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Female Genital Schistosomiasis Lesions Explored Using Circulating Anodic Antigen as an Indicator for Live Schistosoma Worms

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Background: In areas where reinfection with schistosomiasis is rampant, it is not known if the lesions of Female Genital Schistosomaisis are a consequence of live worms, or caused by dead ova. Live schistosome worms regurgitate Circulating Anodic Antigen (CAA). We sought to explore the association between the different lesions of FGS (grainy sandy patches, homogenous yellow patches, rubbery papules and abnormal blood vessels) and the presence of live worms as indicated by *S. haematobium*-derived CAA in blood.

Materials and Methods: In this cross-sectional study, rural high schools were randomly selected from Ilembe, uThungulu and Ugu Districts on the East Coast of South Africa, KwaZulu-Natal Province. Serum samples for CAA analysis were collected from 246 female learners aged 16 - 23 years. Uncorrected chi-square and odds ratio with 95% confidence interval (CI) were used to evaluate the null hypothesis.

Results: CAA was positive in 82/246 (33%) of the participants. Sandy patches were found in 123 (50%) of the study population. Grainy sandy patches were significantly associated with CAA even after controlling for age (Adjusted Odds Ratio (AOR) 4.2, 95% CI 2.3 - 7.9, $p < 0.001$). Likewise, abnormal blood vessels were associated with CAA (AOR 3.0, 95% CI 1.5-4.5, $p = 0.001$) whereas homogenous yellow patches were not associated with CAA ($p = 0.57$). Rubbery papules were not found in this study population.

Conclusion: Grainy sandy patches and abnormal blood vessels are found more commonly in women who harbour live *Schistosoma haematobium* worms whilst homogenous yellow patches may indicate chronic tissue damage due to dead ova.

Keywords: female genital schistosomiasis, FGS, CAA, homogenous yellow patch, grainy sandy patch

INTRODUCTION

Female Genital Schistosomiasis (FGS) is a common complication of schistosomiasis caused by *Schistosoma (S.) haematobium* ova deposited in genital tissue (1). Two different morphologic subtypes of genital sandy patches have been described, namely: grainy sandy patches and homogenous yellow patches at 15 times magnification (1). In addition, women with FGS may have abnormal blood vessels, contact bleeding and rubbery papules. Diagnosis is made by visual inspection of the characteristic genital sandy patches (also called FGS lesions) on the cervix and vaginal wall (2).

Millions of women have domestic, commercial and recreational contact with infested waterbodies and it is estimated that 56 million women in sub-Saharan Africa have FGS (3), and almost 20 million more cases will occur in the next decade unless girls are treated (4). FGS has been linked to abnormal discharge, a burning sensation in the genitals, sub-fertility, ectopic pregnancy, and increased transmission of HIV (3). The control of neglected tropical diseases is gaining momentum; however FGS is underdiagnosed, its burden is significantly underestimated and there is a "huge gap in epidemiological assessment" (3, 5–8).

Worldwide, only one-quarter of the schistosomiasis infected people have been treated and the optimal timing for prevention of morbidity, HIV susceptibility and infertility is unexplored (3). People may receive several rounds of treatment and yet continue to excrete ova, probably due to suboptimal efficacy of praziquantel and reinfection (9). One study found that genital lesions remained unchanged after treatment (9). However, it is not known if these lesions remained because patients were re-infected immediately or, alternatively, if some forms of FGS are irreversible.

Women and children have been found to have FGS from Egypt to Southern Africa and Madagascar (5, 10), and in some areas FGS may be more common than the sexually transmitted infections. Furthermore, a study in South African schools showed that more than 20% of adolescent girls and young women had FGS (11). Another study showed that children, before menstrual and sexual debut, have bloody and malodorous discharge (12), indicating that intra-vaginal morbidity starts in childhood. However, it is not known if FGS lesions are caused by recently laid ova from live worms (13).

The Circulating Anodic Antigen (CAA) is a regurgitate from live schistosome worms (14) and the Lateral Flow test utilizing Up-Converting reporter Particles is a highly sensitive and 100% specific test for this unique carbohydrate structure (15–17). CAA is measurable in serum and urine but would not be present if the worms are already dead.

