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Female genital schistosomiasis in Matta health area, Western Cameroon Region: Prevalence, risk factors and biomarkers profile

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Abstract:

Background: Schistosomiasis is a disease affecting particularly people living in low-income countries. Egg sequestration in the female genitalia, leading to female genital schistosomiasis (FGS), is associated with the release of reactive oxygen species (ROS) that can lead to secondary infertility, which remains underdiagnosed. This study was conducted to determine the prevalence and risk factors associated with FGS and urogenital schistosomiasis (UGS) and to assess some biochemical markers associated with these conditions.
Methodology: This was a descriptive cross-sectional survey of 330 women aged 15 to 50 years, conducted in selected fishing communities in the Matta health area of Western Cameroon. Information on socio-demographic characteristics and risk factors were collected from each participant using a structured questionnaire. UGS and FGS were diagnosed from analysis of urine and vaginal secretion respectively by microscopy and PCR amplification of extracted DNA of *Schistosoma haematobium* and *Schistosoma mansoni*. Biochemical analyses of markers of oxidative stress [levels of malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH), and activities of superoxide dismutase (SOD) and catalase (CAT)] and cytokine profiling (TNF-α, INF-γ, IL-1β, IL-6 and IL-10) were determined in serum samples and vaginal secretions. Univariate and multivariate logistic regression analyses were used to determine associations of FGS, UGS, and potential risk factors.
Results: The prevalence of UGS and FGS was 45.7% and 60.0% respectively. Detection of schistosome DNA by PCR revealed a prevalence of 100.0% and 94.5% for *S. haematobium* and *S. mansoni* infection respectively. Nevertheless, among those infected, just 23.5% shed eggs in urine. Marital status was a risk factor of infection. Analysis of the oxidative stress markers revealed significantly higher levels of NO and MDA in serum and vaginal secretions of infected persons with highest level in women with FGS. Lower levels of GSH, CAT and SOD activity in serum and vaginal secretion in women with UGS and FGS was observed particularly in women with FGS. High IL-10 level was observed in both urine and vaginal secretions.
Conclusion: These results indicate increased oxidative stress in UGS and FGS particularly in the cervicovaginal tract. However, high levels of IL-10 might prevent immunopathology and limit severity of the disease, from a shift to T_{h2} immune response.

Keywords: Schistosomiasis; female genital; urogenital; inflammation; oxidative stress

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Schistosomiase génitale féminine dans la zone de santé de Matta, région de l'Ouest du Cameroun: Prévalence, facteurs de risque et profil des biomarqueurs

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Résumé:

Contexte: La schistosomiase est une maladie qui touche particulièrement les populations des pays à faible revenu. La séquestration d'ovules dans les organes génitaux féminins, responsable de la schistosomiase génitale

féminine (SGF), est associée à la libération d'espèces réactives de l'oxygène (ERO) pouvant entraîner une infertilité secondaire, encore sous-diagnostiquée. Cette étude visait à déterminer la prévalence et les facteurs de risque associés à la SGF et à la schistosomiase urogénitale (SUG), ainsi qu'à évaluer certains marqueurs biochimiques associés à ces affections.
Méthodologie: Il s'agissait d'une enquête transversale descriptive auprès de 330 femmes âgées de 15 à 50 ans, menée dans des communautés de pêcheurs sélectionnées dans la zone de santé de Matta, dans l'ouest du Cameroun. Des informations sur les caractéristiques sociodémographiques et les facteurs de risque ont été recueillies auprès de chaque participante à l'aide d'un questionnaire structuré. L'UGS et la FGS ont été diagnostiquées à partir de l'analyse des urines et des sécrétions vaginales respectivement par microscopie et amplification par PCR de l'ADN extrait de *Schistosoma haematobium* et *Schistosoma mansoni*. Français Des analyses biochimiques des marqueurs du stress oxydatif [taux de malondialdéhyde (MDA), d'oxyde nitrique (NO) et de glutathion réduit (GSH), et activités de superoxyde dismutase (SOD) et de catalase (CAT)] et le profilage des cytokines (TNF-α, INF-γ, IL-1β, IL-6 et IL-10) ont été déterminés dans des échantillons de sérum et des sécrétions vaginales. Des analyses de régression logistique univariée et multivariée ont été utilisées pour déterminer les associations entre le FGS, l'UGS et les facteurs de risque potentiels.
Résultats: La prévalence de l'UGS et du FGS était respectivement de 45,7% et 60,0%. La détection de l'ADN de schistosome par PCR a révélé une prévalence de 100,0% et 94,5% pour l'infection à *S. haematobium* et *S. mansoni* respectivement. Néanmoins, parmi les personnes infectées, seulement 23,5% ont excrété des œufs dans l'urine. L'état matrimonial était un facteur de risque d'infection. L'analyse des marqueurs du stress oxydatif a révélé des taux significativement plus élevés de NO et de MDA dans le sérum et les sécrétions vaginales des personnes infectées, les taux les plus élevés étant observés chez les femmes atteintes de SGF. Des taux plus faibles d'activité du GSH, de la CAT et de la SOD dans le sérum et les sécrétions vaginales ont été observés chez les femmes atteintes de SGF et de SGF, en particulier chez celles atteintes de SGF. Un taux élevé d'IL-10 a été observé dans les urines et les sécrétions vaginales.
Conclusion: Ces résultats indiquent une augmentation du stress oxydatif dans les SGF et les SGF, en particulier au niveau du tractus cervico-vaginal. Cependant, des taux élevés d'IL-10 pourraient prévenir l'immunopathologie et limiter la gravité de la maladie, notamment en favorisant une réponse immunitaire T_{h2}.

Mots-clés: Schistosomiase; génital féminin; urogénital; inflammation; stress oxydatif

Introduction:

Schistosomiasis, also called Bilharzia, is a chronic and debilitating parasitic disease caused by trematode blood flukes of the *Schistosoma* genus. It belongs to the phylum Platyhelminthes and affects a number of vertebrates. Schistosomiasis is a neglected tropical disease that infects over 251.4 million people yearly with about 800 million people at risk of infection (1). This disease is present in over 78 different countries around the globe, precisely low-income countries in Africa, South America and Asia. The disease is ranked second place below malaria in the list of WHO important parasitic diseases due to its impact on public health, causing over 200 thousand deaths annually (1). The species of schistosomes that infect humans reside at specific sites in the human system. *S. mansoni* and *S. japonicum* resides in the intestines of the human host, causing intestinal schistosomiasis whereas *S. haematobium* on the contrary resides in the urinary bladder and urethra of their human definitive host and causes urogenital schistosomiasis (UGS) (2).

Investigations have shown that schistosomiasis is highly endemic in Cameroon, but its distribution is uneven and varies greatly with geographical location across the national territory (3,4). The most affected regions are the Southwest, Adamawa, North, Far North, West and Littoral regions (4). Hotspots of transmission have been observed in several localities around lakes and dams such as Barombi Kotto in the Southwest region and Malantouen in the West region, where water contacts are intense and lead to high reinfection patterns (5). Prevalence of the disease rapidly returns near to the initial level within 6–12 months post-treatment in these localities and the prevalence of the disease within the country ranges from 1.7 to 55.5% (3-6) with rural communities being the most affected. In areas where it is endemic, schistosomiasis can persist for decades without treatment (7). The accumulation of adult *Schistosoma* worm eggs within the host's tissues results in granuloma development, fibrosis, and portal hypertension, and is linked to the chronic morbidity of the disease (8). This is largely due to repeated exposure to freshwater bodies contaminated with cercariae and the longevity of adult worms.

The clinical manifestations of schistosomiasis are associated with the host immune response to the parasite eggs as they circulate within the hosts system (9). The aim of the immune response to eradicate the parasite but it ends up being unsuccessful resulting into an inter-relationship that keeps both parasite and host alive (10). When these schistosome eggs lodged into specific sites in the human system, they elicit an immune response characterized by granuloma formation in the organs and tissues (10). The granuloma formation, which involves the action of white blood cells, results in the release of high levels of reactive oxygen species (ROS) that expose the system to severe oxidative stress. Eggs produced mainly by *S. haematobium* in the urinary system can lodge in the genitals resulting to granulomatous lesions in the genital tract. This causes a form of the disease called genital schistoso-

miasis.

