

Chapter one

Introduction and literature review

1.1 Introduction:

Hundreds of millions of people do not enjoy a healthy, productive life because they are debilitated and unable to achieve the full potential. Parasitic infections contribute significantly to this wide spread deprivation. This assemblage of heterogeneous infections has poverty as its common denominator. Soil transmitted helminthes and schistosomes are the most common infections worldwide. Two billion people are chronically infected with soil- transmitted helminthes and schistosomes. Many suffer from severe morbidity and others from more hidden manifestations of diseases. Almost, all cases occur in areas of poverty in low-income countries in the tropics and subtropics (WHO, 2005).

1.2 The schistosomes:

There are five main *Schistosoma* species that affect humans which are: *S.haematobium*, *S.mansoni*, *S.japonicum*, *S. mekongi* and (Chitsulo *et al.*,2000).

Of these five species, the first three are the most important. *S. haematobium* is the causative agent for urinary schistosomiasis . The other species cause intestinal schistosomiasis and affect the intestine, liver and spleen, and can be fatal (Saconato and Atallah, 2000).

1.3 Classification of schistosomes:

Schistosomes belong to phylum platyhelminthes which is characterized by flattened bodies and without body cavity. The members of the class trematoda have leaf like structure and all species have an alimentary canal. Members of the family schistosomatidae are unisexual and needs a snail intermediate host to complete their life

cycle. The parasite possesses two suckers, the oral and ventral suckers which lack any chitinous support. Each sex has a distinct structure. The male has variable length ranging from 6-22 mm. The body shape is flattened behind the ventral sucker and curves ventrally. The male is shorter and stouter than the female (Markell *et al*, 1999).

The detailed classification of schistosomes according to Schmidt and Roberts (1989) has been given as follows:

Phylum : Platyhelminthes

Class : Trematoda (Rudolphi, 1808)

Subclass: Digena (Van Benden, 1858)

Suborder: Prosostomata (Odhenv, 1905)

Family : Schistosomatidae (Poche, 1907)

Genus : *Schistosoma* (Weinland, 1818)

1.4 Transmission:

Human infection takes place by direct penetration of the cercaria through the skin to invade the circulatory system.

1.5. Life cycle:

The life cycle of schistosomes involves two free living but non feeding larval stages, the intra-molluscan larval stage within the snail and adult stage which reproduce sexually in human. The detailed life cycle of *S. haematobium* has been reviewed by Jordan and Webbe (1982) and Marquardt and Demaree (1986) as follows: the development of *Schistosoma spp* in humans is following a pattern that is similar for all three species i.e. *S. haematobium*, *S. japonicum* and *S. mansoni*. The male and female adults in the

definitive host move against blood flow into small venules until they can move no further. Egg laying begins and may continue for a decade or more. The eggs move towards the lumens of the organ, probably by a combination of muscular action of the organ and release of enzymes through pores in the egg shells. They are laid undeveloped, but have fully formed miracidia. Within several days, they reach the lumen of either the gut or the bladder, and pass to the outside with the faeces or the urine.

The eggs are ready to hatch immediately on reaching the outside and do mostly under the influence of lowered osmotic pressure; eggs hatch at 0.1% NaCl, but are almost completely inhibited from hatching at 0.6% NaCl. Eggs also hatch well at 28°C, but are inhibited from hatching at 4°C and 37°C, light also stimulates hatching. It is said that the miracidia crack the outer membrane of the eggs, but there is little information on the mechanism of hatching (Jordan and Webbe, 1982).

The miracidia that escape from the egg capsules swim in the water and actively seek snail of the genus *Bulinus* for *S.haematobium*. After penetration, the ciliated wall disappears and transforms into the first generation or mother sporocyst. The first generation sporocyst generally remains in a foot of the snail near the point of penetration. They are non-motile convoluted sacs less than 1mm long. Within 2-6 weeks, daughter or second generation sporocysts form in the central cavities of the mother sporocyst. In *S.mansoni*, 200-400 daughter sporocysts form in each mother sporocyst. At this time, the mother sporocyst degenerates releasing the daughter sporocysts, which migrate into digestive gland or ovulates of the snail. The daughter sporocyst is somewhat larger than the mother sporocyst. Cercariae formed in the central cavity of sporocyst leave through a pore at the posterior end. Cercariae may be produced as early as 20 days following infection, but usually 4-7 weeks and they continue to be produced for years. Production of cercariae depends on the size of the snail, the larger the snail the

gerater the production of the cecariae. Cecariae penetrate the host by means of the secretion from post and pre-acetaubular glands. Once the organism penetrates the skin, it loses its tail and is now considered schistosomules which are clearly different from cercariae in their ability to tolerate high smotic pressure, their ability to survive in serum and their appearance which is worm like. The schistosomules are carried by the circulatory system to the lung about a day after penetration. Then, they migrate to the liver. Studies on egg production on schistosomes have been done in both human and animal infections. The data from human infection are limited in many ways but they are consistent with animal studies. Egg production per female per day ranges from 22 to 3500. The highest figure was found in *S.japonicum* infections in hamsters (Marquardt and Demaree, 1986).

1.6 Epidemiology of schistosomiasis:

Human infection is almost exclusively derived from human sources, although monkeys and baboons in endemic areas have occasionally been found infected. In Tanzania, a triple of wild baboons is sustaining the life cycle without intervention (Fenwick,1969). National infection of non primate host was first reported in gerbil in Egypt (Kuntz, 1952). *Schistosoma mansoni* in wild rodents in Brazil and *Rattus rattus* in the Congo are not morphologiclly distinguishable from the form found in man, but in the Congo, there belong different variety rodentorum (Schwtez, 1954). Rodents at time become infected from ingestion of infected snails (Luttermoser, 1963). This disease is perpetuated by infected person who defected in our near water sources containig the appropriate intermediat host. Sewage from homes in endemic foci emptying into streams, also, lead to the propagation of the infection. Infected indiviuals migrating to previously uninfected area having susceptable snails initiate new centers of the disease. Extension of irrigation systems in endemic areas and the relocation of infected persons constitute grave hazards, making control programs more difficult.

The distribution of *Schistosoma haematobium* has been reviewed in world health organization questionnaire (Larotski and Davis, 1981). It is endemic in all countries in Africa, except Rwanda and Burundi. and occurs in islands of African mainland including Zanzibar and Madagascar. It also occurs in parts of the Middle East including Iran, Iraq, Lebanon, Saudi Arabia, Syria, Turkey and North and South Yemen and small focus exists in India. The distribution and population dynamics of the snail host and variation in mass contamination and contact with the water bodies where these snails are present.

