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ARTICLES

Lower gastrointestinal bleeding: Spectrum of colonoscopy findings in Ado-Ekiti, Nigeria
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Trichomonas vaginalis cases presenting at the Komfo Anokye Teaching Hospital, Ghana over a period of 11 years: 1994 to 2004
Lower gastrointestinal bleeding: Spectrum of colonoscopy findings in Ado-Ekiti, Nigeria

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Lower gastrointestinal bleeding (LGIB) is a common ailment seen at emergency departments. It is a significant cause of morbidity and mortality in the elderly worldwide. The aim of this study was to determine the aetiology and management outcome of LGIB in our centre and compare it with results elsewhere. Sixty-eight consecutive patients who underwent colonoscopy for LGIB were recruited into this study. The study was carried out at the Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti, Nigeria from January, 2010 to December, 2012. Ethical approval for the study was obtained from hospital’s Ethics Committee and all the patients gave their individual signed consent. Relevant data were retrieved and analyzed using statistical package for social sciences (SPSS) version 15.0 (SPSS, Inc., Chicago, Illinois, USA) for statistical analysis using the t-test for quantitative variables and χ² test for qualitative variables. Differences were considered to be statistically significant if P value was less than 0.05. The male: female ratio was 1.83:1. The mean age of the studied population was 56.04 ± 10.60 (age range 30 to 75). The indications for colonoscopy were; melena (11.8%), haematochezia (52.9%) and both (35.5%). Findings at colonoscopy were; haemorrhoids (35.3%), colorectal cancer (16.2%), polyps (14.7%), anal fissure (13.2%), arteriovenous malformations (5.9%) and diverticulosis (4.4%). Normal findings were reported in 10.3%. While haemorrhoids, anal fissure, colorectal cancer, polyps and diverticulosis were more prevalent in the male populations, arteriovenous malformation was more prevalent in the females. Co-morbidities found included; diabetes (14.7%), chronic liver disease (14.7%), hypertension (36.7%), diabetes and hypertension (16.2%) and renal disease (5.9%) of the studied population. These findings were found to be statistically significant ($\chi^2 = 68.535$, p = 0.001, $\alpha = 0.05$ that is, 95% confidence interval). Haemorrhoids followed by colorectal cancer are the commonest colonoscopy findings in our environment. It is recommended that colonoscopy should be embraced for routine cancer screening and surveillance in our society.

Key words: Colonoscopy, lower gastrointestinal bleeding, emergency departments.

INTRODUCTION

Lower gastrointestinal bleeding (LGIB) is defined as bleeding that occurs from the bowel distal to the ligament of Treitz (Longstreth, 1997). It is a significant cause of morbidity and mortality in the elderly worldwide. The
incidence of LGIB increases with age and is more common in men than women (Potter and Sellin, 1988). The annual incidence of hospitalization for LGIB is estimated to be 20 to 30 per 100,000 persons in a large, Southern California health maintenance organization (Longstreth, 1997). LGIB is approximately one-fifth as common as upper gastrointestinal bleeding (UGIB) (Kollef et al., 1997; Peura et al., 1997; Velayos et al., 2004). While most patients with LGIB will stop bleeding spontaneously, recurrent bleeding occurs in 10 to 40% of patients (Chaudhry et al., 1998; Das et al., 2003). In contrast to UGIB, predictors of poor outcome in LGIB are not that well defined. Hemodynamic instability, ongoing haematochezia and presence of comorbid illness have been associated with poor outcome (Bhasin and Rana, 2011).