Female genital schistosomiasis (FGS) has a more prominent consequence compared to that of the male, and the WHO estimates its prevalence in about 56 million women around the world (11). FGS in most cases remains undiagnosed due to low index of suspicion among physicians and presents symptoms such as bloody vaginal discharge, bleeding after intercourse or spotting, genital itching or burning sensation (11,12). FGS can result in further complications such as infertility, abortion or ectopic pregnancy, tumors and genital ulcers (12).

Although the presence of the parasite itself in the host is somehow harmless, it results to dysuria and haematuria when infected with *S. haematobium* and occasionally dysentery when infected with intestinal schistosomes (10). The clinical manifestation of schistosomiasis progresses in three phases beginning with a dermatitis reaction following cercariae penetration through the skin that causes an allergic response and later on proceeds to a symptomatic acute phase (Katayama syndrome) characterized by fever, arthralgia and vasculitic skin eruption, eosinophilia and high serum IgM before progressing into a chronic infection (13). During established acute and late chronic stages of the disease, infected persons develop organ-specific morbidity, caused by the accumulation of parasite eggs and granuloma formation around schistosome eggs. The site of inflammation correlates with the infecting *Schistosoma* species as the adult worms of different species localize and lay eggs in specific preferred sites (14).

Adult worms of *S. mansoni* and *S. japonicum* live and lay eggs in the mesenteric veins, some of which get trapped in the intestinal tissues inducing mucosal granulomatous inflammation with micro ulcerations, superficial bleeding and pseudopolyposis during chronic inflammation (15). Some of the eggs released by *S. japonicum* or *S. mansoni* eggs from the mesenteric veins are carried through venous circulation into the small portal branches of the liver through the portal vein, where some get trapped in the pre-sinusoidal periportal tissues inducing the formation of granulomas around the eggs resulting in liver inflammation. In high-intensity, established active infection results in enlargement of the spleen and liver, especially the left liver lobe characteristic of hepatosplenic schistosomiasis (13). In some individuals with prolonged or recurrent infection, egg-induced granulomatous responses may result in severe periportal fibrosis, known as Symmer's pipe stem fibrosis, characterised by deposition of collagen around the portal vein, blockage of the smaller portal branches and severe, often irreversible, pathology (16).

Adult *S. haematobium* worms on the contrary live in the pelvic venous plexus, resulting in UGS symptoms and pathological changes closely associated with the passage of eggs through the urinary bladder wall and egg deposition in bladder tissue and genital organs (13). The passage of egg clusters into the lumen of the bladder is often accompanied by breakage of the epithelial surface of the bladder, ulceration and bleeding which results in haematuria, characteristic of UGS (17). Intense or prolonged egg-induced tissue inflammation can result in bladder wall thickening and development of polypoid protrusions (2) around the ostium, blocking the passage of urine resulting in hydronephrosis which in some cases result in a non-functioning kidney (18). Investigations have shown that UGS is significantly associated with squamous cell carcinoma (SCC) of the urinary bladder, and *S. haematobium* has been classified by the International Agency for Research on Cancer (IARC) as a carcinogen, as infection may result in bladder cancer after many years of exposure, infection and urinary tract inflammation (19).

During established active infection, *S. haematobium and S. mansoni* may migrate from their primary sites through the intercommnections between the different pelvic venous plexuses to the uterovaginal venous plexus where they release eggs that migrate to the genital tract (20). In the genital tract, the eggs cannot be released to the environment, hence, are trapped and their antigens evoke an inflammatory response, resulting in FGS (9). FGS is considered a risk factor for the transmission of sexually transmitted diseases (STDs) such as HIV and HPV because the disease leads to open sores, inflammation and exposure to blood, providing easy access for any virus to enter the body (21). Depending on where eggs are released, the clinical pathology develops in vulva and vagina, cervix, uterus, fallopian tubes, ovaries and all genital organs may be affected simultaneously. Women suffering from FGS may experience infertility, irregular menstruation, pregnancy complications, and problems of sexual intercourse like bleeding (9).

The immune response profile of the disease is attributed to a supervening T_{h1} pro-inflammatory immune reaction and high levels of tumor necrosis factor (TNF-α) and interferon gamma (IFN-γ), in concert with a building up of a T_{h2} modulatory profile during the chronic phase of the infection (9). This switch takes place gradually over a long period and coincides with egg over-deposition during which inflammatory cells are gradually being replaced by fibroblasts and granulomas calcified as a result of humoral mediators of the host and parasite in the presence of deposited eggs. The most serious manifestations of the disease such as fibrotic lesions, glomerulo-

nephritis, amyloidosis and malignancy can take place over several weeks or years after infection (10).

Schistosomes depend on human host for its life processes by feeding on red blood cells during which hemoglobin is catabolized. Hb is broken down by schistosomes to produce dipeptides or free amino acids that are ingested by the parasite and broken down by proteolytic enzymes, producing waste products that are regurgitated into the bloodstream of the host as toxic heme and cellular debris (22). Accumulation of these substances within the bloodstream and organs of the host, particularly the liver induces oxidative stress and also inhibits mechanisms set in place to defend against ROS (9).

Toxic heme, also known as free iron (II)-heme, has earned this name because of its capacity to oxidize para- site biomolecules and reduce molecular oxy- gen to produce superoxide anion (O_2), which is crucial for the generation of reactive oxygen species (ROS), which have a direct impact of lipid peroxidation (22). Reactions between lipids, intracellular H_2O_2, and the active heme exacerbate this. The free iron (II)-heme (Fe^{2+}) is converted to non-functional ferric heme (Fe^{3+}) and O_2 that in turn generates H_2O_2 via the Fenton (iron-catalyzed Haber-Weiss) reaction. H_2O_2 produced has the potential to react with Fe^{3+} in the bloodstream to produce the ferryl heme (Fe^{4+}) intermediate.

Extremely hazardous, these products and their intermediates are known to oxidize lipids, amino acids, and nucleic acids (22). They also create toxic heme, which in turn causes the loss of heme and the release of free iron. There is therefore the need to have more data about the complications of schistosomiasis in women in particular, the impact on the immune system and the development of oxidative stress among infected women

Materials and method:

Study site and design:

This study was a descriptive cross-sectional study carried out in selected fishing communities in the Matta health area (5°57'N 11°13'E) which is one of the 18 health areas of the Malantouen Health District in the West Region of Cameroon. These communities included Matta Barrage, Matta Village, Mabonkor Bore, Mabonkor Village and Makossa. These communities were chosen based on their presence within the Matta health area, higher population size, proximity to the lake, prevalence of *S. haematobium* infection and the socioeconomic activities (fishing and farming) carried out among the people as described by Masong et al., (23).

The Matta health area is made up of one Government Integrated Health Centre and two private health centers (4). According to the socio-economic statistics of Cameroon in 2015, the Matta health area has an estimated population of 5000 people with women representing 51% or 2,550 of the population and age group 15–64 years representing 54.6% or 1,400 of the female population (28% of the total population).

Ethical considerations:

The study was approved by the Institutional Review Board of the Faculty of Health Science of the University of Buea (IRB-FHS) (Ref N° 2022/1790-04UB/SG/IRB/FHS). An administrative authorization was gotten from the Regional Delegation of Public Health for the West Region of Cameroon (Enregistrement S/N° 163 /2022).

Written and verbal informed consent was provided by each participant to take part. Participants under the age of eighteen gave their approval after receiving permission to participate from their parents, spouses, or guardians. Furthermore, as required by cultural rules, husbands of married women or partners had to give their verbal consent before they were approached.

Study participants and method of selection:

The participants for this study were females aged 15-50 years residing in the Matta Health area. These participants were selected from 5 rural fishing communities surrounding the Mape Dam, a known transmission hotspot for *S. haematobium*, with most participants living within 200 meters of the dam and relying on it for household water and income-generating activities such as fishing.

Participants for the study were selected by simple random sampling technique. After selection, participants (or their guardians for minors) were informed about the study objectives, procedures, and potential risks. Only those who gave consent were included in the study.

Sample size determination:

The sample size was determined using the formula described by Charan and Biswas (24), predicated on achieving a 95% precision rate with a 5% error margin, as follows; $n = \frac{Z^2 p(1-p)}{d^2}$, where Z=standard normal variate (at 5% type 1 error = 1.96), p = expected proportion of 0.59 in population based on a previous study by Masong et al., (23), and d = absolute error or precision = 0.05. Thus, $n = \frac{1.96^2 \times 0.59\ (1-0.59)}{0.05^2}$, which gave the calculated sample size of 372.