In this study of adolescent girls and young women of KwaZulu-Natal Province of South Africa, we sought to explore the association between the different lesions of FGS and the presence of live worms as indicated by CAA in blood.

MATERIALS AND METHODS

Ethical Considerations

The study was approved by the Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal (Ref BF029/07), KwaZulu-Natal Department of Health (Reference HRKM010-08) and the Regional Committee for Medical and Health Research Ethics (REC), South Eastern Norway (Ref 46907066a1.2007.535). The ethical committees, BREC (annual renewal) and REC, were aware that minors (aged 16 and 17 years) were participating in the study and specifically approved independent minor consent without parental consent. According to South African legislation, persons over the age of 12 may consent independently to participate in research. Each participant received a detailed explanation of the gynaecological examination procedure for identifying lesions, and questions they had were answered. All study participants were offered anti-schistosomal and, if applicable, sexually transmitted diseases' treatment, and/or referral to the local health system for treatment of HIV when needed. STI treatment was offered to participants with clinical signs and symptoms, and their partners in accordance with the South African syndromic treatment protocol (18).

Study Subjects and Area

The study was conducted between 2011 and 2013 in the KwaZulu-Natal Province of South Africa. The participants were female learners, aged 16 - 23 years, from randomly selected high schools in Ilembe, uThungulu and Ugu Districts on the East Coast of South Africa that had not undergone anti-schistosomal mass-treatment the last year before investigation. The participants were recruited from schools that were classified as rural by the Department of Education and were below the altitude of 400 meters above sea level, with an estimated prevalence of *S. haematobium* of 10% or more based on an initial show of hands for red urine in Ugu District and a haematuria dipstick survey in Ilembe and uThungulu districts (11). Only those who completed the gynaecological examination were included; virgins, pregnant, and severely ill females were excluded. Schools with prevalence below 10% were excluded.

Questionnaires and Clinical Examinations

The investigation has been described previously but briefly, a questionnaire on water contact, reproductive history, genital and abdominal symptoms was administered individually to participants in isiZulu prior to gynaecological examination (11). The clinician performing the exams was blinded to the childhood origin schistosomal status. Examination was commenced by cervico-vaginal lavage. Saline (10 ml) was sprayed on the vaginal wall and cervix twice, whereupon it was drawn back into a syringe and deposited into four tubes. This was followed by photocolposcopic examination (Leisegang Photocolposcope, Germany, Magnifications 7.5; 15; 30 or Olympus OSC 500 photocolposcope, Olympus America Inc., Center Valley, PA, USA with a mounted camera Olympus E420, 10.0 megapixels) using an autoclaved metal speculum after which Pap (Papanicolau) smears were collected from all consenting women. The cervix, the fornices, the entire vaginal wall and vulval surfaces were inspected section by section according to a predefined protocol (1). Acetic acid and/or iodine application for colposcopic examination was always done last. Typical lesions of FGS are shown in **Figure 1**.

Laboratory Analyses

Serum samples were collected as has been described elsewhere and stored at minus 80 degrees celcius (19). The samples were analysed for CAA (14). After thawing, 0.5 mL 4% trichloro-acetic acid (TCA) was added to 0.5 mL of serum samples in 1.5mL microfuge tubes and mixed well using a vortex. The TCA/serum mixture was centrifuged for 15 minutes (13,000 rpm) using a Biofuge Pico 21 Heraeus and Biofuge Pico Heraeus centrifuges. 0.5mL clear TCA-supernatant was transferred to a concentration device (Amicon® Ultra 0.5 mL Centrifugal Filters; Merck-Millipore) and centrifuged for 30 minutes at 13,000 rpm. Assay buffer was dissolved and 80 µL added to hydrate dry UCP conjugate in microtiter wells. A 20 µL of the concentrated TCA-supernatant was mixed with hydrated UCP conjugate and incubated on thermos-shakers (60 min). LF was initiated by placing a UCP-LF CAA strip in the microtiter wells and left overnight. The UCP-LF CAA strips were read using the UCP-Quant reader (ESE Quant) and analysed with matched software, LateralFlowStudio version 3.3.7 (QIageN Lake Constance GmbH, Stockach, Germany). A sub-sample of the population (the early participants, before full sample was reached) had been investigated by Schistosoma PCR, as described previously (20).