The causes of LGIB vary from one region of the world to the other. In the countries of Western Europe and the United States where diverticulosis coli is common, it is one of the most common causes of LGIB unlike in Asia, diverticulosis coli is uncommon and is much less responsible as a cause of LGIB in the region (Longstreth, 1997). Colonoscopy when performed within 12 to 24 h of bleeding or admission is the preferred diagnostic procedure after stabilization in patients with lower gastrointestinal (GI) bleeding. The diagnostic yield of colonoscopy is more than radiographic tests, which require active bleeding at the time of the radiological examination. The diagnostic yield of urgent colonoscopy in acute lower GI bleed has been reported to be between 75 to 97% depending on the definition of the bleeding source, patient selection criteria and timing of colonoscopy (Barnert and Messmann, 2009; Wong and Baron, 2008). Literature is very scanty as regards the aetiology of LGIB in Nigeria. The aim of this study is to determine the aetiology and management outcome of LGIB in our centre and compare it with results elsewhere.

MATERIALS AND METHODS

Study location

This study was carried out at the Ekiti State University Teaching Hospital (EKSUTH), Ado-Ekiti, Nigeria from January, 2010 to December, 2012.

Study population

Sixty eight consecutive patients who underwent colonoscopy for LGIB were recruited into this study.

Inclusion and exclusion criteria

All patients age 18 years and above with LGIB were included in the study, while patients with severe cardiopulmonary instability/failure were excluded.

Data collection

The following were extracted from the patients or their relations: age, gender, previous history of LGIB, use of aspirin or nonsteroidal anti-inflammatory drugs (NSAIDS), alcohol ingestion, use of native concoctions, melena and haematochezia.

Procedure

Colonoscopy was performed within 48 h after adequate resuscitations were carried out using intravenous normal saline, blood transfusion and parenteral omepraprazole. The procedure was carried out using video-colonoscopes (CF 130 Olympus). Colon preparation was achieved by the oral administration of 3 liters of Movicol® and Ducolax® suppository, given 12 to 18 h before the examination. Blood pressure and oxygen saturation were monitored with mercury sphygmomanometer and pulse oxymeter, respectively. Warm water (37°C) infusion method was used instead of the traditional air insufflations. This method significantly gave a better patient procedure tolerance, better evaluation of the mucosal wall and adenoma detection rate. Findings at endoscopy were documented.

Ethical clearance

Ethical approval for the study was obtained from the hospital’s Research and Ethics Committee and all the patients gave their individual written consent.

Statistical analyses

SPSS version 15.0 (SPSS, Inc., Chicago, Illinois, USA) was deployed for statistical analysis using the t-test for quantitative variables and χ² test for qualitative variables. Differences were considered to be statistically significant if P value was less than 0.05.

RESULTS

The male: female ratio was 1.83:1. The mean age of the studied population was 56.04 ± 10.60 years (age range 30 to 75). Majority of the patients were in the age group 51 to 70 years (Table 1). LGIB was found to increase steadily with age up to the seventh decade of life when a sharp decline was noticed. The indications for colonoscopy were; melena (11.8%), haematochezia (52.9%) and both (35.5%) (Table 2 and Figure 1).

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Table 1. Age group distribution among the study participants.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Frequency</th>
<th>Percentage</th>
<th>Cumulative %</th>
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<td>1</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>31-40</td>
<td>7</td>
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<td>3</td>
<td>4.4</td>
<td>100.0</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Table 2. The indications for colonoscopy among the study participants.

<table>
<thead>
<tr>
<th>Indication</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melena</td>
<td>8</td>
<td>11.8</td>
</tr>
<tr>
<td>Haematochezia</td>
<td>36</td>
<td>52.9</td>
</tr>
<tr>
<td>Melena/Haematochezia</td>
<td>24</td>
<td>35.3</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Figure 1. Indications versus age group.

Findings at colonoscopy were; haemorrhoids (35.3%), colorectal cancer (16.2%), polyps (14.7%), anal fissure (13.2%), arteriovenous malformations (5.9%) and diverticulosis (4.4%). Normal findings were reported in 10.3% (Figure 2). While haemorrhoids, anal fissure, colorectal cancer, polyps and diverticulosis were more prevalent in the male populations, arteriovenous malformation was more prevalent in the females. These
findings were not statistically significant ($\chi^2 = 8.867$, $p = 0.181$, $\alpha = 0.05$ that is, 95% confidence interval). Co-morbidities found included: diabetes (14.7%), chronic liver disease (14.7%), hypertension (36.8%), coexistence of diabetes and hypertension (16.2%) and renal disease (5.9%) of the studied population (Figure 3). These findings were found to be statistically significant ($\chi^2 = 68.535$, $p = 0.001$, $\alpha = 0.05$ that is, 95% confidence interval). None of the patients had a previous history of LGIB.