Sample and data collection:

Urine, stool, vaginal swab and blood samples were collected from girls 15 years and above and women within the target communities and codes assigned to each participant.

The sample collection exercise was supervised by trained medical professionals working within the Matta health area.

On the day of enrollment, each participant was given 2 sterile, wide mouthed, screw capped plastic bottles carrying their identification information. About 250 ml urine were collected from each participant and a small scoop of faeces. The samples were stored in cooling boxes containing cooler ice packs to prevent the eggs from hatching during transportation to the laboratory of the Integrated Health Center of Matta Barrage. Questionnaires were administered to participants during sample collection to obtain information on frequency and history of fresh water contact and related symptoms of FGS.

Sample processing:

The urine samples collected were used for microscopic examination to detect eggs of *S*. *haematobium* excretion, from which we inferred the possibility of UGS in correlation with microhematuria from dipstick tests. A single dose (40 mg/kg) of Praziquantel was offered and administered to those who had microhematuria from the dipstick tests (12).

Laboratory identification of *Schistosoma* by microscopy:

The urine samples were tested for microhematuria and proteinuria using urine reagent strips (Siemens Multistix 10). In the laboratory, the urine samples were processed using the membrane filtration technique and examined microscopically for the presence of eggs of *S*. *haematobium* using Binocular compound light microscope. Terminal-spined eggs, characteristics of *S*. *haematobium* were identified and counted manually. Fecal samples were processed and examined microscopically for the presence of *S. mansoni* infection by identifying lateral-spined eggs characteristic of *S. mansoni*.

Molecular detection of *Schistosoma* species:

Polymerase chain reaction was used to amplify schistosomes DNA extracted from dried blood spot and vaginal secretions to detect schistosomiasis and FGS respectively.

DNA extraction:

DNA was extracted from dried blood spotted on Whatman paper number 2 using the Chelex method as described by Ategeka (25) with minor modifications. Each coded dried blood spot was cut and transferred into their corresponding 1.5µl Eppendorf tube using sterile equipment and 1ml of 0.5% saponin was added into the tubes and incubated overnight at 4℃. The brown solution was discarded and replaced with 1ml of 1x PBS and the tubes incubated at 4℃ for 30 minutes. A stock 20% Chelex resin (50µl) of and 150 µl of autoclaved distilled water were both transferred into newly labelled tubes and heated at 99℃ for 30 minutes on a heat block. The tubes containing PBS were centrifuged at 4000 rpm for 2 minutes and each filter paper was carefully transferred into their corresponding hot Chelex tubes. The tubes were vortexed for 30 seconds and placed on a heat block at 99℃ for 10 minutes. The tubes were then centrifuged at 4000 rpm for 2 minutes and 100µl supernatant were transferred into freshly labelled tubes. The supernatant contained the DNA template and was stored at -20°C for PCR analysis.

DNA was extracted from vaginal secretion using the Qiagen kit following the manufacturer's protocol. Lysis buffer AL, 100µl, 50µl proteinase K and 100µl lysis buffer ATL were pipetted respectively into 1.5ml Eppendorf tubes containing 100 µl vaginal secretion. The tubes were vortexed for 15 seconds then, incubated for 10 minutes followed by a brief centrifugation to remove the drops from the inner lid. Ethanol (100 µl, 95%) was added to the samples and vortexed for 15 seconds. Following a brief centrifugation of the tubes, the mixtures was carefully transferred to respective QIAamp spin columns placed in a 2ml collection tubes and centrifuged at 8000 rpm for 1 minute. The QIAamp spin column was removed and placed in a new 2ml collection tube and 500µl washing buffer (AW1) carefully pipetted in the QIAamp spin column and centrifuged at 14000 rpm for 1 minute.

The QIAamp spin column was removed for the second time and placed in a new 2 ml collection tube and 700µl washing buffer (AW2) was carefully pipetted in the QIAamp spin column and centrifuged at 14000 rpm for 3 minutes. The QIAamp spin column was removed for the third time, placed in a new 2 ml collection tube and was centrifuged at 14000 rpm for 1 minute. Finally, the QIAamp spin column was placed in a clean 1.5 ml micro centrifuge tube, 100 µL of distilled water were added followed by incubation at room temperature for 1 minute and centrifugation at 8000 rpm for 1 minute and the solution collected stored at -20℃ until used.

DNA amplification and detection:

The DNA of *S. haematobium* and *S. mansoni* in the DNA extract was detected by amplification of tandemly arranged 121bp long units of *Dra*1 repeats gene of *S. haematobium* and *Sm*1-7 repeats gene of *S. mansoni* (26) in a PCR machine (Bio-Rad T100™ Thermal Cycler, Berkeley California, USA). These repeats genes constitute approximately 15% of *S. haematobium* genome and 12% of *S. mansoni* genome respectively.

Amplification was carried out in a total volume of 10 µL including 5 µL of One Taq Hot Start 2x Mastermix (New England Biolabs),

Table 1: Primer sequences for *Schistosoma* species identification by polymerase chain reaction

Schistosome species	Primer sequence	Expected band size	Reference
Schistosoma haematobium	SHF: 5' -GATCTCACCTATCAGACGAAAC- 3'	97 bp	Lodh et al., (27)
	SHR: 5' -TCACAACGATACGACCAAC- 3'		
Schistosoma mansoni	SMF: 5'-GATCTGAATCCGACCAACCG- 3'	110 bp	
	SMR: 5'-ATATTAACGCCCACGCTCTC- 3'		

Table 2: Polymerase chain reaction conditions for *Schistosoma* species identification

Schistosome species	Initial denaturation	Denaturation	Annealing	Extension	Number of cycles	Final extension
Schistosoma haematobium	95℃ for 10 minutes	95℃ for 30 seconds	53℃ for 90 seconds	72℃ for 1 minute	33	60℃ for 5 minutes
Schistosoma mansoni	95℃ for 10 minutes	95℃ for 30 seconds	60℃ for 90 seconds	72℃ for 30 seconds	35	72℃ for 5 minutes

Lodh et al., (27)

0.5 µL of both forward and reverse primers (primer sequence listed in Table 1), 1 µL of the DNA template and 3 µL DNase free water, with reaction conditions as set in Table 2 (27). The amplified fragments together with a 100bp molecular weight marker were separated in a 2% agarose gel and observed using a gel documentation system (Molecular Imager® Gel DocTM XR+ System with Image LabTM Software, Bio-Rad, Berkeley California, USA).

Biochemical assay:

Using a 5 ml syringe, 4 ml of venous blood was obtained from each participant and poured into sterile plain blood tubes carrying participants' identification numbers and centrifuged to obtain serum. The serum was transferred to clean sterile Eppendorf tubes and then transported in cooling boxes containing cooler ice parks from Matta to the University of Buea Molecular and Cell Biology Laboratory (MCBL) for storage at -20℃ (28). Biochemical analysis of markers of oxidative stress (malondialdehyde (MDA), nitric oxide (NO) and reduced glutathione (GSH) levels, superoxide dismutase (SOD) and catalase activities) and cytokine profiling (TNF-α, INF-γ, IL-1β, IL-6 and IL-10) was then carried out using vaginal secretion and serum samples.

Analysis of oxidative stress markers:

Assay of malondialdehyde level:

Malondialdehyde (MDA) level, a measure of lipid peroxidation based on the reaction of malondialdehyde (MDA) with thiobarbituric acid (TBA); forming an MDA-TBA2 adduct that absorbs strongly at 532 nm was determined as described by Hamburger et al., (26). The reagents, 1.5 mL acetic acid (20% at pH 3.5), and 1.5 ml thiobarbituric acid (0.67%) were added to 0.1 mL of sample, and then heated at 90°C for 60 minutes. The mixture was cooled, centrifuged at 3000 rpm for 10 min, the supernatant collected and absorbance measured at 532 nm using a spectrophotometer (Tecan, Infinite M200). The concentration of MDA was quantified by the extinction coefficient (ε) of 1.56×10^5 M/cm using the formula; C=A/ℰl, where A is the absorbance at 532 nm, ε is the extinction coefficient, C is the concentration of MDA and l is the length of the cuvette. The concentration of MDA was expressed in nmol/ml of sample.

