comparison with the pre-treatments levels as shown in Figure 10.

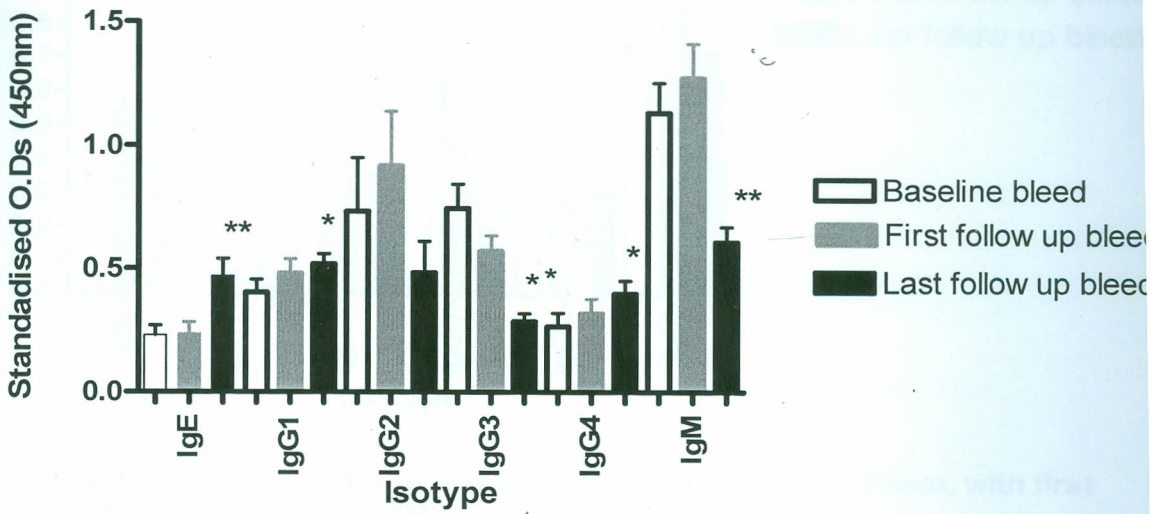

Figure 9. Baseline comparisons of antibody levels before treatment, with first ar last bleed follow up in carwashers.

Bars represent standard error of the mean. * represents P<0.05 and ** represents P<0.

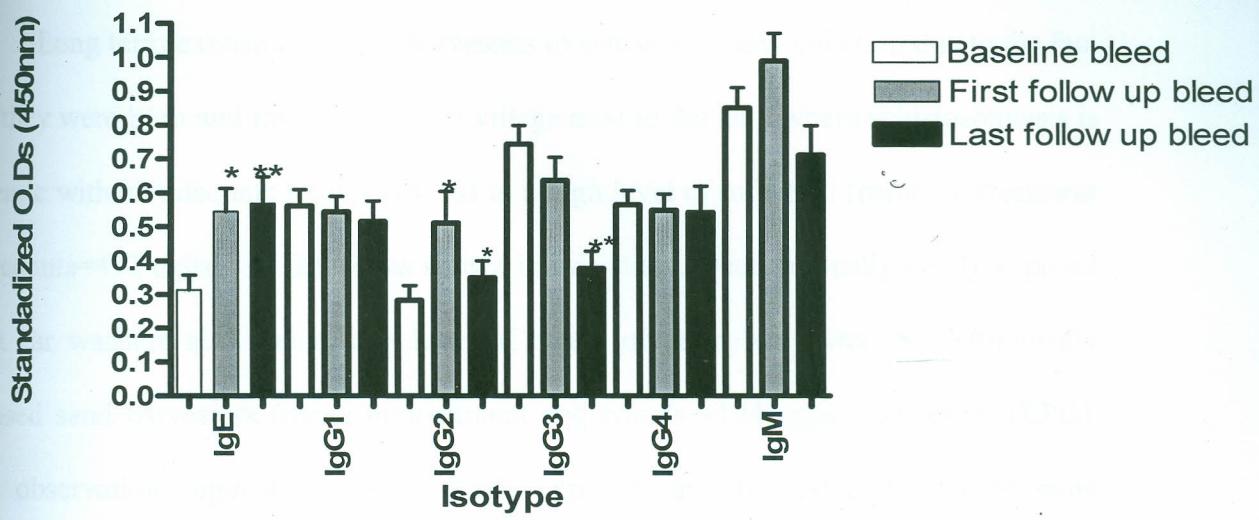

Figure 10. Baseline comparisons of antibody levels before treatment, with first follow up and at last follow up bleed in sandharvesters.
Bars represent standard error of the mean.* represents P<0.05, ** represents P<0.01.