The epidemiology of *Schistosoma haematobium* infection shares many features with that of the other species of schistosomes including the over dispersed distribution of intensity of infection, as measured by egg counts, and the patchy, focal distribution within an endemic area. Water resource development leads to the spread of schistosome infection, including *Schistosoma haematobium*. Its endemism has been changed by large scale civil engineering works like Aswan dam (Mobarak, 1982) or Volta lake (Scott *et al.*, 1982) and by the creation of water bodies as small as single borrow pit beside a road (Wilkins *et al.*, 1986). Environmental change may also lead to a decrease in prevalence which was observed during drought conditions in the Sahel (Pugh and Gilles, 1978). As in the other species, intensity and prevalence of *Schistosoma haematobium* infection are interrelated. Nevertheless, in a few studies, surprisingly low mean egg counts have been found, in association with significant levels of prevalence, perhaps, due to technical factors such as the time of specimen collection. However, the epidemiology of *Schistosoma haematobium* may differ from that of the other species in respect to relationship between age and the prevalence and intensity of infection. *Schistosoma haematobium* infection characteristically has peak intensity and prevalence of infection in the 5 to 16 years old age group after which there is a fall to much lower levels of intensity prevalence in middle age (El Almay and Cline, 1977).

The disease is increasing in distribution, as snails are carried from infected foci into new irrigation projects. Appropriate snails in previously uninfected areas, become infected from eggs discharged into the streams, pools, or canals by infected individuals (Beaver *et al.*, 1984).

Farmers, women washing clothes in the streams, and children bathing or wading in the water are all subject to exposure. In most endemic foci, children are more frequently and more heavily exposed and infected than adults. In endemic foci of certain countries, religious practices tend to increase pollution of the water and encourage to infection (Beaver *et. al* 1984).

1.7.Distribution of schistosomiasis in Sudan:

The first epidemiological survey by the Blue Nile Health Project (BNHP) in Aljazeera Irrigated Scheme (AIS), showed the overall prevalence rate of schistosomiasis to be 51 %, ranging between 30% and 70% (Blue Nile Health Project, 1989). Urinary schistosomiasis was reported in Darfur and Kordofan provinces. Wright (1973) estimated that, urinary schistosomiasis ranged between 9% in Darfur and 35% to 47% in Kordofan. These figures were confirmed by other studies (Eltom, 1976; Dafa Allah and Suleiman, 1988). In Khartoum state, the prevalence of both types of human schistosomiasis has been reported (Malek, 1985). However, a marked increase in their prevalence was reported after the extension in the irrigated areas around Khartoum (Hilali, 1992). In certain villages in Sharg Alneel, Sittelbanat (2006) reported an overall prevalence rate of 9.9% which reflects the impact of the extensions of irrigated areas around Khartoum North.

During 14-month study, Babiker *et al.*, (1985) collected 128 765 *Bulinus truncatus* snails from canals located near four villages in the northern part of the Gezira irrigated areas, and were examined for patent trematode infections, by exposure to light. In all, 903 shedded cercariae, of which 424 were identified as *S.haematobium*, and the highest

density of snails were found between March to May while the peak of snail infections occurred from June through August. One village, Bashagra, was the source of 80 of these *S. haematobium* infections. The residents of the four villages and any adjacent small camps were examined for infection with *S.mansoni* and or *S.haematobium* by collecting and examining stool and urine samples. The overall prevalences were 50% for *S.mansoni* and 20 for *S.haematobium*. Their observations at the human water contact sites suggested that the transmission of *S.haematobium* increased when the canals contained shallow stagnant water, when the temperature was above 26 , and when the site was frequented by small children particularly boys aged 5-15 years old.

Deganello *et al.*, (2007) conducted a survey of schistosomiasis among school children in 2 villages in Southern Sudan. In Lui (west Equatoria region), the prevalence of *Schistosoma mansoni* infection was 51.5% and no cases of *S.haematobium* infection were detected. In Nyal (upper Nile region), the prevalence of *S.haematobium* infection was 73%.

Bakhit *et al.*, (2008) studied the association, if any, between *S.heamatobium* infection and bacteria in Keryab village. The study revealed that the overall infection rate was 25% (78 out of 312 urine sample examined). The prevalence in females was higher than that of males (34 and 22% respectively). The highest infection rate (30%) was reported among the 11-20 years age group while no infection was found among the 21-30 years age group.

Mohammed (2009) studied schistosomiasis in Assalaya camps, White Nile state. He examined 447 basic school pupils. He found that the overall prevalence of *S. haematobium* was found to be 21 % with a mean of 50 eggs per 10 ml of urine and the prevalence of *S. mansoni* was found to be 19% with a mean of 100 eggs per gram of faeces. The prevalence was found to be higher in males than females and the most infected age group was 10-15 years.

Ahmed (2009) studied the prevalence of schistosomiasis in Ommahani village in White Nile state. In this study, stool samples were collected from 200 individuals (116 males and 84 females) and urine samples from 200 individuals (106 males and 94 females). The stool samples were examined using kato-katz technique while urine samples were examined using the sedimentation technique. The overall prevalence of *S. haematobium* and *S. mansoni* were found to be 10.5% and 0% respectively. For the positive cases of *S. haematobium*, the age group 6-16 years had the highest rate of prevalence (57.1 %).

1.8. Pathology and pathogenesis:

Eggs are passing through the bladder wall or intestinal wall cause hemorrhage. The pathogenesis of schistosomiasis is complicated and results from host immune reaction against deposited eggs. Adult worms induce limited pathological changes and are thus the major pathogenic agents. According to Markell *et al.*, (1999), localized reaction, perhaps mediated by cercariae provokes histamine release by mast cell and this may be seen in persons who had no previous exposure. In the previously infected immunocompetent host, schistosomulae are subjected to two kinds of immune responses; dependant cell mediated cytotoxicity and anti schistosomular antibody.