Assay of reduced glutathione level:

Reduced glutathione (GSH) was assayed by Ellman method as described by Fotio et al., (29). The method is based on a thiol-exchange reaction between GSH and Ellman's reagent (5 mg of Disthio-bis 2-nitrobenzoic acid (DTNB) in 250 ml of phosphate buffer) forming one equivalent of 5-thio-2-nitrobenzoic acid (TNB). The produced TNB is detected by spectrophotometry in the visible region at 405 nm.

The sample (20µl) was added to 3 ml of Ellman reagent. The mixture was kept at room temperature for 1 hour and the absorbance read using a spectrophotometer (Tecan, Infinite M200) at 405nm. The concentration of GSH was quantified with the extinction coefficient (ε) of 14,150 $M^{-1}cm^{-1}$ using the formula; C=A/ℰl, where, A is the absorbance, l is the path length in centimeters, C is the concentration in moles/liter (M) and ε is the extinction coefficient. The obtained concentration was converted to µg of protein per ml of sample.

Measurements of nitric oxide:

The method for nitric oxide levels was based on the Griess reaction as described by Hamburger et al., (26). It is based on the reaction between nitrite and Griess reagent (sulfanilamide and N-naphthyl ethylene diamine) in acidic medium leading to the formation of a pink/magenta azo product that

absorbs light at 540nm (30). 100 µl of sample were measured and mixed with an equal volume of Griess reagent and the absorbance determined spectrometrically at 540nm. Absorbance readings obtained were compared with a standard curve plotted with known concentrations of nitrite and the results obtained expressed in µg/ml of sample.

Measurement of superoxide dismutase (SOD) activity:

Superoxide dismutase activity was determined according to the method of Misra and Fridovich (31); a method which is based on the superoxide dismutase inhibition of epinephrine autoxidation to adrenochrome at pH 10.2. Presence of superoxide dismutase prevents the conversion of epinephrine to adrenochrome which can absorb light at 480 nm. Sample (134 µl) was mixed with 1666 µL of carbonate buffer (0.05M, pH 10.2) followed by 200µl of epinephrine (0.06 mg/ml). Absorbance was recorded at 20 and 80 seconds. A blank was prepared by adding 1800 µl of the carbonate buffer to 200 µl of epinephrine and absorbance recorded at 20 and 80 seconds. The change in absorbance (ΔA) within one minute (absorbance at 20 S – absorbance at 80 S) was calculated for the blank and for each sample.

The activity of SOD was determined as percentage inhibition of epinephrine autoxidation as; % inhibition = 100 – [(Δ A sample x 100)/Δ A blank]. One unit of SOD was defined as the enzyme amount causing 10% inhibition in the epinephrine oxidation rate (U), and results were expressed in units of SOD per ml of mixture.

Measurement of catalase activity:

The catalase activity was measured following the spectrometric method by Fotio et al., (32) which is based on the fact that hydrogen peroxide (H_2O_2) is broken down into oxygen (O_2) and water (H_2O) by the enzyme catalase (CAT). Catalase in the sample is allowed to split H_2O_2 and the remaining hydrogen peroxide is measured spectrometrically at 340 nm. A volume of 250µl of sample was added to 250µl of phosphate buffer and 1ml of H_2O_2 (30 mM) added to the mixture and the absorbance read at 340 nm at 30, 60, and 90 S consecutively after the addition of the hydrogen peroxide in the medium. The catalase activity was expressed as units of catalase per ml of sample.

Cytokine profiling:

The values of inflammatory cytokines (TNF-α, INF-γ, IL-1β, IL-6 and IL-10) in both serum and vaginal secretion samples were determined using Quantikine ELISA kit (R&D Systems, USA) following the manufacturer's instructions. This assay employs the quantitative sandwich enzyme immunoassay technique. The concentration of cytokines was determined and recorded in pg/ml by comparing the absorbances with those of a standard curve for the respective recombinant cytokine as described by Misra and Fridovich (31). The intensity of the colour measured is proportional to the amount of the specific cytokine bound in the initial step.

Monoclonal antibodies specific for human TNF-α, INF-γ, IL-1β, IL-6 and IL-10 were pre-coated onto the wells of separate 96 well microplate and 100 µl of sample pipetted into the wells. The plates were incubated for 2 hours at room temperature such that any cytokine (TNF-α, INF-γ, IL-1β, IL-6 and IL-10 respectively) present should be captured by the immobilized antibody. Washing buffer (400 µl) was used to remove any unbound substances, followed by the addition of 100 µl of enzyme-linked polyclonal antibody specific for the human cytokine present in the wells, respectively and incubated at room temperature for 2 hours. Following a wash to remove any unbound antibody-enzyme reagent, 100 µl of substrate solutions were added to the wells of each plate and incubated for 30 minutes at room temperature away from light. The stop solution (50µl) was then added. This yielded to an enzyme reaction that turned the blue products to yellow. The optical densities were determined within 30 minutes, using a microplate reader (Thermo Scientific; MultiskanTM FC Microplate Photometer) set at 450 nm.

Data analysis:

Data obtained were entered into Microsoft Excel 2019 and checked, with tabulations, graphs and statistical analyses conducted using IBM Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) and GraphPad Prism version 9.

Chi-square and Fisher's Exact tests were used to check for associations between UGS, FGS, site, risk factors and morbidity. Univariate and multivariate logistic regression analyses were conducted for *S. haematobium* and *S. mansoni* associations with independent variables. Age group, village site, marital status and economic activity were included as core variables in regression models, and a stepwise regression approach was used to arrive at the most parsimonious adjusted model, applying a 5% level of statistical significance.

Results:

Socio-demographic characteristics of the study participants:

A total of 350 females (girls and women) from five (5) mainland communities in the Matta health area were considered from which 165 participants were enrolled into the

study (Table 3). The age range of the enrolled participants is 15 to 50 years old (median age: 23 years), 60.6% were married and 39.4% were single women. Majority of the participants use the lake (95.8%, n=158) for domestic activities, and most of them use it on daily bases (49.7%, n=79) due to lack of access to improved water source (72.7%).

Prevalence of urogenital schistosomiasis (UGS) by microscopy:

After urine analysis for *S. haematobium* egg excretion, and microhematuria test, the prevalence of UGS (with or without eggs in urine) was 45.7% (n=75) from all selected sites.

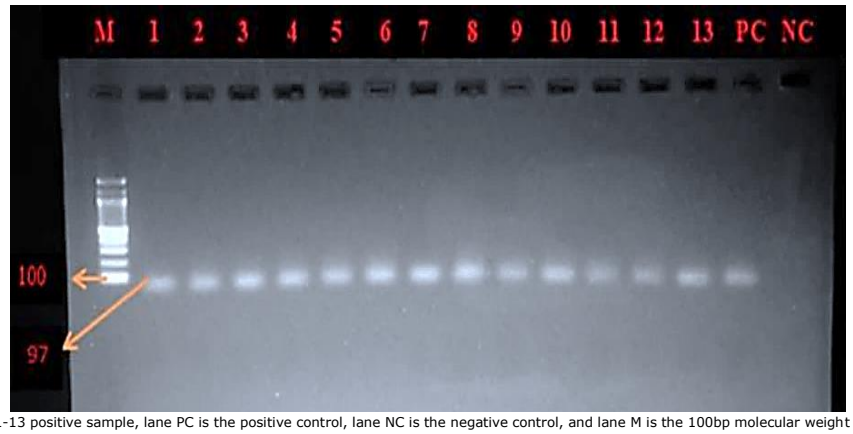
Prevalence of schistosomiasis by molecular detection method (PCR):

PCR detection of *S. haematobium* and *S. mansoni* DNA extracted from both dried blood and vaginal secretion revealed a band of 97 bp and 110 bp for positive samples respectively (Figs 2 and 3). All participants blood samples (100.0%, n=165) tested positive for *S. haematobium* by PCR, with 94.5% (n=158) co-infected with *S. mansoni* (schistosomiasis).

Most of the participants (86.0%, n= 142) provided vaginal secretion samples but no *S. mansoni* was detected in the vaginal secretion while *S. haematobium* was detected in 59.9% (n=85) of vaginal secretion by PCR (FGS).