DISCUSSION

LGIB is a significant cause of morbidity and mortality in the elderly worldwide. It is also one of the most common gastrointestinal indications for hospital admission. The incidence increases with age and is more common in men than women (Potter and Sellin, 1988). Our study equally confirmed this statement. The male: female ratio was 1.83:1 with a male preponderance in all age groups. This male preponderance is similar to what was reported by (Olookoba et al., 2013) in the North Central region of Nigeria. LGIB was found in this study to increase steadily with age up to the seventh decade of life. This is similar to similar studies elsewhere outside African continent by Chait (2010), Comay and Marshall (2002) and Longstreh (1997). This increase in incidence of LGIB with increasing age can be adduced to two factors commonly found in the elderly: (1) the increased incidence of gastrointestinal disease specific to elderly patients and (2) co-morbid diseases. Co-morbid diseases found in this study were; diabetes mellitus (14.7%), hypertension (36.8%), diabetes and hypertension (16.2%), chronic liver disease (14.7%) and renal disease (5.9%). Majority of our patients have at least one coexistent illness. This was similar to the findings of (Al Qahtani et al., 2002; Schmulewitz et al., 2003).

Findings at colonoscopy in our study were; haemorrhoids (35.3%), colorectal cancer (16.2%), polyps (14.7%), anal fissure (13.2%), arteriovenous malformations (4%) and diverticulosis (4.4%). Haemorrhoids were the commonest cause of LGIB in this study similar to the findings by Alatise et al. (2012), Dakubo et al. (2008) and Olookoba et al. (2013). This was contrary to the pattern in the Western world where diverticular diseases, colorectal cancer and angiodyspasias were the common findings at colonoscopy (Strate, 2005). Contrary to the general belief that colorectal cancer is not common in our environment, with the availability of colonoscopy, this had been debunked as shown in this study where colorectal cancer ranked as the second commonest finding (16.2%). This may in part be due to increased

![Figure 2. Findings at colonoscopy among the study participants.](image-url)
Westernization among the populace. The findings in this study that showed haemorrhoids topping the list of the findings at colonoscopy might be due in part to frequent or chronic constipation, straining to have a bowel movement, diets low in fiber and pregnancy. Little information exists as regards racial differences in LGIB. However, this geographic variation may be due in part to dietary and lifestyle factors. In this study, haemorrhoids, anal fissure, colorectal cancer, polyps and diverticulosis were found to be more prevalent in the male populations while arteriovenous malformation was more prevalent in the females. The reasons for these findings are not known. The indications for colonoscopy in this study were; melena (11.8%), haematochezia (52.9%) and both (35.5%).

Most patients with LGIB have favorable outcomes despite advanced age and comorbid conditions (Boley et al., 1979). While most patients with LGIB will stop bleeding spontaneously, continued or recurrent bleeding during an acute episode occurs in 10 to 40% of patients (Das et al., 2003). All the patients studied were managed conservatively with fluid replacements, parenteral omeprazole and blood transfusions. Those that required advanced interventional endoscopy therapy were duly referred after stabilization to other facilities. Endoscopic polypectomy was carried out in those that had polyps. Among those that had haemorrhoids, 25% had haemorroidal banding while 75% had haemorroidectomy successfully carried out. In all, seven patients died, giving a mortality rate of 10.3%. These deaths were recorded among those having colorectal cancers. Most of these patients presented in the late advanced form.