Anti schistosomular antibodies cover the parasite and the FC portion of the IgG antibodies attach to the FC receptor on the eosinophils, which are degranulated with release of eosinophilic major basic protein that results in damage on schistosomular membrane and possible death of the parasite. Macrophage may also contribute to the elimination of the parasite at this stage through the action of the specific IgE. Effectively, the release of lysosomal enzymes from this cell, after a few days within host, will lead to the schistosomes either-becomes covered with host antigens or produce antigens that trigger the production of antibodies mentioned above (Markell *et al.*, 1999).

Many infections are asymptomatic. Acute schistosomiasis (Katayama fever) may occur weeks after the initial infection, especially by *S. mansoni* and *S. japonicum*. Manifestations include fever, cough, abdominal pain, diarrhea, hepatosplenomegaly, and eosinophilia. Occasionally, central nervous system lesions occur: cerebral-granulomatous disease may be caused by ectopic *S.japonicum* eggs in the brain, and granulomatous lesions around ectopic eggs in the spinal cord from *S.mansoni* and *S. haematobium* infections may result in a transverse myelitis with flaccid paraplegia. Continuing infection may cause granulomatous reactions and fibrosis in the affected organs, which may result in manifestations that include: cystitis and ureteritis (*S.haematobium*) with haematuria, which can progress to bladder cancer; pulmonary hypertension (*S.mansoni*, *S.japonicum*, more rarely *S.haematobium*; glomerulonephritis; and central nervous system lesions (CDC , 2008).

1.9 Symptomatology:

According to Markell *et al.*, (1999), urinary schistosomiasis is seen with *S. haematobium* infection and the two stages (miracidia and cercaria) present in the common environment provide the essential links in the chain of infection. Light infection is usually asymptomatic. Dysuria and haematuria are early symptoms and signs (Markell *et al.*, 1999). Haematuria is as common among adult boys in the Nile river valley as to have been widely considered a phenomenon analogous in girls. Terminal haematuria is usually the first sign of infection. Eosinophilia is a constant finding in urinary schistosomiasis, but what is more common with *S.haematobium* infection is the visible blood in urine, i.e. haematuria (WHO, 2002).

1.10 Immunology of schistosomiasis:

There is longstanding epidemiological and clinical evidence that people living in endemic areas acquire some form of immune resistance after years of exposure (Butterworth, 1993). In terms of parasite population dynamics, host- related factors such as innate or acquired immunity are likely to have an important role in truncating the

encormous reproduction of shistosomes (Gryseels, 1996). The acquisition of effective immunity is difficult to prove, because the decrease in infection rates after adolescence can also be explained by reduced water contact. immunological advances, new epidemiological approaches, and mathematical modeling corroborate the existence of aquired immunity (Mountford, 2005). Compative studies of reinfection after curative treatment have shown that childern are far more susceptible than adults and that these differences cannot be explained by differing water- contact patterns. Observations in people and in animals suggest that acquired immunity is mediated by IgE against antigens of larvae and adult worms. which trigger eosinophils to release cytokines targeting schistosomulae (Butterworh, 1993). The slow development of acquired immunity is thought to be due to blockage of the IgE receptors by excess antischistosome IgG4 and possibly other immunoglobulin isotypes in the first year of infection. Most schistosomiasis- related pathology is induced by cellular immune responses. The granulomatous reactions around the eggs are orchestrated by CD4-positive T cells and involve eosinophils, monocytes, and lymphocytes (Cheever *et al*, 2000). In mice, a predominantly T helper-1 reaction in the early stages of infection shifts to an egg-induced T helper-2 biased profile, and imbalances between these responses lead to severe lesions (Pearce, 2005). Although these observations could be readily extrapolated, similar mechanisms could lead to the basis of fibrotic pathology in human beings. Much effort has been devoted to the development of vaccines againt schistosomiasis (Capron *et al.*, 2005).

1.11 Diagnosis of *S. haematobium*:

1.11.1. Direct diagnosis of the urine:

Diagnosis of infection with members of the genus *Schistosoma* is based on clinical findings and history of living in the endemic areas, serological testing and finding characteristic egg (Marquarat and Demaree, 1986). Method of finding eggs depends on the species and the stage of infection. If *S. haematobium* is suspected, a urine sample

should be taken and centrifuged. The sediment is examined microscopically for presence of the characteristic terminal spine egg. Occasionally, the hatched meracidia of *S. haematobium* in urine can be found if the urine is diluted or has been left to stand for few hours before being examined. Filtration technique to detect the egg of *S. haematobium* is recommended because it has been simple, more sensitive, rapid and reproducible for detecting and quantifying *S. haematobium* eggs in urine (WHO, 2002).

1.11.2. Serological tests:

The most common serological tests used are circumoval precipitation test' (COPT), indirect fluorescent antibody test (IFA), indirect haemagglutination test (IHA), enzyme linked immunosorbent assay (ELISA), and immunoblot technique. Acute and chronic schistosomiasis may now be differentiated by the level of immunoglobulin G and immunoglobulin M directed against keyhole limpet hemocyanin (KLH) and immunoglobulin A against soluble egg antigen (SEA). The sensitivity of salivary fluid to detect IgG antibody against SEA is comparable to serum and offers an alternative blood collection. Detecting of circulating anodic antigens (CAA) and circulating cathodic antigens (CCA) in serum and urine for diagnosis of active schistosomal infection appears promising as complimentary diagnosis method, with faecal and urine microscopy and antibody detection (WHO, 2002).

Van Gool *et al.*, (2002) studied the serodiagnosis of imported schistosomiasis by a combination of commercial indirect haem agglutination test with *Schistosoma mansoni* adult worm antigen (WA) and an enzyme-linked immunosorbent assay with *S. mansoni* egg antigens (SEA). A commercial indirect haemagglutination (IHA) test using erythrocytes coated with *Schistosoma mansoni* adult worm antigens (WA) and an enzyme-linked immunosorbent assay (ELISA) with *S. mansoni* egg antigens (SEA) were assessed for their use in serodiagnosis of important schistosomiasis. Thereafter, these tests were designated WA/IHA and SEA/ELISA respectively. The sensitivity of the tests was evaluated with sera from 75 patients with proven *S. mansoni* infection, 25