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5.0 DISCUSSION

Long term exposure of sand harvesters to schistosomiasis infection due to the fact that they were born and raised in Usoma village next to the lake where schistosomiasis is endemic without adequate treatments, led to a high level of infection (mean pretreatment egg counts=492 eggs/g of feaces) as shown in this study. Occupationally newly exposed adult car washers also had a high level of mean infection but lower than chronically exposed sand harvesters (mean pretreatment egg counts =124 eggs/g of feaces (EPG). This observation suggests that chronically exposed sand harvesters harboured more worms than car washers as the number of *S. mansoni* worms present in an individual was positively correlated with the level of egg excretion in the feaces as also previously reported by Cheever (1986).

The results of this study demonstrated that the intensity of infection at pre-treatment, as measured by egg output significantly positively correlated with specific IgG4 responses against SWAP in both car washers and sand harvesters groups. This observation was in agreement with the previous studies in *S. mansoni* infection reports (Viana *et al.,* 1995; Hagan *et al.,* 1991) which suggested a possible blocking role of IgG4 interference with IgE mediated degranulation of mast cells (Holfsetter *et al.*, 1982; Hussain *et al.*, 1992). This observation provided an appropriate explanation of rare occurrences of signs and symptoms of immediate hypersensitivity in conditions of chronic schistosomiasis and constant intense cercarial challenge via the skin in individuals living in schistosomiasis endemic areas.

IgG1 responses against SWAP in sand harvesters too positively correlated with egg counts at baseline. This observation together with the IgG1 induction of a high

eosinophil mediated killing of schistosomula *in vitro* (Khalife *et al.*, 1989) suggests a protective role of IgG1 responses mounted by chronically exposed sand harvesters before treatments and supports observation of a link between resistance to reinfection in chronically infected canal cleaner who had a higher levels of IgG1 to WWH response than newly recruited canal cleaners (Satti *et al.*, 1996).

The intensity of infection at pre-treatment in car washers showed a significant inverse correlation with IgG2 response against SWAP with high egg counts being associated with low IgG2 response. This observation coupled with the correlation of specific IgG4 response with egg counts at baseline in car washers, made it difficult to suggest that IgG4 responses against SWAP at baseline suppressed blocking IgG2 responses or weather enhanced production of IgG4 had an overriding protective effect and represented the development of immunity in this naïve group of individuals who only got exposed to schistosomiasis at adult stage of life due to car washing occupation. At first follow up bleed 6 months later, a significant inverse correlation was observed between IgG3 response against SWAP and the intensity of the infection in sand harvesters. The immunological meaning of this observation is difficult to suggest because it could not be attributed to a decreased worm burden as the levels of IgG3 responses reduced with the increase in egg counts which positively correlated with worm burden.

Even though resistance was not looked at directly, the absence of clear protective variations in the antibody isotypes between baseline and first follow up until after multiple rounds of treatments and cures in the last bleed might be an indicator that these differences are due to development of resistance to infection and not just an indicator that antibody responses change after a single or a few treatments. Schistosome worms have a

long life span of between 3-7 years (Fulford *et al.,* 1995) and so it may take several years for the antigens to be released naturally in sufficient amount to evoke immune responses (Mutapi, 2001). This knowledge was important in this study because by considering the history of infection, sand harvesters group had been exposed to schistosomiasis infection from an early age due the fact that they were born and raised in Usoma village next to the lake where schistosomiasis is endemic as opposed to car washers who only got exposed to the infection in adult age due to their nature of occupation. As a result of longer experience of the infection, sand harvesters might have been sensitized by the experience of *in vivo* dying worms.

This study found a significant mean increase of IgE responses against SWAP in sand harvesters 6 months post treatments and a mean increase in car washers at the same time though not significant. In the last follow up after multiple rounds of treatments, there were significant mean increase in the levels of IgE response against SWAP in both car washers and sand harvesters. In human schistosomiasis, IgE elevated levels is usually associated with resistance to reinfection after treatment (Butterworth *et al.,* 1988; Dunne *et al.,* 1992), and this finding strongly supported the idea hypothesised by Woolhouse and Hagan (1999) that sufficient amount of worm antigens resulting from worm death repeated over many years could be the basis of slow development of adult protective immunity in human populations living in schistosomiasis endemic areas.

Another related study conducted in China where antibody responses of *S. japonicum* infected individuals living in endemic area were compared with infected individuals from a new focus demonstrated that in endemic area, worm -IgE and –IgG4 increased by 8wks post treatment and were positively correlated with the number of

previous treatments (Li *et al .,* 2000). Similarly, Caldas *et al.*, (2000) study conducted in Brazil found that individuals classified as resistant to *S. mansoni* infection had an elevated levels of IgE responses against SWAP post treatment, suggesting that worm IgE elevated by treatment was associated with development of resistance to reinfections. All these studies seem to suggest that multiple episodes of infections and worm death may be required to generate the elevated IgE responses against worm antigen which are associated with reinfection resistance. Furthermore, Karanja *et al.,* (2002) also reported the development of increased resistance to *S. mansoni* infection/reinfection after multiple episodes of treatments.