Sample Size Calculation and Statistical Analyses

We planned a study of independent CAA positive and negative females. Prior data was not available for CAA, however as a proxy, in urine microscopy negative the probability of sandy patches is 40 percent. If the true probability of sandy patches among CAA positive is 60 percent, we needed to study 97 CAA positive and 97 CAA negative patients to be able to reject the null hypothesis that the sandy patch prevalence for CAA positive and CAA negative are equal with probability (power) 80%. To cater for uncertainties in the calculations we added 52 cases. The Type I error probability associated with the test of this null hypothesis is 0.05. We used an uncorrected chi-square statistic and odds ratio (OR) with 95% confidence interval (CI) to evaluate this null hypothesis; to study the association between CAA and different lesions of FGS (after controlling for age and treatment). Adjusted odds ratio was calculated using logistic regression to control for age and treatment. The SPSS version 27 (IBM) was used.

RESULTS

A total of 246 young females were included; 82 (33%) of these were positive for CAA. The mean age of the study participants was 19 years (standard deviation (SD) 1.8). Sandy patches were found in 123 (50%) of the study population, grainy sandy patches were found in 63 (26%) (**Figure 1**). Homogenous yellow patches were found in 75 (30.5%) females, and 118 (48%) had current risk water contact. Urine microscopy results were available for 243 participants, 39 (16%) were positive by microscopy of one urine sample, geometric mean 12.1 eggs/10mL (SD 43.9). A sub-

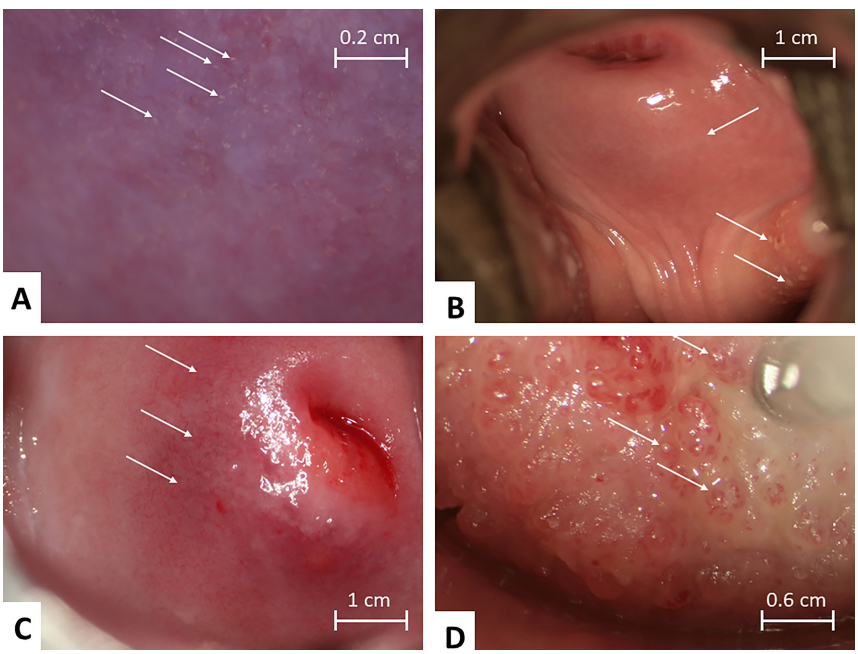

FIGURE 1 | Mucosal colposcopic findings of clinical manifestations of Female Genital Schistosomiasis. **(A)** Grainy sandy patches appearing as single grains and clusters of grains. **(B)** Rubbery papules in both fornices (left and right), abnormal blood vessels (circular) are seen on cervix 6 -7 o'clock. **(C)** Homogenous sandy patch on the cervix (clearly seen on left side of image), abnormal blood vessels are seen especially clearly 7 - 10 o'clock outside the transformation zone, mottled yellowish areas can be seen in the background. **(D)** Rubber papules on cervix found in a 22 year old women in Madagascar. Photos by: Elisabeth Kleppa, Bodo Raniandrasolo and Eyrun Kjetland.

sample of 178 study participants were tested by schistosoma PCR. **Table 1** shows the association between demographic variables, the lesions and the CAA in univariate analysis.