with proven *S. haematobium* infection, and 10 with clinical Katayama fever. The specificity was assessed with sera from 283 patients with various parasitic, bacterial, viral, and fungal infections and sera containing autoimmune antibodies. Sensitivities of the WA/IHA with cutoff titer of 1: 160 (WA/IHA160) in detecting *S. mansoni*, *S. haematobium*, *S. mansoni* and *S. haematobium* combined, and clinical Katayama fever were 88.00/0, 80.0%, 86.0% and 70.0%, respectively, with a specificity of 98.9%. The WA/IHA with a cutoff of 1:80 (WA/IHA 80) showed sensitivities of 94.7%, 92.00/0, 94.0% and 90.0% respectively, with a specificity of 94.70/0. The comparable values of SEA/ELISA were 93.30/0, 92.0%, 93.0% and 50.0% respectively, with a specificity of 98.20%. Combined use of ELISA and WA/IHA 80 gave sensitivities of 100% for *S. mansoni*, *S. haematobium*, and *S. mansoni* and *S. haematobium* combined and 90% for Katayama fever. The specificity of this combination in detecting schistosomiasis was 92.9%. Combination of SEA/ELISA with WA/IHA 160 gave sensitivities of 98.7%, 96%, 98 % and 80% with a specificity of 97.2%. Their findings suggested that WA/IHA and SEA/ELISA are each sensitive and specific serological tests that are easy to use for the diagnosis of imported schistosomiasis. The combined use of these two tests enabled the serological diagnosis of schistosomiasis to be achieved with very high degrees of both sensitivity and specificity (Van Gool *et al.*, 2002).

De Jonge *et al.*, (1989) studied the presence of the schistosome circulating anodic antigen (CAA) in urine of patients with *S.mansoni* or *S.haematobium* Infections. This genus specific antigen was demonstrated only in the serum of schistosomiasis patients. They studied urine of 80 patients with *Schistosoma mansoni* infections, 33 patients with *S. haematobium* infections, and 2 patients with mixed *S. haematobium* and *S.mansoni* infections. They were screened by a quantitative enzyme-linked immunosorbent assay (ELISA). CAA was demonstrated in 81 % of those with intestinal schistosomiasis and in 97% of those with urinary schistosomiasis.

Rationale

Schistosomiasis remains one of the increasingly significant health issues. Symptoms and signs of the infection are quite effecting both the health and social status of the individual especially children. On the other hand, the disease is basically associated with terminal haematouria, which may lead to anemia due to chronic blood loss. The seriousness of the complications of the disease such as cancer of bladder and secondary bacterial infection prompted thorough investigation of the disease especially in the endemic areas.

Diagnosis is usually performed using the routine wet preparation, which may lead to false negative results especially in mild infections. The use of more sensitive techniques becomes a necessity .

Objectives

General objectives:

To determine the prevalence rate of *S.haematobium* infection in Al-Lamab, Khartoum state and to evaluate the efficiency of different techniques used for the diagnosis of urinary schistosomiasis.

Specific objectives:

- To study the prevalence of *S.haematobium* infection in the area according to gender, age group and water contact.
- To compare the efficiency of urine centrifugation technique , filtration technique and ELISA.

Chapter two

Material and methods

2.1 Study design:

It is a cross sectional study.

2.2 Study area:

The study was conducted in Al-Lamab area in Khartoum state. Al-lamab is located far away from Khartoum center.

2.3 Study population:

The study was carried out on randomly selected person from the area.

2.4 Sample size:

270 persons (200 males and 70 females) living in Al-Lamab were examined for the presence of *S. haematobium* infection. The population was divided in to 4 age groups (≤ 9 , 10-15, 16-21, and ≥ 22 year).

2.5 Study period:

The study commenced in August 2013 and ended in December 2013.

2.6 Ethical consideration:

Approval was taken from the college of Medical Laboratory Science_ Sudan University of Science and Technology. A consent was taken from each individual before being enrolled in the study . Each patient was informed on the nature of the study.

2.7 Sample collection:

2.7.1 Urine sample:

A total of 270 urine samples were obtained from randomly selected people live in Al-Lamab. Labeled, sterile, wide mouthed, screw capped plastic containers were distributed to the selected patients with the instruction to deposit urine after physical exercise between the hours of 10.00 and 14.00.

2.7.2 Blood sample:

Based on the result of urine examination 3-5 ml of blood were taken from study population after been categorized into those who are proved positive (30) and those who are proved negative (30) for *Schistosoma heamatobium* . The blood was collected in sample tubes containing EDTA and transferred to laboratory in boxes filled with ice. The sample were centrifuged at 4000 RPM for 3minutes, the supernatant (plasma) was pippetted into vials and frozen at -20 C until analyzed.

2.8 Data collection

A questionnaire was designed to collect data on sex, age group, contact with water, haematouria and proteinuria (Appendix).

2.9 Methodology

2.9.1 Sedimentation technique (centrifugation):

Ten ml of urine sample was centrifuged at 2000 rpm for 5 minutes, and the sediment was then examined microscopically for each individual for the presence of *S.haematobium* eggs under the low power (10x) (Cheesbrough, 2000).

2.9.2 Filtration technique:

After gentle agitation, 10 ml of urine was drawn from each container and filtered through apolycarbonate membrane following the standard method of filtration (WHO, 1991). Each filter was placed on a clean glass slide and then examined under a light microscope using 40X objective. Any urine sample with ova of *S. haematobium* was recorded as positive.

2.9.3 ELISA technique:

Apolystyrene microtitre plates coated by SEA, 100 µl of the diluted (1:40) test samples were added to the wells and then incubated at room temperature for 10 minutes. After washing 3 times with diluted wash buffer, 2 drops of Enzyme Conjugate were added to each well. And was incubated at room temperature for 10 minutes. Then washing 3 times with wash buffer and 2 drops of Chromogen were added to every well. Incubated at room temperature for 5 minutes.

After that, 2 drops of stop solution were added. and the absorbance was read at 450 nm after Zero ELISA reader on air.

Absorbance reading greater or equal to 0.2 OD units recorded as positive case.

Sensitivity and specificity of the technique were calculated according to the following formula:

Method		Centrifugation technique	
ELISA Technique	Results	Positive	Negative
	Positive	A (True positive)	B (False positive)
	Negative	C (False negative)	D (True negative)

Sensitivity:

$$\frac{A}{A+B} \times 100$$

Specificity

$$\frac{D}{D+C} \times 100$$

2.10 Data analysis:

Statistical analysis was done using SPSS program using chi square test.

Chapter three

Results

The result revealed that out of 270 urine samples collected from Al-Lamab area in Khartoum state, 30 samples were found positive for *Schistosoma haematobium* using the two techniques (sedimentation and filtration). This constituted an overall prevalence rate of 11.1% (table 1, figure 1).