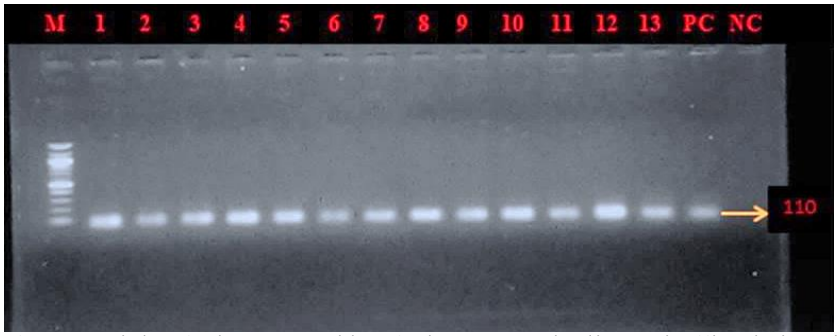
Table 3: Socio-demographic characteristics of the study participants in the Matta Health Area, Western Region of Cameroon

Variables	Category	Frequency (%)
Area of residence	Matta barrage	74 (44.8)
	Matta village	32 (19.4)
	Mambonkor bord	28 (17.0)
	Mambonkor village	14 (8.5)
	Makossa	17 (10.3)
Age group (years)	15-24	88 (53.3)
	25-34	42 (25.5)
	≥35	35 (21.2)
Marital status	Single	65 (39.4)
	Married	100 (60.6)
Level of education	Primary	112 (67.9)
	Secondary	53 (32.1)
Occupation	Housewife	35 (21.2)
	Farmer	58 (35.2)
	Fishing	12 (7.3)
	Pupil/students	53 (32.1)
	Civil servants	7 (4.2)
Religion	Christianity	107 (64.8)
	Islam	58 (35.2)



Lane1-13 positive sample, lane PC is the positive control, lane NC is the negative control, and lane M is the 100bp molecular weight marker

Fig 2: Amplicons gel visualization of representative *Schistosoma haematobium* in dried blood and vaginal secretion



Lane1-13 positive sample, lane PC is the positive control, lane NC is the negative control, and lane M is the 100bp molecular weight marker

Fig 3: Amplicons gel visualization of representative *Schistosoma mansoni* in serum

Self-reported clinical symptoms of female genital and urogenital schistosomiasis associated with *S. haematobium* egg excretion:

Self-reported symptoms and descriptions from the questionnaire survey included lower abdominal pain, foul-smelling vaginal discharges, inconsistent/painful menstruation, genital itch or burning sensation, difficulty in conceiving, frequent miscarriage and pelvic pain or pain during intercourse. The frequency of these self-reported clinical symptoms of FGS by the participants include; vaginal itches (20%, n=33), burning sensation (12.1%, n=20), bleeding after sex (1.2%, n=2), stress incontinence (12.7%, n=21), hypermenorrhoea (21.8%, n=36), difficulty in conceiving (12.1%, n=20), frequent miscarriage (7.3%, n=12), foul-smelling vaginal discharge (42.4%, n=70), irregular/painful menstruation (37.8%, n=61) and abdominal pain (32.7%, n=54) (Table 4).

Relating the participants reported FGS symptoms with prevalence of egg excretion, there was significant association between egg shedding and abdominal pain (p=0.003), vaginal itches (p=0.002) and burning sensation (p=0.03). Other reported symptoms such as smelly vaginal discharge, stress incontinence, pain during/after sex, burning sensation, irregular menstruation, difficulty in conceiving, frequent miscarriage and hypermenorrhea that may be associated with FGS were not significantly related (p>0.05) with egg excretion (Table 4).

Table 4: Summary of self-reported FGS symptoms and their relationship with *Schistosoma haematobium* egg excretion

Reported FGS symptoms	Category	Number (%) egg excretion	*p* value
Abdominal pain	Yes (n=54) No (n=111)	20 (37.0) 18 (16.2)	0.003*
Vaginal itches	Yes (n=33) No (n=132)	14 (43.4) 24 (18.2)	0.002*
Burning sensation	Yes (n=20) No (n=145)	10 (50.0) 28 (19.3)	0.003*
Bleeding after sex	Yes (n=2) No (n=163)	0 38 (23.3)	0.431
Stress incontinence	Yes (n=21) No (n=144)	4 (19.1) 34 (23.6)	0.609
Smelly vaginal discharge	Yes (n=70) No (n=95)	11 (15.7) 27 (28.4)	0.689
Hypermenorrhoea	Yes (n=36) No (n=129)	10 (27.8) 28 (21.7)	0.420
Irregular menstruation	Yes (n=61) No (n=104)	11 (18.0) 27 (26.0)	0.274
Difficulty in conceiving	Yes (n=20) No (n=145)	3 (15.0) 35 (24.1)	0.340
Frequent miscarriage	Yes (n=12) No (n=153)	4 (33.3) 73 (47.7)	0.564

* = statistically significant; FGS = female genital schistosomiasis

Table 5: Association of self-reported UGS symptoms and *Schistosoma haematobium* egg excretion

UGS reported symptom	Category	Number (%) of egg excretion	*p* value
Painful urination	Yes (n=51)	25 (49.0)	0.01*
	No (n=114)	13 (11.4)	
Blood in urine	Yes (n=41)	12 (29.3)	0.260
	No (n=124)	26 (21.0)	
Frequent urination	Yes (n=50)	11 (22.0)	0.770
	No (n=115)	17 (14.8)	

* = statistically significant; UGS = urogenital schistosomiasis

The frequency of UGS symptoms reported by participants include blood in urine (24.8%, n=41), painful urination (30.9%, n=51) and frequent urination (30.3%, n=50). Associating symptoms reported by participants and *S. haematobium* egg shed in urine showed that among the UGS clinical manifestation reported, only painful urination was significantly associated (p=0.01) with egg shedding as shown in Table 5. Blood in urine and frequent urination had p>0.05, therefore not significantly associated with egg excretion

Association of UGS and FGS with sociodemographic factors of participants:

Differences in prevalence rate of UGS and FGS were considered along several variables such as area of residence, educational level, age, marital status, religion, occupation, infertility and sub-fertility. The relationship between UGS and FGS with the sociodemographic factors is summarized in Table 6. Factors such as the area of residence, religion, marital status and age group shows no significant relationship with the prevalence of UGS. However, it was observed that level of education and occupation had significant relationship with UGS (p<0.05) with the disease being more prevalent among participants with only primary education (43.8%), housewives (51.4%) and fishing (83.3%) compared to other groups (Table 6).

No significant association was observed when relating FGS with sociodemographic factors such as age (x^2=0.758, p=0.158), marital status (x^2=3.715, p=0.156), area of residence (x^2=13.685, p=0.188), level of education (x^2=4.269, p=0.640), occupation (x^2 = 1.211, p=0.997), and religion (x^2=0.113, p= 0.945). However, the prevalence of UGS was significantly higher in participants with primary level education (43.8%, n=49, p=0.038) and fishing (83.3%, n=12, p=0.000) as occupation.

There was no significant association between intensity of egg excretion and FGS. Further analysis revealed that the prevalence of FGS only was 45.8% and prevalence of FGS with egg excretion in urine was 14.1%. Females between the ages of 15–24 and 25–34 years had the highest prevalence of FGS with egg excretions, while the prevalence of FGS increased with age (Fig 1).

Table 6: Association of UGS and FGS with socio-demographic characteristics of the study participants

Variable	Category	No (%) with UGS	Chi square & *p* values	No (%) with FGS	Chi square & *p* values
Area of residence	Matta Barrage (n=74)	31 (41.9)	x^2 = 1.453	32 (43.2)	x^2 = 13.685
	Matta Village (n=32)	12 (37.5)	p = 0.918	18 (56.3)	p = 0.188
	Mambonkor Bord (n=14)	5 (35.7)		7 (50.0)	
	Mambonkor Village (n=28)	11 (39.3)		17 (60.7)	
	Makossa (n=17)	5 (29.4)		11 (64.7)	
Age group (years)	15 – 24 (n=88)	34 (38.6)	x^2 = 19.563	43 (48.9)	x^2 = 0.758
	25 – 34 (n=42)	14 (33.3)	p = 0.927	23 (54.8)	p = 0.158
	≥ 35 years (n=35)	16 (45.7)		19 (54.3)	
Marital status	Married (n=100)	39 (39)	x^2 = 0.039	52 (52.0)	x^2 = 3.715
	Single (n=65)	25 (38.5)	p = 0.843	33 (50.8)	p = 0.156
Level of education	Primary (n=112)	49 (43.8)	x^2 = 8.446	53 (47.3)	x^2 = 4.269
	Secondary (n=53)	15 (28.3)	p = 0.038*	32 (60.4)	p = 0.640
Occupation	Housewife (n=35)	18 (51.4)	x^2 = 21.042	18 (51.4)	x^2 = 1.211
	Farmer (n=58)	15 (25.9)	p = 0.000*	31 (53.4)	p = 0.997
	Fishing (n=12)	10 (83.3)		5 (41.7)	
	Pupil/students (n=53)	21 (39.6)		27 (50.9)	
	Civil servant (n=7)	0		4 (57.1)	
Religion	Christianity (n=107)	43 (40.2)	x^2 = 0.115	56 (52.3)	x^2 = 0.113
	Islam (n=58)	21 (36.2)	p = 0.734	29 (50.0)	p = 0.945