Colonoscopy was carried out in this study within 12 to 48 h of admission and it was found to be safe and effective. This was similar to the findings in the studies of Strate and Syngal (2003). Generally, the diagnostic yield of colonoscopy ranges from 45 to 95% (Al Qahtani et al., 2002), the diagnostic yield in this study was 89.7%. The high yield obtained here was similar to the findings of Olookaba et al. (2013), much higher than that of Dakubo et al. (2008), Ismaila and Misauno (2011) and Mbengue et al. (2009). This finding was contrary to the low yield.
found by Al-Shamali et al. (2001) (21%) in the Saudis and Sahu et al. (2009) (48%) in the Indian patients. These observed differences may be due to the varying spectrum of colonic diseases across the world regions and the water method used in this study as against the traditional air inflation.

Conclusion

Haemorrhoids followed by colorectal cancer are the commonest colonoscopy findings in our environment. It is recommended that colonoscopy should be embraced for routine cancer screening and surveillance in our society.

REFERENCES


Expression of toll-like receptor (TLR)-2 and TLR4 in monocytes following stimulations by genital secretions of HIV infected and uninfected women with symptomatic vulvo-vaginal candidiasis

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Vulvo-vaginal candidiasis (VVC) is a common condition in human immunodeficiency virus (HIV)-infected women. Toll-like receptor (TLR) 2 and TLR4 are key pattern-recognition receptors of the innate immune system in sensing Candida albicans. The aim of this study was to assess the expression of TLR2 and TLR4 signaling pathways in HIV-infected and uninfected women with VVC. Cervico-vaginal fluids (CVF) were obtained from 7 HIV infected and 11 HIV uninfected clinic attendees in KwaZulu-Natal between June, 2011 and December, 2011. VVC was diagnosed clinically and confirmed by Gram stain and culture of genital samples. Monocytes were isolated from a healthy adult volunteer, pre-incubated with anti-TLR2, anti-TLR4 and a combination of anti-TLR2/anti-TLR4 monoclonal antibodies. Monocytes were then stimulated by CVF. Levels of cytokines were measured by Luminex® multiplex immunoassays. Compared with baseline concentrations, stimulation with CVF of HIV+VVC+ women post-TLR2 blockage increased IL-6, IL-10 and IL-13 production by 165.5, 162.5 and 106.7%, respectively. Using paired T-tests, there was a significant difference in the increase of the concentrations of IL-6 (P = 0.04), IL-10 (P = 0.003), and IL-13 (P = 0.031) when comparing stimulation by CVF of HIV+VVC+ versus stimulation by CVF of HIV-VVC+ patients. There was a linear correlation between genital HIV RNA loads and mean level production of IL-6 (r = 0.722; R² = 0.679; P = 0.067) as well as IL-8 (r = 0.910; R² = 0.833; P = 0.004). Findings suggest potential roles of TLR2 in the pathogenesis of VVC among HIV-infected women.

Key words: Symptomatic VVC, HIV, TLR2 and TLR4.

INTRODUCTION

In human immunodeficiency virus (HIV) infected women, symptomatic vulvo-vaginal candidiasis (VVC) is
seen to be frequent and less effectively responsive to conventional anti-fungal therapy. Reasons are not well understood. *Candida albicans* has been reported as the cause of VVC in 85 to 95% of cases (Sobel, 2007). The cell wall of *C. albicans* is composed of pathogen-associated molecular patterns (PAMPs), especially polysaccharides like chitin, 1,3-β-glucans and 1,6-β-glucans and proteins that are heavily mannosylated with mannann side-chains.

Pathogen recognition receptors (PRRs), such as the toll-like receptors (TLRs) and C-type lectins (CLR)s on the surface of antigen presenting cells (APCs) are able to recognize PAMPs. Studies have shown that TLR2 recognizes phospholipomannans; TLR4 recognizes O-linked mannans and macrophage mannose receptor (MMR) recognizes N-linked mannans (Jouault et al., 2003). Whilst the CLR dectin-1 recognizes β-glucan, CLR dectin-2 recognizes mannose residues (McGreal et al., 2006; Brown and Gordon, 2001).