Following chemotherapy 6 months post treatments, there was a significant increase in the levels of IgG1 response against SWAP in car washers though in sand harvesters group the levels remained unchanged. This interesting observation was in accordance with the report where the newly recruited Sudanese canal cleaners exposed to *S. mansoni* infection had elevated levels to whole worm antigen (WWH) 3 months post treatment (Satti *et al.,* 1996). This observation could be interpreted as an acute acceleration of protective immunity development response of a naïve group, as car washers only got exposed to the infection at adult stage when they start washing cars in the lake as compared to sand harvesters who got exposed at an early ages. The levels increased significantly from the pre-treatment levels at last follow up 3 years post treatments in car washers but no variation in the levels of IgG1 responses in sand harvesters, though the levels were maintained high. The exact significance of this response in a naïve group remains to be determined, but the results reported in this study indicated that IgG1 responses against SWAP were affected by duration of exposure rather

than the intensity of the infection as car washers group had a mean pre-treatment intensity of infection of 124 EPG as opposed to 492 EPG in sand harvesters. The increasing trend following multiple episodes of chemotherapy in car washers in this study demonstrated the development of protective immunity because IgG1 has been shown to induce a high eosinophil mediated killing of schistosomula *in vitro* (Khalife *et al.,* 1989).

IgG2 responses against SWAP showed an increasing trend in both car washers and sand harvesters with a significant mean increase in the level in sand harvesters 6 months post treatments. The levels then decreased, even though not significantly in both groups in the last follow up bleed. It was a somewhat puzzling observation that 6 months post-treatments, IgG2 responses showed an increasing tendency with a significant increase in the levels in sand harvesters. This in particular was surprising because of distinct roles of IgG2 antibodies in immunity to infection having been reported as having a blocking effect on immunity to schistosomiasis (Butterworth *et al.,* 1988). This observation could be attributed to a similar epitope-recognition pattern with other isotypes upon release of worm antigens following treatments and this is supported by Naus *et al.,* (1998) report where a strong association between IgE to AWA and blocking antibodies such as IgG2 and IgG4 isotypes was reported before treatment and 1 year post treatments.

A decline in the mean levels of IgG2 response in car washers and sand harvesters in the last follow up could have been as a result of elimination of adult worms and mature eggs following multiple episodes of treatments, consequently removing the source of stimulus and also a possible indicator of the development of resistance to reinfection as a

result of the diminishing levels of the blocking antibodies after multiple episodes of treatments.

In this study, chemotherapy was followed by a decreasing tread of IgG3 responses from baseline through first to last follow up in both car washers and sand harvesters, with a significant decline in responses against SWAP in sand harvesters between pre-treatment and 6 months post treatments and between baseline and last bleed in car washers. IgG3, IgM and IgG2 productions have been associated with responses to polysaccharide egg antigens (Butterworth *et al.,* 1992), due to the fact that schistosome eggs only live for a few weeks in host tissues before dying and losing their structural integrity coupled with constant treatments which results into the elimination of adult worm and mature eggs thus absence of continued exposure to complete antigens required for maintaining the response, consequently leading to a steady decline in IgG3 response. Anti-schistosome IgG3 responses in Booma and other areas endemic for schistosomiasis and malaria has been shown to be largely driven by chronic exposure to malaria, perhaps via cross-reactive antigens (Naus *et al.,* 2003), and frequent treatment of malaria infection as was done in this study could also have led loss of cross-reactive antigens responsible for maintaining the isotype high levels in these individuals and thus steady decline in responses.

IgG4 responses against SWAP showed no change following chemotherapy in sand harvesters group during the study period. However, there was an increasing trend in car washers with significant increase in the mean level of the response in the last follow up bleed. This observation made it difficult to suggest that the increasing trend observed in the car washers was related to antigenic stimulation by the dead worms because sand

harvesters had the highest pre-treatment egg count as a result of longer exposure which translates to higher worm load as compared to car washers. This observation is in agreement with the Sudanese report (Satti *et al.,* 1996) where they found a significant increase in the level of IgG4 to WWH in newly recruited canal cleaners exposed *Schistosoma mansoni* infection after chemotherapy and no change in the levels of IgG4 in chronically infected individuals. This observation suggested the possibility of IgG4 antibodies contributing to immune complexes with IgE antibody in a way that potentiates or mediates schistosomula killing without anaphylactic consequences.