A total of 126 people reported that they had never been treated and 68 did not know. Of the 52 who had been treated, 26 reported their age at treatment was 10 years (SD 4). The time between previous treatment and the clinical investigation was mean 9 years (SD 4.8).

FGS Lesions: Grainy Sandy Patches and the Presence of Live Worms

In the sub-group of people with grainy sandy patches, 59% (37/63) were positive for CAA; the association was significant even after controlling for age (Adjusted Odds Ratio (AOR) 4.2, 95% Confidence Interval (CI) 2.3 - 7.9, p < 0.001), indicating the presence of live worms. Of those with grainy sandy patches, 42% (16/38) were schistosomiasis PCR positive (p < 0.001). Many had both sandy patch types, however, in a sub-analysis of those with grainy sandy patches only, 56% (27/48) were CAA positive (AOR 4.4, 95% CI 2.1 - 9.1, p < 0.001). Amongst those who had grainy sandy patches but were CAA negative, 5/26 (19%) had eggs in their urine upon microscopy.

FGS Lesions: Homogenous Yellow Patches and the Presence of Live Worms

In the sub-group of people who had homogenous yellow patches only (excluding the ones with grainy sandy patches), 30% (18/60) were positive for CAA (AOR 1.7, 95% CI 0.8 - 3.5, p = 0.15). Of those who had homogenous yellow patches only, 20% (9/45) were positive by PCR (p < 0.001).

FGS Lesions: Abnormal Blood Vessels and the Presence of Live Worms

Abnormal blood vessels were strongly associated with CAA (p = 0.001). Amongst those with abnormal blood vessels, 21% (15/73) were positive by Schistosoma PCR (p = 0.061).

CAA as a Continuous Variable

Confirming the finding above, CAA as a continuous variable was found to be significantly associated with grainy sandy patches after controlling for age (AOR 4.2, 95% CI 2.3 – 7.8, P <0.001), and abnormal blood vessels (AOR 2.7, CI 1.5 – 4.7, p = 0.001). However, CAA was not associated with homogenous yellow patches (AOR 1.4, CI 0.8 – 2.5, p = 0.26). CAA concentrations were not significantly associated with urinary egg count (p = 0.17).

Other Lesions, Symptoms and Live Worms

Red urine and thick and lumpy discharge were the only symptoms that were associated with CAA, indicating the presence of live worms (**Table 2**). No association was observed between CAA and other symptoms and lesions such as warts, polyps or ulcers (p > 0.4 for all).

Prior Treatments and Current Water Contact

Unexpectedly, CAA was positive significantly more often in those who had been treated (**Table 2**). The associations between CAA and grainy sandy patches or abnormal blood vessels were not influenced by prior treatment (Adjusted OR 3.0, 95% CI 1.4 - 6.6, p = 0.004 and Adjusted OR 2.8, 95% CI 1.4 - 5.5, p = 0.004, respectively). Likewise, CAA was not associated with homogenous yellow patches after controlling for prior treatment and age (Adjusted OR 1.2, 95% CI 0.6 - 2.6, p = 0.57).

Current risk water contact was not associated with grainy sandy patches (OR 1.2, 95% CI 0.7 - 2.1, p = 0.60), abnormal blood vessels (OR 1.6, 95% CI 0.9 - 2.6, p = 0.076), and homogenous yellow patches (OR 0.2, 95% CI 0.9 - 2.5, p = 0.17).

DISCUSSION

The current study shows, for the first time, that genital schistosomiasis - grainy sandy patches and abnormal blood

TABLE 1 | Association between demographic variables, sandy patches (FGS) and CAA (live worms).