Immune cell populations involved in recognition of *C. albicans* during the innate immune response include monocytes, macrophages and neutrophils. Dendritic cells are crucial for processing of and antigen presentation to T cells, and therefore for activation of specific immunity. This recognition of *C. albicans* by immune cells is done mainly through TLRs. The latter are involved in inflammatory responses induced by *C. albicans*, of which TLR2 and TLR4 are the most studied (Jouault et al., 2003). They are expressed by monocytes, macrophages, dendritic cells, neutrophils, CD4+ T cells and epithelial cells (Weindl et al., 2007). Studies have shown that the activation of TLR2 signal pathways in these antigen-presenting cells (APCs) by ligation of *C. albicans* cell-wall components such as phospholipomannan leads to the production of cytokines that are able to induce a Th2 cellular response (Weindl et al., 2007; Weis et al., 1998; Belloccio et al., 2004; Miyazato et al., 2009). Hence, blocking TLR2 with a TLR2-specific antibody before stimulation of monocytes by *C. albicans* was shown to result in diminished release of Th2-associated cytokines (van de Veerdonk et al., 2008). In contrary, the activation of TLR4 signal pathways during candidiasis will result in the production of cytokines able to induce a Th1 cellular response. Mannans of *C. albicans* are recognized by TLR4 leading to the production of pro-inflammatory cytokines (Roeder et al., 2004).

It is however unclear whether immune changes observed at vaginal mucosal surfaces of HIV infected women interfere with the pattern recognition process of *C. albicans* by innate immune cells. Hence, the aim of this study was to assess the expression of TLR2 and TLR4 on monocytes following stimulations by genital secretions of HIV infected and uninfected women presented with symptomatic VVC. We hypothesized that HIV infection alters TLR2 (but not TLR4) dependent responses to *Candida* antigens by monocytes, resulting in symptomatic VVC.

**MATERIALS AND METHODS**

**Study participants**

A total of 18 women (7 HIV-infected and 11 HIV-uninfected), aged ≥18 years, all black Africans, attending Umlazi D clinic, a primary healthcare facility in KwaZulu-Natal between June, 2011 and December, 2011, were consecutively enrolled by informed consent. Patients aged < 18 years as well as those menstruating or having visible blood contamination of genital samples were excluded. All patients presented initially with signs and symptoms suggestive of lower genital tract infections (LGTIs) and were thereafter screened for the presence of LGTIs caused by *Chlamydia trachomatis, Neisseria gonorrhoeae, Mycoplana genitalium, Trichomonas vaginalis*, herpes simplex virus type 2 and bacterial vaginosis as described elsewhere (Zimba et al., 2011; Apalata et al., 2009). The selected 18 participants were retained in the study because they were free from the aetiological agents causing LGTIs. The study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (Ref. BE 224/11).

**Diagnostic criteria of symptomatic vulvo-vaginal candidiasis**

Vaginal swab (Becton Dickinson) taken from the anterior fornix was directly plated onto Sabouraud Dextrose agar with chloramphenicol (BBL™ Becton Dickinson) and incubated at 29°C, 48 h to estimate the relative vaginal fungal burden. The numbers of yeast colonies were recorded as the number of colonies per plate (Sherrard et al., 2011). Cases of symptomatic VVC were defined according to clinical and laboratory criteria as described by the 2011 European (IUSTI/WHO) guideline on the management of vaginal discharge (evidence level III, recommendation grade B) (Sherrard et al., 2011).