* = statistically significant; FGS = female genital schistosomiasis; UGS = urogenital schistosomiasis

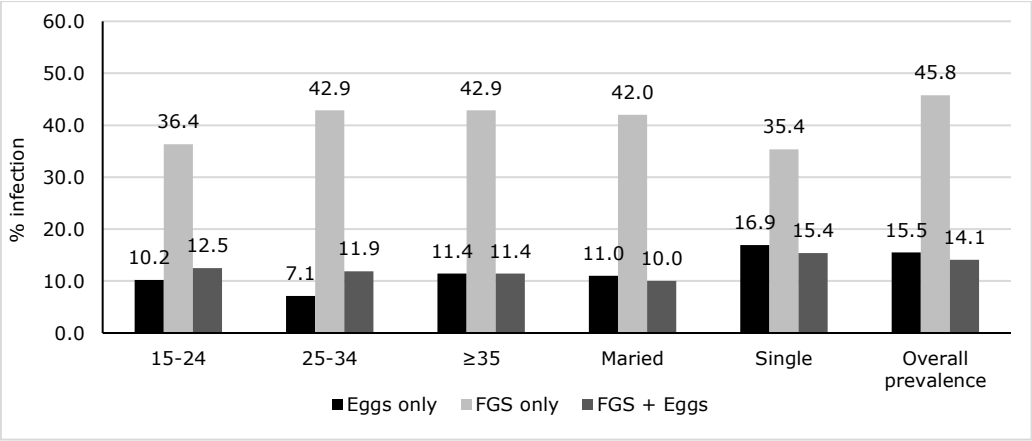


Fig 1: Distribution of participants with female genital schistosomiasis (FGS) across age groups and marital status

Table 7: Association between urogenital schistosomiasis (UGS) and water contact behavior

Variable	Category	No (%) with UGS	Chi square and *p* values
Lake usage	Yes (n=158) No (n=7)	64 (40.5) 0	x^2 = 4.880 *p* = 0.027*
Lake usage frequency	Daily (n=79) Weekly (n=53) Monthly (n=33)	40 (50.6) 18 (34) 6 (18.2)	x^2 = 9.583 *p* = 0.008*
Bathing	Yes (n=70) No (n=95)	37 (52.9) 27 (28.4)	x^2 =8.571 *p* = 0.003*
Laundry	Yes (n=139) No (n=26)	61 (43.9) 3 (11.5)	x^2 = 8.897 *p* = 0.003*
Crossing	Yes (n=100) No (n=65)	43 (43) 21 (32.3)	x^2 = 1.924 *p* = 0.165
Swampy farming	Yes (n=113) No (n=52)	47 (41.6) 17 (32.7)	x^2 = 0.829 *p* = 0.363
Fishing	Yes (n=24) No (n=141)	13 (54.2) 51 (36.2)	x^2 = 2.361 *p* = 0.124
Playing in the lake	Yes (n=43) No (n=122)	17 (39.5) 47 (38.5)	x^2 = 0.049 *p* = 0.824
Access to improved water source	Yes (n=120) No (n=45)	47 (39.2) 17 (37.8)	x^2 = 0.047 *p* = 0.828

* = statistically significant

Association between UGS and water contact behavioral pattern of the population:

The association between UGS and water contact behavior is represented in Table 7. It was observed that lake usage, frequency of lake usage and domestic lake activities had significant relationships with UGS ($p<0.05$), with the disease being more common in those who use the lake compared to those who do not. Other factors such as access to improved water source, crossing, swampy farming, fishing and recreational lake activities (playing) show no significant relationship with UGS with $p>0.05$.

Markers of oxidative stress:

Table 8 shows the effects of UGS and FGS on the levels of oxidative stress markers in serum and vaginal secretion. GSH levels observed was significantly lower ($p<0.05$) in the serum of participants with UGS and FGS compared to the uninfected. There was no significant ($p=0.085$) difference in serum GSH levels of participants with UGS compared to those with FGS. The level of GSH in vaginal secretions was significantly lower ($p<0.05$) in participants with UGS and FGS compared to the uninfected (mean=7.85±0.464). There was a significant difference in the levels of GSH between participants with FGS and UGS ($p=0.043$).

Catalase activity was observed to be significantly higher in serum of uninfected participants (mean=2.13±0.41) compared to participants with UGS (mean=0.99±0.31) ($p=0.019$). Catalase activity was also significantly higher in serum of uninfected participants (mean=2.21±0.32) compared to participants with FGS (mean=0.88±0.25) ($p=0.011$). However, there was no significant differ-

Table 8: Mean values of oxidative stress markers in women with different *Schistosoma* infection status

		Serum				Vaginal secretion			
Parameter	**Pairs**	**Mean ± SD (µg/mL)**	**SE of mean**	***t***	***p* value**	**Mean ± SD (µg/mL)**	**SE of mean**	***t***	***p* value**
GSH	Uninfected	11.85±0.784	0.105	3.375	0.041	7.65 ± 0.682	0.110	3.715	0.037
	UGS	9.05 ± 0.713	0.153			5.19 ± 0.701	0.113		
	Uninfected	11.77 ± 0.612	0.113	5.681	0.037	7.85 ± 0.464	0.106	6.616	0.031
	FGS	8.21 ± 0.442	0.120			3.45 ± 0.492	0.112		
	UGS	9.12 ± 0.575	0.144	0.633	0.085	5.00 ± 0.637	0.146	2.456	0.043
	FGS	8.21 ± 0.442	0.120			3.45 ± 0.492	0.112		
CAT	Uninfected	2.13 ± 0.41	0.020	3.175	0.019	1.52 ± 0.28	0.015	3.575	0.003
	UGS	0.99 ± 0.31	0.010			0.89 ± 0.23	0.009		
	Uninfected	2.21 ± 0.32	0.019	3.899	0.011	1.49 ± 0.27	0.021	5.856	0.003
	FGS	0.88 ± 0.25	0.008			0.42 ± 0.11	0.008		
	UGS	0.94 ± 0.33	0.009	0.726	0.058	0.91 ± 0.21	0.009	3.456	0.003
	FGS	0.88 ± 0.25	0.008			0.42 ± 0.11	0.008		
NO	Uninfected	39.03 ± 4.73	0.767	4.736	0.001	37.34 ± 5.30	0.679	3.136	0.001
	UGS	70.6 ± 7.77	1.261			67.1 ± 8.93	0.991		
	Uninfected	38.2 ± 5.23	1.169	5.123	0.001	36.1 ± 5.23	1.041	4.326	0.001
	FGS	74.63 ±3.38	0.757			78.37 ± 5.82	0.821		
	UGS	72.34 ± 4.01	0.897	0.377	0.103	66.2 ± 4.91	0.897	.775	0.031
	FGS	74.63 ± 3.38	0.757			78.37 ± 5.82	0.821		
SOD	Uninfected	3.97 ± 0.41	0.038	2.736	0.011	3.02 ± 0.38	0.031	6.236	0.001
	UGS	3.09 ± 0.36	0.031			2.08 ± 0.33	0.019		
	Uninfected	3.83 ± 0.43	0.036	3.236	0.032	3.83 ± 0.43	0.036	12.316	0.032
	FGS	2.88 ± 0.45	0.027			1.52 ± 0.30	0.017		
	UGS	3.17 ± 0.36	0.029	0.377	0.091	2.10 ± 0.31	0.022	1.177	0.04
	FGS	2.88 ± 0.45	0.027			1.52 ± 0.30	0.014		
MDA	Uninfected	0.599 ± 0.111	0.031	2.136	0.001	0.634 ± 0.114	0.031	2.736	0.001
	UGS	1.143 ±0.265	0.019			1.253 ± 0.210	0.013		
	Uninfected	0.587 ± 0.102	0.036	2.636	0.032	0.587 ± 0.102	0.056	4.236	0.032
	FGS	1.168 ± 0.247	0.017			1.625 ± 0.231	0.017		
	UGS	1.101 ± 0.210	0.022	0.375	0.04	1.201 ± 0.190	0.011	1.377	0.041
	FGS	1.168 ± 0.247	0.014			1.625 ± 0.231	0.014		

ence in serum catalase activity in participants with UGS and FGS (p=0.058). The catalase activity in vaginal secretion was significantly lower in participants with FGS and UGS compared to the uninfected (p<0.05). It was also observed that the difference in catalase activity in the secretions of participants with UGS and FGS was statistically significant (p= 0.003).