	CAA[a] positive (N=82)	CAA negative (N=164)	p-value	Odds Ratio[b]	95% Confidence Interval
Mean age in years [Standard deviation (SD)]	19.1 (1.8)	18.5 (1.5)	0.003	1.3	1.1 - 1.5
Mean number of adults in the home (SD)	4.0 (1.7)	4.0 (1.8)	0.8	0.9	08-1.1
Mean number of children in the home (SD)	2.3 (1.7)	2.8 (1.8)	0.4	0.9	0.8-1.1
		CLINICAL FINDINGS			
Grainy sandy patch (%)[c]	37/82 (45)	26[d]/164 (16)	<0.001	4.4	2.4-8.0
Homogenous yellow patch (%)	28/82 (34)	47[e]/164 (29)	0.38	1.3	0.7-2.3
Superficial grainy sandy patch (%)[c]	26/82 (32)	17/164 (10)	<0.001	4.0	2.0-8.0
Deep grainy sandy patch (%)[c]	12/82 (15)	10/164 (6)	0.032	2.6	1.1-6.4
Any sandy patch (%)[f]	55/82 (67)	68/164 (42)	<0.001	3.0	1.7-5.0
Abnormal blood vessels (%)	53/82 (65)	68[g]/164 (42)	0.001	3.0	1.5-4.5
Pre-contact bleeding (%)	1/82 (1)	1/164 (1)	1.00	1.0	0.1-11.1
Contact bleeding (%)	5/82 (6)	9/164 (6)	0.85	1.1	0.4-3.5
Dilated blood vessels (%)	9/82 (11)	14/164 (9)	0.54	1.3	0.5-3.2
Genital ulcer (%)	3/82 (4)	4/164 (2)	0.59	1.5	0.3-7.0
Cervical intra-epithelial neoplasia (%)	4/82 (5)	9/164 (6)	0.84	0.8	0.3-3.0

Some had several types of lesions concurrently.

[a]CAA is an indicator for the presence of live worms, [b]Unadjusted, [c]Constituting grains of 0.05 × 0.2 mm long grains shaped like minuscule rice grains in clusters of up to 300, [d]Sub-sample tested by PCR: 3/18 were Polymerase Chain Reaction (PCR) positive, [e]Sub-sample tested by PCR: 2/34 were PCR positive, [f]Summary of all the sandy patch types, [g]Sub-sample tested by PCR: 2/44 were PCR positive.

TABLE 2 | Association between laboratory results, symptoms and patient history and CAA (live worms).

	CAA[a] positive (N=82)	CAA negative (N=164)	p-value	Odds Ratio[b]	95% Confidence Interval
LABORATORY TECHNIQUES					
Schistosoma PCR vaginal lavage positive (%)[c]	6/49 (12)	4/124 (3)	0.032	4.2	1.1-15.5
Schistosoma PCR urine positive (%)[d]	16/48 (33)	8/124 (7)	<0.001	7.3	2.8-18.5
Urinary egg excretion (microscopy) (%)[e]	29/82 (35)	10/161 (6)	<0.001	8.3	3.8-18.1
Mean urinary egg count (SD)	8.3 (17.9)	3.0 (21.6)	0.11	1.0	0.9-1.0
SYMPTOMS					
Red urine previously or now (%)	35/82 (43)	37/164 (23)	0.001	3.0	1.4-4.5
Experienced thick/lumpy discharge (%)	18/82 (22)	20/164 (12)	0.05	2.0	1.0-4.1
Bloody discharge (%)	11/81 (14)	19/164 (12)	0.31	1.2	0.8-1.8
Lower abdominal pain (%)	26/82 (32)	55/164 (34)	0.55	1.0	0.7-1.2
Irregular menstruation (%)	25/53 (47)	58/103 (56)	0.28	0.7	0.4-1.4
OTHER PATIENT HISTORY					
Previously treated for schistosomiasis (%)	32/65 (49)	20/113 (18)	<0.001	5.0	2.3-8.9
Mean number of pregnancies (%)	52/82 (63)	87/164 (53)	0.15	1.4	0.9-2.2
Father in the home (%)	17/82 (21)	35/163 (22)	0.89	1.0	0.5-1.8
Mother in the home (%)	51/82 (62)	104/164 (63)	0.85	1.0	0.5-1.6
Water contact at any time point (%)	78/82 (95)	149/164 (91)	0.25	2.0	0.6-6.1
Any risk water contact the last year (%)[f]	41/82 (50)	77/164 (47)	0.65	1.1	0.7-1.9