**Isolation of monocytes from peripheral human blood**

Using Histopaque® 1077 and Histopaque® 1119 (Sigma-Aldrich®) per manufacturer’s instructions, we isolated neutrophils and monocytes from fresh human blood collected from a healthy donor (neutrophils were used for other experiments not discussed here) (Rubin-Bejerano et al., 2003). After centrifugation and different wash steps, the peripheral blood mononuclear cells (PBMCs) appeared as a dense white band above the Histopaque® 1077 and granulocyte layer. This was removed with a 5 ml plastic pipette. Monocytes were separated from lymphocytes on the basis of their differential adherence to plastic (Rubin-Bejerano et al., 2003). The

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cells were finally resuspended into 2 ml RPMI-1640 medium supplemented with D-glutamate (HI Media Laboratories, Mumbai, India). The cell count was done using the dye exclusion test. Briefly, a total of 90 μl of isotonic phosphate buffered saline (PBS; Oxoid Limited Basingstoke, UK) (pH = 6.9) and 10 μl of monocytes was mixed and added to 100 μl of Trypan blue solution, 0.4% (Gibco®). The number of cells was counted with a haemocytometer under an inverted microscope and adjusted to 1 x 10⁶ cells/ml.

Collection of and stimulation of monocytes with cervicovaginal fluids (CVF)

A vaginal tampon (8 Ka, Tampax Regular® (Compak) was inserted into the vagina, left in situ for 3 min and then placed into a sterile container containing 10 ml of phosphate buffered saline (PBS; Oxoid Limited Basingstoke, UK) (pH = 6.9). Vaginal fluid was expressed using an autoclaved wooden tongue depressor and filtered through a 0.22 μm Costar Spin-X cellulose acetate filter membranes (Sigma).

In testing the roles of TLR 2 and TLR 4, monocytes were pre-incubated (1 h at 37°C) separately with anti-TLR2 (Abcam®) and anti-TLR4 (Abcam®) specific monoclonal antibodies before stimulation with CVF or sterile normal saline (negative control) into 96 wells tissue culture plates. A total of 500 μl of 1 x 10⁶ monocytes/ml were pre-incubated with anti-TLR2 (50 μl). Another 500 μl of 1 x 10⁶ monocytes/ml were pre-incubated with anti-TLR 4 (50 μl). Another 500 μl of 1 x 10⁶ monocytes/ml were pre-incubated with a mixture of 50 μl of anti-TLR 2 and 50 μl of anti-TLR 4 antibodies. We also used 500 μl of 1 x 10⁶ monocytes/ml pre-incubated with 50 μl of sterile normal saline (no anti-TLR antibodies) as controls. At the end of the pre-incubation period, 50 μl of 1 x 10⁶ monocytes/ml were mixed with 50 μl of CVF obtained from HIV infected and HIV uninfected women diagnosed with symptomatic VVC. The mixture was incubated into 96 wells plate at 37°C and supernatant was collected after 4 and 24 h following stimulation in order to measure cytokines and chemokines.

Measurement of cytokines/chemokines

Concentrations in pg/ml of IL-1β, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, G-CSF, GM-CSF, IFN-γ, MCP-1, MIP-1β, TNF-α, TGF-β1, TGF-β2 and TGF-β3 were measured by a multiplex microbead system (Invitrogen, UK) using a Luminex platform. Multiplex cytokine fluorescent bead-based immunosassays were performed using two different commercially available multiplex luminex kits: Bio-plex pro human cytokine 17-plex assay and Bio-plex pro TGF-β 3-plex assay (Bio-Rad Laboratories, Inc., Parkwood). The assay sensitivity or limit of detection (pg/ml) was: IL-1β (0.6), IL-2 (1.6), IL-4 (0.7), IL-5 (0.6), IL-6 (2.6), IL-7 (1.1), IL-8 (1.0), IL-10 (0.3), IL-12 (3.5), IL-13 (0.7), IL-17 (3.3), G-CSF (1.7), GM-CSF (2.2), IFN-γ (6.4), MCP-1 (1.1), MIP-1β (2.4), TNF-α (6), TGF-β1, TGF-β2 and TGF-β3; and a 5 PL regression formula was used to calculate cytokine/chemokine concentrations from the standard curves (Bio-Plex Manager software, version 4). Cytokines/chemokine concentrations below the lower limit of detection were reported as the midpoint between the lowest concentrations measured for each cytokine and zero.