The level of nitric oxide in serum of the uninfected participants (mean=39.03±4.73) was observed to be significantly lower (p= 0.001) than that of participants with UGS and FGS (mean=70.6±7.77) as shown in Table 8. However, there was no significant difference in the serum NO levels between participants with UGS and FGS (p=0.103). The level of NO in vaginal secretions of participants with UGS and FGS were significantly higher than that of the uninfected (p<0.05). NO levels were also significantly higher in vaginal secretions of participants with FGS than in those with UGS (p=0.031).

The SOD activity in serum of the uninfected participants was observed to be significantly higher than those of participants with UGS and FGS (p<0.05) as shown in Table 8. No significant difference in serum SOD activity was observed between participants with UGS and FGS (p=0.091). However, there was a significant difference in SOD activities of vaginal samples of participants with UGS and FGS compared to the SOD activity levels in the uninfected participants as shown in Table 8.

SOD activity was also significantly lower in those with FGS compared to those with UGS.

Results from pairwise comparison of MDA levels in participants with different infection status showed significantly lower levels of serum MDA in uninfected participants compared to those of participants with UGS and FGS. MDA levels in participants with UGS showed no significant difference to those with FGS ($p< 0.05$). There were significantly higher MDA levels in vaginal secretions of participants with UGS and FGS compared to the uninfected. There was also a significantly higher MDA level in vaginal secretion of participants with FGS compared to secretion of participants with UGS.

Levels of inflammatory cytokines in serum and vaginal secretions:

The mean concentration of levels of inflammatory cytokines in both serum and vaginal secretion were determined and FGS effect on the cytokine levels is shown in Table 9. Results obtained shows that there was no significant correlation between FGS infected TNF-α, INF-γ, IL-1β, IL-6 and IL-10 levels in serum and vaginal secretion. There was no significant difference ($p>0.05$) in the cytokine profiles in serum and vaginal secretion between participants with FGS and non-infected participants.

Discussion:

The molecular identification of *S. haematobium* and *S. mansoni* infection gave a prevalence of 100% and 94.5% respectively. This reveals the co-endemicity of the area with both schistosome species. However, the prevalence of *S. haematobium* infection in this study is higher than the 63.8% UGS prevalence reported by Masong et al., (23). This higher prevalence may be due to the use of a more sensitive diagnostic method that was able to detect schistosomes in all its stages of development in blood. Nevertheless, among those infected, just 23.5% shed eggs in urine which is relatively lower compared to that reported by Njunda et al., (4) in Matta secondary and primary schools (41.1%) and that reported by Masong et al., (23) in some fishing communities in the Matta health area (63. 8%).

The prevalence of UGS (haematuria + egg shed) in this study was observed to be 45.7%, which is similar to that of a study carried out by Njunda et al., (4) across Matta Barrage community alone. The prevalence observed was relatively similar to that reported in other schistosomiasis foci in the South West region; 40.27% in Munyenge and 41.3% in Marumba (33). However, the prevalence of UGS observed in this study is higher compared to the overall prevalence of schistosomiasis reported in the West Region by Tchuem-Tchuente et al., (34) and in other areas of Cameroon including 37% in Tiko (33), 1.7% in Kékem (6), and 22.9% in Maroua (35). The higher prevalence of UGS reported in our study might be due to the difference in geographical settings, while Tiko, Kekem and Maroua are semi-urban/urban areas with improved water sources which limits the frequency of human water contacts (35), Matta health area is a rural area with natural and artificial ponds, and the presence of a lake on which their daily activities rely. Because Matta health area is an endemic area that has been targeted regularly for control of schistosomiasis and soil transmitted helminths through mass drug administrations, this may have accounted for the lower prevalence of urine egg excretion reported in the current study. Independent of the lower egg excretion prevalence, the higher prevalence with results from PCR revealed that either the infection was still at its early stage or some eggs were trapped in body tissues.

The overall estimated prevalence of FGS

Table 9: Association of inflammatory cytokines and FGS status

		Serum			Vaginal secretion		
Parameter	**Pairs**	**Mean ± SD (pg/ml)**	***t***	***p* value**	**Mean ± SD (pg/ml)**	***t***	***p* value**
TNF-α	Uninfected	13.75 ± 1.45	0.06	0.995	20.31 ± 1.64	-0.538	0.592
	FGS	13.71 ± 2.05			21.80 ± 2.32		
INF-γ	Uninfected	15.58 ± 2.22	0.195	0.845	22.51 ± 2.32	-0.198	0.843
	FGS	14.85 ± 3.10			23.25 ± 2.99		
IL-1β	Uninfected	13.92 ± 0.24	-0.99	0.922	13.52±0.24	-0.593	0.554
	FGS	13.98 ± 0.69			13.85±0.57		
IL-6	Uninfected	134.95 ± 21.36	0.546	0.586	151.54±21.86	0.435	0.664
	FGS	116.20 ± 26.27			136.94±24.81		
IL-10	Uninfected	277.99 ± 40.77	0.910	0.365	347.81± 40.61	-0.06	0.995
	FGS	220.05 ± 45.83			348.20±49.1		

(59.9%) in this study is similar to Masong et al., (23), who reported a prevalence of 58%. This similarity could be as a result of the sensitivity of the diagnostic techniques and the signs and symptoms on which diagnosis was based. Our study explored PCR identification of schistosome DNA and signs such as vaginal itches, burning sensation and abdominal pain for diagnosis whereas vaginal lesions by colposcopy, egg load in urine and other similar criteria, which were used in the study by Masong et al., (23). FGS prevalence was solely due to *S. haematobium* infection and no association with *S. mansoni* was observed. FGS appeared to be higher in married women than in unmarried women and was also higher in females aged ≥ 35 years compared to other age groups. This is similar to Masong et al., (23) who reported a higher FGS prevalence in those aged ≥29 years and those married. Unlike Masong et al., (23) which reported that single females did not show any "FGS + eggs", our current study reported a higher prevalence of "FGS + eggs" in singles (15.4%) than that in married women (10%).

Painful urination was identified as the most common symptom associated with *S. haematobium* infection which is not very different from the findings of a similar study by Saotoing et al., (35) on urinary schistosomiasis in the Northern regions of Cameroon and Njunda et al., (4) in the Matta Barrage community. Frequent contact with the lake was identified as the associated factor for infection with the *Schistosoma* parasite. Many inhabitants in Cameroon especially in rural areas have limited access to portable water for domestic use leaving them with the option of using natural water bodies such as lakes, rivers, ponds and dams which may be infested with the parasite (34). As the lake was the main source of water for fishing, irrigation and domestic use, it is more likely that the people became infected as they come in contact with the infested water.

The study participants appeared to have good knowledge on schistosomiasis with most of the information coming from community health workers. This result is different from that reported by Njunda et al., (4) and Kamga et al., (36) which reported lack of knowledge of the disease in Cameroon. This improvement in knowledge can be attributed to the mass drug administration of Praziquantel under the National Program for the Control of Schistosomiasis and Soil Transmitted Helminths which is being conducted yearly and usually involves sensitization of the population (37). This mass drug administration has made it possible for some rural areas to receive education concerning schistosomiasis and its clinical manifestations.

Given that there is need for the human host to maintain a critical balance between antioxidant defence mechanisms and the generation of reactive oxygen species and other free radicals, the measurement of the antioxidant capacity, level of oxidative damage and level of reactive oxygen species were carried out. The measurement of the antioxidant capacity was done using the levels of reduced glutathione, superoxide dismutase and catalase, the level of oxidative destruction was assessed using malondialdehyde, and the level of reactive oxygen species was carried out by measuring nitric oxide. Catalase is responsible for the conversion of hydrogen peroxide to water and oxygen and it was observed that the levels of catalase activity was significantly lower in serum and vaginal secretions of women with UGS and FGS compared to the uninfected. This result is in line with that reported by Aloho-Bekong et al., (38) which evaluated the level of oxidative stress is serum of school children with schistosomiasis and other helminthiasis.