[a]CAA is an indicator for the presence of live worms, [b]Unadjusted, [c]Only 173 were tested for PCR, [d]Only 172 were tested for Polymerase Chain Reaction, [e]Only 243 were tested for urinary schistosomiasis via microscopy, [f]River, lake or dam.

vessels - are found in women who harbour live *Schistosoma haematobium* worms. However, homogenous yellow patches in the genitals, although associated with urinary egg excretion, seem not to be associated with live worms; indicating that homogenous yellow patches may be a result of a long-standing infection or chronic tissue damage caused by constant irritation from dead ova. We found that the presence of CAA was associated with having received anti-schistosomal treatment previously. This was expected as study participants live in an endemic area with continuous risk of exposure and developing morbidity, it may be an indication of continuous re-infection (21).

Current exposure to schistosomiasis infested water was, however, not associated with any of the lesions, indicating that lesions may have been established in childhood and possibly maintained by a continuous flow of new ova (21). Laboratory technicians did not differentiate between calcified and viable-looking ova during microscopy of urine and therefore we cannot preclude that *S. haematobium* positive women with homogenous yellow patches lesions may have had dead ova. Schistosoma PCR may also be an indication of recent egg deposition, however, only a sub-sample had been tested by PCR, therefore results should be interpreted with caution. A lack of association between homogenous yellow patches and CAA in this study could also represent a Type 2 error. Therefore, further studies are needed to confirm the chronicity of homogenous yellow patches.

In this study area, mass-treatment had not yet been rolled out (22). Therefore, participants had only been treated upon presentation at the local clinics. Such individual therapy is often not repeated, the surrounding community members are still infesting the waters, and infection control is not sustained, resulting in new live worms and genital lesions in these young women. South Africa should implement mass-treatment in endemic areas (23). Furthermore, as recommended by UNAIDS, WHO, many scientists, and programme managers, prevention of FGS lesions and screening should be integrated into sexual and reproductive health programmes, such as HPV vaccination, cervical cancer screening, antiretroviral therapy, and pre-exposure prophylaxis (PrEP) for HIV/AIDS (3, 7).

The findings indicate that some lesions may be untreatable and further studies are needed to explore the significance of the different lesions and the living worms on symptoms, cervical cancer and HIV susceptibility. Furthermore, it is still not clear for how long homogenous yellow patches, symptoms, and risks persist after the worms are dead. CAA may be effective to do this research but CAA is not yet available as a diagnostic tool, it may not be affordable in routine health care.

For now, patients should be informed that lesions and symptoms may be refractory to treatment. Nevertheless, they should be treated to kill the living worms and prevent further morbidity. Further investigations and a follow-up study using CAA and PCR, with a larger sample size, several urine samples and biopsies, differentiating between viable-looking and dead ova by microscopy, are needed.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Biomedical Research Ethics Committee (BREC), University of KwaZulu-Natal (Ref BF029/07), KwaZulu-Natal Department of Health (Reference HRKM010-08) and the Regional Committee for Medical and Health Research Ethics (REC), South Eastern Norway (Ref 46907066a1.2007.535). The ethical committees, BREC and REC, were aware that learners

who are 16 - 17 years old were participating in the study and specifically approved the consent procedure (independent minor consent, no parental consent). According to South African legislation, persons over the age of 12 may consent independently to participate in research. STI treatment was offered to participants with clinical signs and symptoms, and their partners in accordance with the South African syndromic treatment protocol. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

TN conceived the presented idea, carried out the laboratory and statistical analysis, contributed to the writing and finalization of the manuscript. EFK helped conceive the presented idea, supervised the project, carried out statistical analysis, contributed to the writing and finalization of the manuscript. SN helped supervise the project and contributed to writing. GD and PC supplied laboratory test kits and contributed to writing. PP contributed to writing. EK, HG-A, SG, BJV, PN, and MT contributed to conceptualization and writing. All authors contributed to the article and approved the submitted version.

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