IgM responses against SWAP following chemotherapy showed an increasing trend in both car washers and sand harvesters groups 6 months post-treatments and a declining tendency in both groups during the last follow up with the car washers showing a significant mean decrease in IgM response. IgM antibodies have been shown to be predominantly produced in response to polysaccharide egg antigens (Butterworth *et al.,* 1992). This study observation suggests that praziquantel treatment resulted into disintegration of adult worms, and since adult female worms also contain egg antigens, the death of these worms following treatments resulted into the release of both adult worm and egg antigens and it is possible that disintegrating female worm egg specific antigens cross-reacted with SWAP thus elevating the IgM levels in both groups 6 months post- treatments.

Frequent multiple episodes of treatments in the last follow up led to elimination of worms before getting established and this resulted into the absence of appropriate stimulating antigens to maintain the IgM isotype levels thus a decline in IgM levels against SWAP in sand harvesters and a significant decline in car washers group. High

levels of IgM are known to be associated with susceptibility to reinfection in children (Butterworth *et al.,* 1988; Khalife *et al.,* 1986). IgM antibodies in the sera of infected children have been shown to compete for the same antigens expressed by both eggs and schistosomulum surface with the protective (ADCC-positive) IgG antibodies, thereby blocking the binding of protective antibodies. The declining trend in the levels of IgM antibodies after multiple treatments in the last follow up suggest the loss of a blocking response leading to the development of protective immunity in the infected individuals.

This study clearly demonstrated that at pre treatment, IgG4 responses against SWAP in both sand harvesters and car washers groups were significantly positively correlated with the intensity of infection irrespective of the length of time spent in the area of endemicity. It was also observed in this study that at pre-treatment, a significant positive correlation between the intensity of infection and potentially protective IgG1 responses against SWAP in chronically infected sand harvesters was attributed to longer experience of infection in sand harvesters having experienced more *in vivo* dying worms than car washers. Isotypic responses against SWAP also differed between car washers and sand harvesters, and in regard to a few or multiple treatments with praziquantel, car washers made more IgE against SWAP only after multiple treatments in the last follow up as opposed to sand harvesters who made more IgE after one or a few treatments in first follow up. Car washers also responded with more IgG1 after multiple treatments in the last follow up while sand harvesters maintained high levels of IgG1 regardless of treatments from baseline to last follow up.

The impact of frequent repeated treatments and reinfections on SWAP specific antibody responses in this study resulted into some changes which were associated with

the acceleration of development of protective mechanisms. These included significant elevations of potentially protective IgE responses in both groups at last follow up together with increasing trend of protective IgG1 in car washers and elevated levels in sand harvesters at last follow up also demonstrated immunity development. These findings after multiple episodes of treatments have further confirmed strongly the idea hypothesised by Woolhouse and Hagan (1999) that worm death repeated over many years could be the basis of slow development of adult protective immunity in human populations living in schistosomiasis endemic areas, and Karanja *et al.*, (2002) report which showed that the development of increased resistance to *S. mansoni* infection/reinfection occurs after multiple episodes of treatments.

The results of this study also demonstrated that following multiple episodes of treatments, the levels of IgE, IgG1 and IgG4 against SWAP showed an increasing tendency suggesting that they were stimulated by the antigens released from dying worms after chemotherapy, and that multiple praziquantel chemotherapy favoured the production of antibodies to peptide antigens. A steady decline in the levels of IgG3 responses against SWAP in both groups suggests that IgG3 do not usually stay elevated in the absence of stimulating antigens. These observations are in agreement with the idea that immunity to schistsomiasis could be attributable to not only the existence of antibodies with defined effector functions, but also to the absence of blocking antibodies.

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

In conclusion, the findings from this study led to suggestion that multiple episodes of treatments in *Schistosoma mansoni* infection could have a potential immunizing effects resulting into humural changes associated with the development of protective immunity to infection/reinfection in exposed individuals. Significantly high levels of antischistosome SWAP IgE levels in both car washers and sand harvesters groups following multiple episodes of treatments, strongly support the idea hypothesised by Woolhouse and Hagan (1999) that worm death repeated over many years could be the basis of slow development of adult protective immunity in human populations living in schistosomiasis endemic areas. These findings and similar ones reported by others which are associated with specific antibodies responses associated with development of protective immunity suggest that it is possible to define vaccine capabilities that have responses associated with resistance to reinfection, and this could involve the identification of the specific responses associated with the expression of immunity under conditions of natural infection and exposure.

6.2 Recommendations and Suggestions for Further Research

Further research into the precise roles of the blocking antibody isotypes in human schistosomiasis in relation to expression of immunity is also necessary. It would also be of interest in further studies to assess if both IgE and IgG4 target the same epitope by measuring isotype-specific antibody responses to individual purified antigens that together make up SWAP.

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