Statistical analysis

Data were expressed as means ± standard error of the mean (SEM) for the continuous variables and proportions (percentages) for the categorical variables. When data were normally distributed, analysis of the variance (ANOVA) was used to examine differences between groups. However, non-parametric Mann-Whitney U or Kruskal-Wallis tests were used when data were asymmetrically distributed. Multiple comparisons of means of cytokine/chemokine levels displaying significant differences in univariate analyses across the study groups were performed using Post Hoc Bonferroni pairwise tests considering a type I error rate of 0.05. For normally distributed variables, Paired T-tests were used to determine if two sets of variables were significantly different from each other. Data were analysed using SPSS® statistical software version 21.0 (SPSS Inc; Chicago, IL). All tests were two sided and a p value < 0.05 was considered as significant.

RESULTS

Of the 18 participants, symptomatic VVC was diagnosed from 7/7 (100%) of HIV infected and 6/11 (54.5%) of HIV uninfected women. Following blockage of TLR2 with an anti-TLR2 monoclonal antibody, monocytes were stimulated with CVF of HIV-VVC-, HIV-VVC+ and HIV+VVC+ women. Table 1 depicts the mean concentrations of cytokines/chemokines displaying significant differences across those 3 study groups. Bonferroni multiple comparison tests were performed for variables that showed significant univariate associations (Table 1). Of the 5 cytokines that showed significant differences across the study groups, 2 anti-inflammatory (IL-10 and IL-13) and 1 pro-inflammatory (IL-6) cytokines were confirmed by Bonferroni tests. The mean level of IL-6 was significantly higher in HIV+VVC+ group as compared to HIV-VVC+ group (P = 0.03). In addition, there were significantly higher mean levels of IL-10 (P = 0.003) and IL-13 (P = 0.019) in HIV+VVC+ group as compared to HIV-VVC- group.

Compared with baseline concentrations, stimulation with CVF of HIV+VVC+ women post-TLR2 blockage increased IL-6, IL-10 and IL-13 production by 165.5, 162.5 and 106.7%, respectively (Figure 1). However, stimulation with CVF of HIV+VVC+ women only increased IL-6, IL-10 and IL-13 by 36.8, 65.9 and 66.7%, respectively (Figure 2). Using paired T-tests, there was a significant difference in the increase of the concentrations of IL-6 (P = 0.04), IL-10 (P = 0.003) and IL-13 (P = 0.031) when comparing stimulation by CVF of HIV+VVC+ versus stimulation by CVF of HIV-VVC+ patients. Stimulation post-TLR4 blockage by CVF of HIV-VVC+ and CVF of HIV+VVC+ women did not show significant differences of the mean concentrations of all tested cytokines across the study groups. After blocking TLR2 and TLR4 simultaneously with specific monoclonal antibodies, only IL-6, IL-10 and IL-13 were significantly increased when monocytes were stimulated with CVF of HIV+VVC+ as depicted in Figure 3. Findings also showed a linear correlation between genital HIV RNA loads and mean level production of IL-6 (r = 0.722; R² = 0.679; P = 0.067).
Table 1. Comparison of cytokine/chemokine mean levels across the groups post-LTR2 blockage.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Study group (mean ± SEM)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV- and VVC- (n=5)</td>
<td>HIV- and VVC+ (n=6)</td>
</tr>
<tr>
<td>Anti-inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10</td>
<td>0.02±0.001</td>
<td>0.42±0.12</td>
</tr>
<tr>
<td>IL-13</td>
<td>0.15±0.001</td>
<td>0.25±0.001</td>
</tr>
<tr>
<td>TGF-β2**</td>
<td>60±0.001</td>
<td>47.37±4.23</td>
</tr>
<tr>
<td>Pro-inflammatory</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6</td>
<td>0.35±0.001</td>
<td>0.26±0.08</td>
</tr>
<tr>
<td>MCP-1**</td>
<td>1.05±0.001</td>
<td>0.93±0.004</td>
</tr>
</tbody>
</table>

Figure 1. Variations of cytokine levels in HIV infected women.