In this study, the population comprised 200 males and 70 females. The positive cases within males group was 28 (14 %) and 2 (2.8 %) were found positive in female group (table 2, figure 2). These rates were found to be statistically significant at $p = 0.01$

For age groups, the highest prevalence rate (16.4%) was reported among the 10-15 years age group, while the lowest prevalence rate (5%) was reported among the ≥ 22 years of age (table 3, figure 3)

29 (14.2%) of the positive cases had direct contact with water and only 1 (1.4%) had no contact with water (table 4, figure 4). The differences in rates between water contact and infection were statistically significant at $p = 0.02$.

The proteinuria and haematuria were found in 27 (90%) of the positive cases and 3 (10%) of them were without proteinuria and haematouria (table 5, figure 5). The differences in rates was highly significant at $p = 0.00$

Assuming the centrifugation technique as the gold standard, the sensitivity and specificity of the ELISA were 90.6% and 96.4% respectively (table 6).

Table (1): The prevalence of *S.haematobium* by using two different methods among the study population:

Method	Total examined	Positive cases	Percentage
Sedimentation technique	270	30	11.1%
Filtration technique	270	30	11.1%

Figure (1): Overall prevalence of *S.haematobium* :

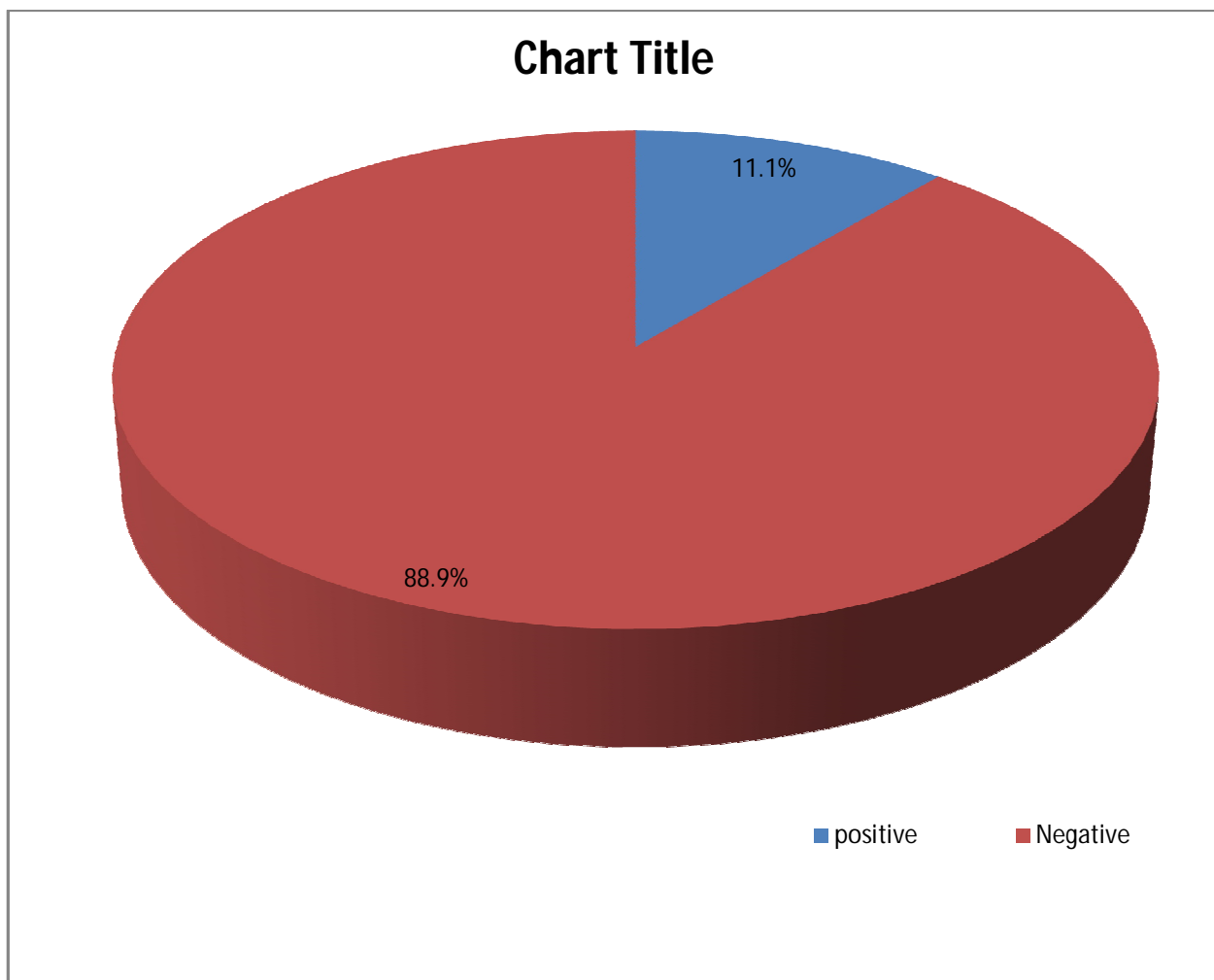


Table (2): The prevalence of *S.haematobium* according to gender using the centrifugation method:

Gender	No. examined	Positive cases	Percentage
Male	200	28	14%
Female	70	2	2.8%
Total	270	30	11.1%

P=0.01

Figure (2): The prevalence of *S.haematobium* according to gender using the centrifugation method