The level of catalase activity was also seen to be significantly lower in vaginal secretions of women with FGS compared to those with UGS. This significant reduction of catalase activity can be attributed to the effect of the *Schistosoma* parasite on the liver. The liver is the major organ involved in the production of catalase and schistosomiasis is known to induce liver fibrosis (39), which can account for the decreased catalase level during infection. The significant reduction of catalase activity in vaginal secretion of women with FGS compared to those with UGS can also be attributed to the fact that the parasite feeds on red blood cell which is one of the cells with the most amount of catalase (9). Also, since accumulation of schistosome egg in host tissue causes inflammation and granuloma characterized by reactive oxygen species production, it can be said that the reactive oxygen species produced in FGS has resulted to the modification of the catalase enzyme and its synthesis. This is because reactive oxygen species has been shown to have several dysfunctional effects on various cellular components that are involved in the transcription and translation of enzymes including catalase (15).The reduced activity of catalase will result to severe oxidetive stress due to the accumulation of hydrogen peroxide within host tissues.

Superoxide dismutase (SOD) acts as the first line of defence against oxidative stress by converting superoxide to hydrogen peroxide and the hydrogen peroxide is further converted to water and oxygen by catalase (40). The level of SOD activity was observed to be significantly lower in serum and vaginal secretion of women with UGS and FGS compared to the uninfected. This result is similar to that reported by Aloho-Bekong et al., (38) which evaluated the level of oxidative stress is serum of school children with schistosomiasis

and other helminthiasis. The results obtained in this study can be attributed to the fact that hydrogen peroxide is released by phagocytic cells as a means to fight against helminthic infections resulting to high levels of hydrogen peroxide which will exert a negative feedback mechanism on the activity of SOD (41). Also, schistosomiasis has been shown to interfere with the activity of antioxidant enzymes thr-ough a mechanism that affects the expres-sion of the enzymes (42).

Nitric oxide (NO) produced by the enzyme nitric oxide synthase during inflam-mation act as a signalling molecule and a vasodilator for rapid blood flow to the inflamed organ (43). The level of NO was observed to be significantly higher in serum and vaginal secretion of women with UGS and FGS com-pared to the uninfected, which is similar to the findings of a study conducted by Nkam et al., (44) on the implication of serum NO and IgE in urinary schistosomiasis in Bamendjing, West region of Cameroon. The higher level of NO can be attributed to the ability of schisto-somal eggs to induce inflammation in various tissues and organs and investigations have shown that during schistosomiasis induced inflammation there is increased nitric oxide synthase expression in macrophages which are not normally expressed in resting cells (22). This nitric oxide acts as a precursor of other reactive species like nitroxyl anion, nitrosonium cation and higher oxides of nitrogen, S-nitrosothiols and dinitrosyl iron complexes. These substances have the ability to induce oxidative damage to other biomole-cules (43).

Malondialdehyde (MDA) is known to be a characteristic product of lipid peroxidation induced by reactive oxygen species during oxidative stress conditions (38). The level of MDA in serum and vaginal secretion of UGS and FGS infected persons was observed to be higher than that of the uninfected. This result is in line with that reported by Aloho-Bekong et al., (38) which investigated on the level of oxidative stress in serum of school children with schistosomiasis and other helminthiasis in Makenene, Centre Region of Cameroon. The results are also similar to the findings of Facundo et al., (45) which studied the levels of lipids peroxidation in patients with schistoso-miasis. These results can be attributed to the increased release of reactive oxygen species in schistosomiasis principally by phagocytic macrophages as a means to fight infection (22). There was also a significantly higher level of MDA in vaginal secretion of women with FGS compared to those with UGS indi-cating severe oxidative stress induced in the urogenital tract due to the presence of parasite eggs.

Reduced glutathione (GSH) plays a very important role in removing hydrogen per-oxide produced to prevent oxidative stress. The enzyme glutathione peroxidase catalyses the reduction of GSH by hydrogen peroxide to oxidised glutathione (GSSG) which is reversed by the enzyme glutathione reductase (46). The level of GSH in both serum and vaginal secretion was observed to be higher in the uninfected compared to those with UGS and FGS. This reduction in GSH in infected persons can be attributed to the increased cytotoxicity associated with hydrogen peroxide release which has an inactivation effect on the enzyme glutathione reductase responsible for GSH turnover from GSSG (47). The measurement of these biomarkers of oxidative stress was done in both serum and vaginal secretion due to the fact that serum levels do not give a clear indication of the level of tissue specific damage (46). This was observed to be so given that though there was no significant difference in levels of the various biomarkers in serum of women with UGS compared to those with FGS, there exist a significant difference in these biomarkers in vaginal secretions of the women with UGS when compared to those with FGS.

In this study, the mean TNF-α, INF-γ, IL-1β, IL-6 and IL-10 levels between women with FGS and uninfected for both serum and vaginal secretion was not statistically different from each other. The presence of other helminths in the study site might have infected the participants together with schistosomes and this might be responsible for these find-ings. No correlation was observed between TNF-α, INF-γ, IL-1β, IL-6 and IL-10 levels in serum and vaginal secretion of women with FGS. This agrees with the study by Njaanake et al., (48) which reported no correlation between levels of urinary and serum IL6, IFN-γ, TNF-α and IL-10 among children. These findings suggest that there may be differences between systemic and local mucosal cytokine secretion during infections in the genital tract. T_{h1} immune cytokines (TNF-α, INF-γ, IL-1β and IL-6) favour the production of proinflam-matory responses to schistosomes infection and activate immune cells while T_{h2} immune cytokines (IL-10) tend to produce anti-inflam-matory response.

IL-10 plays a crucial role in immuno-regulation and inflammation. It represents an important element in the down-regulation of the T_{h1} response by preventing excessive inflammation in the early stages of the infec-tion limiting tissue damage. However, this may also allow the schistosome to persist in its host, as the immune response is not strong to eliminate it, leading to chronic disease. This study shows a significantly higher IL-10 cyto-kine level compared to that of T_{h1} cytokines levels. The review done by Masamba et al., (22) indicate that a shift towards a more anti-inflammatory immune response of schistoso-miasis infection may contribute to the severity

of the disease. TNF-α, INF-γ and IL-1β are proinflammatory cytokines that are typically elevated during the acute phase of the disease (22). They play a role in activating the immune cells, promoting inflammation and recruiting immune cells to the site of infection. As the infection progresses, there is a shift toward a more anti-inflammatory immune response leading to a decrease in these cytokines level as seen in the current study.

IL-6, is a pleotropic cytokine that has both anti-inflammatory and proinflammatory response (49). It is produced in response to the infection and tissue damage. In this study, its level was significantly higher compared to other proinflammatory cytokines evaluated. This might be due to the fact that it also helps to mobilise immune cells to the site of infection thereby facilitating the pathogenesis of the disease (50).

Conclusion:

The present study reports a *S. haematobium* prevalence of 23.5% by microscopic and morphological identification. Molecular identification gave prevalence of 100.0% and 94.5% in bloodspot for *S. haematobium* and *S. mansoni* respectively, and the prevalence of FGS in the Matta Health area was 60%. Lake usage, marital status and symptoms such as abdominal pain, painful urination, vaginal itches and burning sensation were significantly associated with UGS in the Matta Health area. Marital status was identified as a key risk factor among participants, with single women at higher risk of contracting schistosomiasis compared to married women.

Higher levels of malondialdehyde and nitric oxide, and lower levels of reduced glutathione, catalase and superoxide peroxidase activities in UGS and FSG indicate oxidative stress in women living in the Matta Health area. High levels of IL-10 in serum and vaginal secretion compared to proinflammatory cytokines (IL-1β, IL-6, INF-γ and TNF-α) might prevent immunopathology and also limit the severity of the disease, with a shift to T_{h2} immune response.

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Contributions of authors:

MSDN conceived and designed the study; SIU, MDE, FIA, and CDN collected samples and carried out the major part of study; MSDN, SIU, CDN, and PAN analyzed the data and wrote the manuscript; MSDN, MBT, and AFL made provision of reagents and proofread the manuscript. All authors reviewed and approved the manuscript submitted for publication.

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