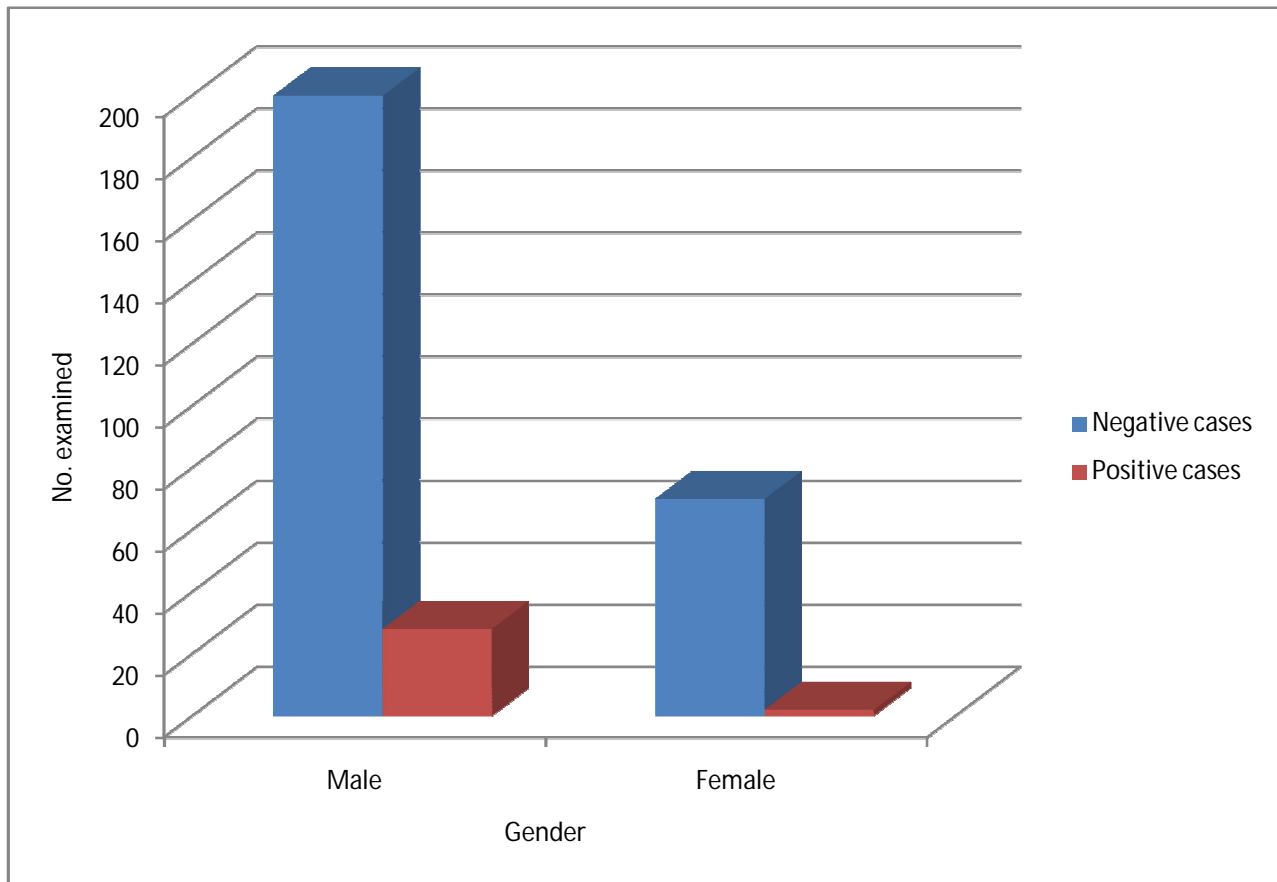


Table (3): The prevalence of *S.haematobium* according to age groups by the urine centrifugation method:

Age groups	No. examined	Positive cases	Percentage
≤ 9year	70	5	7.1
10-15 year	85	14	16.4
16-21 year	57	8	14
≥ 22 year	58	3	5
Total	270	30	11.1

Figure (3): The prevalence of *S.haematobium* according to age groups by the urine centrifugation method:

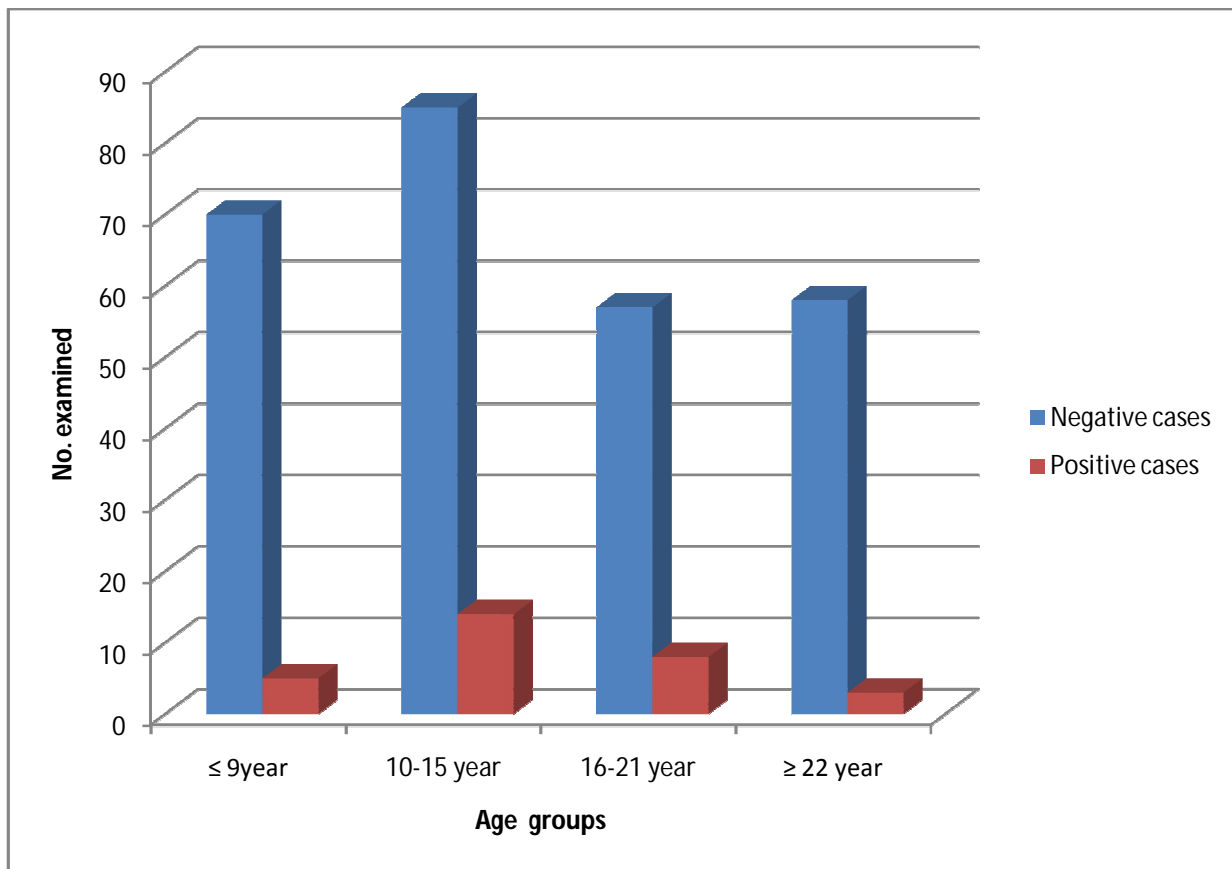


Table (4): The prevalence of *S.haematobium* according to contact with water:

Contact with water	No. examined	Positive cases	Percentage
Yes	203	29	14.2%
No	67	1	1.4%
Total	270	30	11.1%

P=0.02

Figure (4): The prevalence of *S.haematobium* according to contact with water:

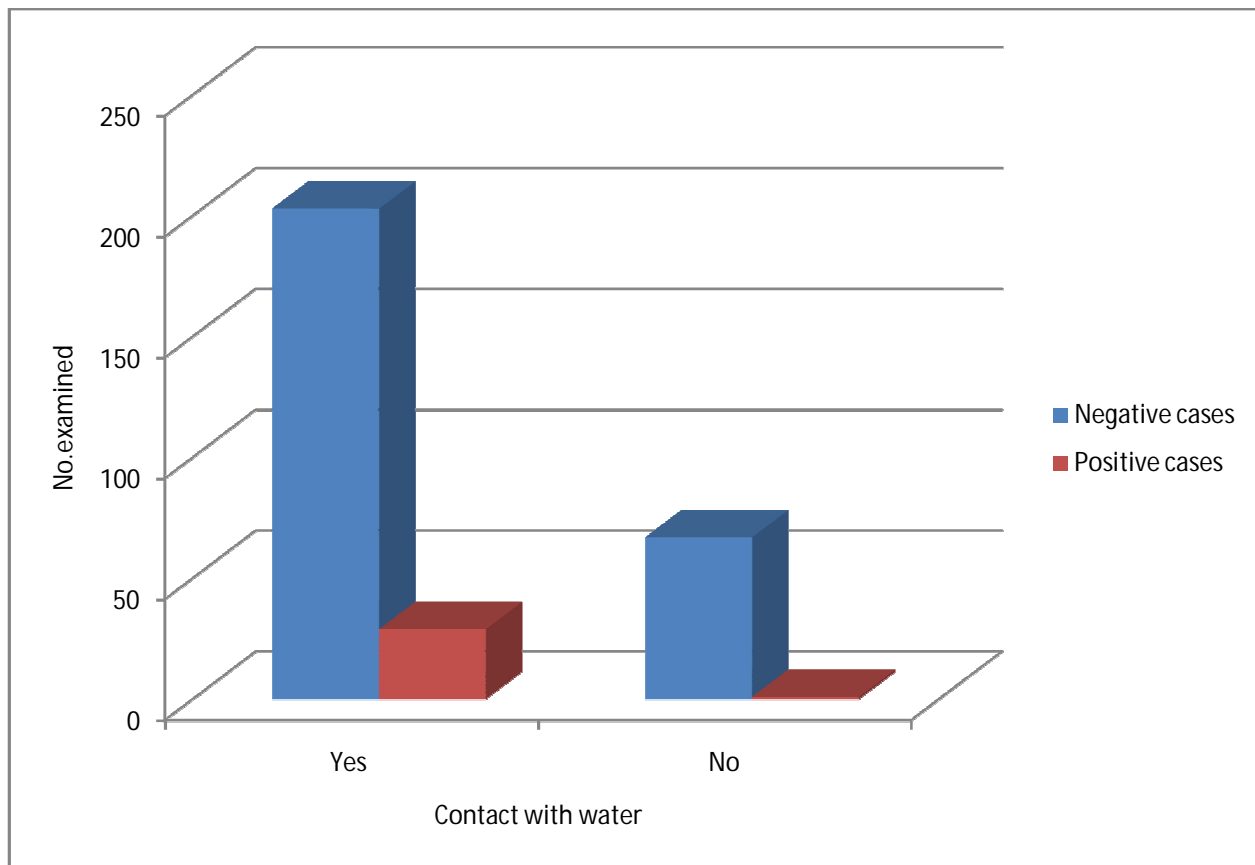


Table (5): *S.haematobium* infection with relation to haematouria and proteinuria:

Haematouria and proteinuria	Negative cases	Positive cases	Percentage
Yes	4	27	90%
No	236	3	10%

P=0.00

Figure (5): *S.haematobium* infection with relation to haematouria and proteinuria:

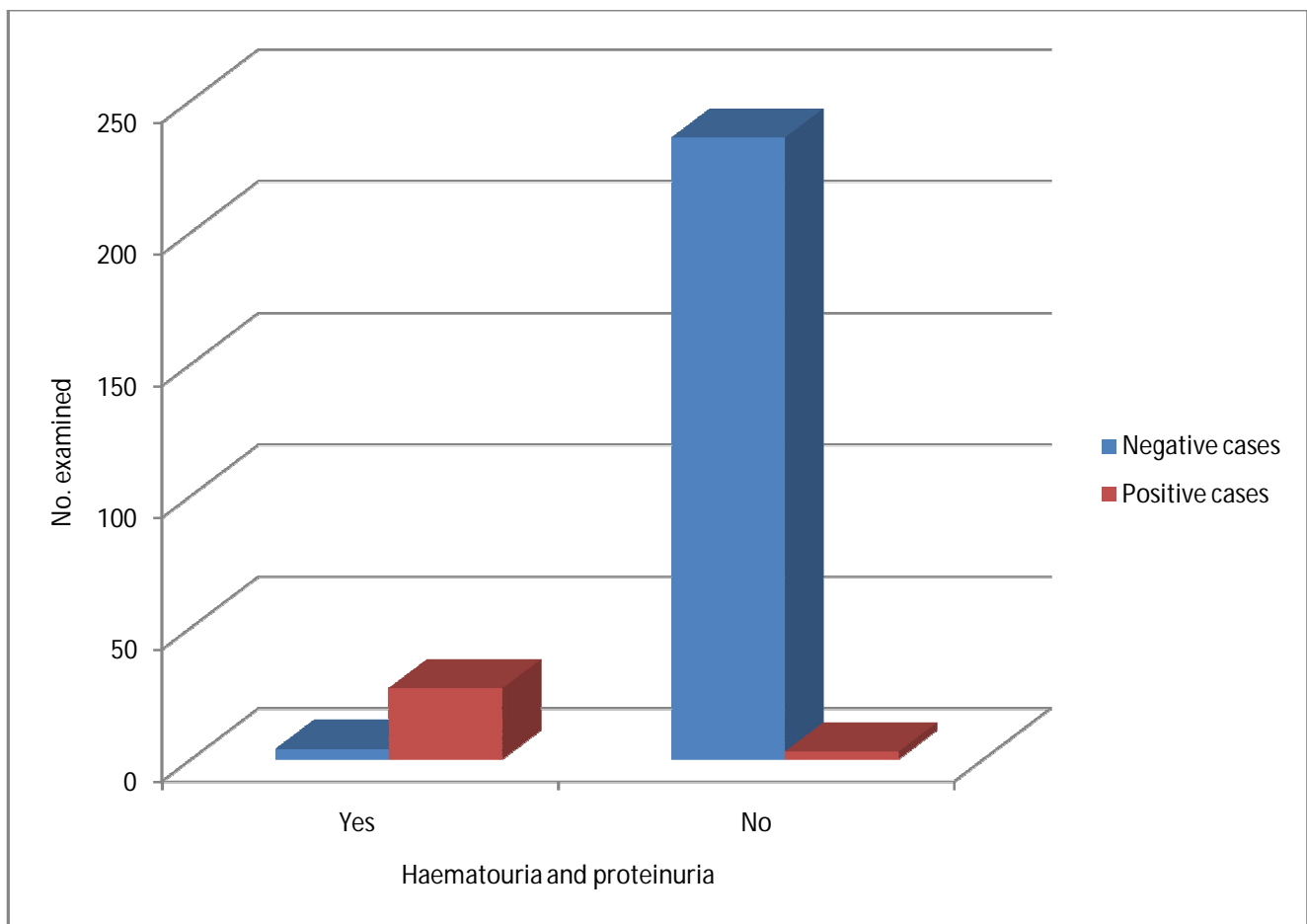


Table (6): Sensitivity and specificity of the ELISA technique:

Method		Centrifugation technique	
ELISA technique	Results	Positive	Negative
	Positive	29	3
	Negative	1	27

Sensitivity:
$$\frac{29}{29+3} \times 100 = 90.6\%$$

Specificity:
$$\frac{27}{27+1} \times 100 = 96.4\%$$

Chapter four

Discussion

A prevalence rate of 11.1% for urinary schistosomiasis was reported in this study. According to the classification of the WHO Expert Committee on the control of schistosomiasis (WHO, 1985), prevalence rates greater than 25% are moderate while those below are low.

This rate was greater than the rate reported by Naeem (2009) in Omshoka village in Sinnar (5.2%) and was also greater than the rate reported by Ahmed (2009) in Omhani village near Kosti in the White Nile (10.5%) and the rate reported by Sitealbanat (2006) in certain villages in Sharg Elnil (9.9%).

However, the prevalence rate reported in this study was lower than the rate reported by Ezz Alarab (2011) in Dar Alsalam - Khartoum state (17.6%). It was also less than the rate reported by Mohammed (2009) in Asalaya campus (20%) and Bakhit (2008) in keryab village (25%).

From the investigation, it was obvious that the rate of infection in males (14%) was higher than that the rate reported in females (2.8%). This finding is in the line with the finding of Mohammed (2009) who found the prevalence to be higher in males than in females. However, our finding contradicts the finding of Bakhit (2008) who reported higher prevalence (34%) among females compared to 22% among males. Our finding might probably be due to less contact of water by females.

The significance of contact with water is evidenced by the high prevalence rate (14.2%) in those who had contact with canal water compared to the infection rate (1.4%) of those who had no contact with water.

In this study, the highest prevalence rate (16.4%) was reported among the age group (10-15) years old and the lowest rate (5%) was reported among the age group more than 22 years old. This was also confirmed by Mohammed (2009) and Ahmed (2009) who reported the highest infection rate among the age 10-15 years old.

The present revealed that the sedimentation technique was as efficient as the centrifugation technique in detecting *Schistosoma haematobium* infection among the population. 30 positive cases were detected by both techniques.

Meshinda *et al.*, (1989) concluded that urine filtration technique is the standard field technique for detection of urinary schistosomiasis, but using of reusable filters may give false positive finding because filter can not be washed adequately.

Richards *et al* (1984) stated that urine sedimentation by centrifugation gave higher values than filtration technique for detection of infection and intensity of urinary schistosomiasis through the number of eggs recovered in the preserved urine. The preserved urine caused obstruction of filters. The results of sedimentation obtained in the present study disagree with that obtained by Richards *et al.*, (1984) because both techniques gave same results which may be due to fact that the study used fresh and unpreserved urine sample.

From table 6, the results revealed high sensitivity of the ELISA test in detecting *S.haematobium* antibody and also high specificity rate (90.6% and 96.4% respectively). However, our finding was confirmed previously by Ezzalarab (2011) who reported relatively higher sensitivity and specificity for ELISA (88% and 92% respectively) and van Gool *et al.*, (2002) who also reported higher sensitivity and specificity for ELISA test in detecting *S.haematobium* antibodies (96% and 97.2% respectively).

The ELISA test was performed on already known cases (positives and negatives) confirmed by the centrifugation technique, however. Despite the high sensitivity of any

serological tests, this undoubtedly does not reflect the active infection as the *Schistosoma haematobium* remain in the circulation months after treatment .

Chapter five

5.1 Conclusion:

The study concludes that:

- 1- *Schistosoma heamatobium* is more prevalent among school children age especially those who used to contact with canal water.
- 2- The infection is more prevalent among males compared to that in females.
- 3- Urine filtration technique is as efficient as the sedimentation technique in detecting *Schistosoma heamatobium* infection.
- 4- The ELISA test showed relatively high specificity and sensitivity rates (96.4% and 90.6% respectively).

5.2 Recommendations:

A control program should be launched in the area of study. It should include:

- 1- Mass treatment of the population.
- 2- Irradication of the snail intermediate host.

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Sudan University of Science and Technology

College of Graduates studies

M.Sc. in Parasitology and Medical Entomology

Questionnaire form

No:.....

Name:.....

Sex:

Male ()

Female ()

Age group:

≤ 9 ()

10-15 ()

16-21 ()

≥ 22 ()

Water contact:

Yes ()

No()

Proteinuria and haematouria:

Yes ()

No()