Figure 2. Variations of cytokine levels in HIV uninfected women.
as well as IL-8 ($r = 0.910; R^2 = 0.833; P = 0.004$).

**DISCUSSION**

Findings from this study have shown that blocking TLR2 with TLR2-specific antibody followed by stimulation of monocytes with CVF of HIV-infected women who were also co-infected with symptomatic VVC resulted in higher increase in the concentrations of IL-6, IL-10 and IL-13. HIV+VVC+ patients produced unusually high levels of one inflammatory cytokine (IL-6) and 2 potent anti-inflammatory cytokines (IL-10, IL-13).

Studies in mice have shown that the development of protective anticandidal Th1 responses requires the concerted actions of several pro-inflammatory cytokines in the relative absence of inhibitory Th2 cytokines, such as IL-4 and IL-10, which inhibit development of Th1 responses (Tonnetti et al., 1995). In the present study we found that despite increased mean levels of IL6, there was a parallel excess production of IL-10 and IL-13 post-TLR2 blockage. These findings confirm that in HIV-infected patients, Th1 activation results in phagocyte-dependent immunity and might represent an important mechanism of anticandidal resistance; but subsequent Th2 reactivity, triggered by *Candida* infection would be mostly associated with the pathology (Romani et al., 1995). This can suggest that Th2 reactivity overcame the Th1 responses in HIV positive women leading to candidiasis. Thus, the Th cell dichotomy to *Candida* may have important implications particularly in contributing to the dominance of Th2 responses in cases of recurrent VVC observed among HIV positive women (Romani et al., 1995).

IL-13 is recognized for its effects on monocytes, where it upregulates class II expression, promotes IgE class switching and inhibits inflammatory cytokine production. In general, activation of TLR2 signal pathways during candidiasis should lead to the production of Th2 cytokines, thus blocking TLR2 with a TLR2-specific monoclonal antibody followed by stimulation of monocytes by *C. albicans* should result in diminished release of these Th2 cytokines. Netea et al. (2004) showed that TLR2+/− mice are more resistant to disseminated *Candida* infection and this is associated with increased chemotaxis and enhanced candidacidal capacity of TLR2−/− monocytes/macrophages. Whilst the production of pro-inflammatory cytokines can be normal, levels of anti-inflammatory cytokines are severely impaired in the TLR2−/− mice. The authors found that this was accompanied by a substantial decrease in the CD4+CD25+ regulatory T (Treg) cell population in TLR2−/− mice (Netea et al., 2004). Furthermore, in vitro studies confirmed that enhanced survival of Treg cells was induced by TLR2 agonists; *C. albicans* induces immunosuppression through TLR2-derived signals that mediate increased anti-inflammatory cytokine (that is, IL-10) production and survival of Treg cells, playing a critical role in the pathogenesis of symptomatic VVC.

**Conclusion**

The present study demonstrated that in HIV-infected
individuals, there might be an upregulation of TLR2 during stimulation of monocytes by *Candida* spp. leading to an over production of anti-inflammatory cytokines. This might suggest an underlying role played by Th2/Treg cell populations during HIV infection.

**LIMITATION**

The limitation of this study is mainly the small sample size that might not allow generalization of our findings. A further study is thus warranted.

**REFERENCES**


Full Length Research Paper

Trichomonas vaginalis cases presenting at the Komfo Anokye Teaching Hospital, Ghana over a period of 11 years: 1994 to 2004


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The study aims at establishing the trend of Trichomonas vaginalis infections diagnosed at the Komfo Anokye Teaching Hospital (KATH) in Kumasi over an eleven (11) years period (1994 to 2004). The retrospective study involves the yearly distribution of the infection as well as the monthly distribution relating them to the ages and sex. The mean ages of males and females infected were 28.8 and 26.0 years, respectively. The prevalent age groups mostly infected were found to be 18 to 31 for both males and females. The age distribution of T. vaginalis cases from the high vaginal swab (HVS) records gave a standard deviation of 3.47 and the standard deviation of the age distribution for males from the urine routine examination (R/E) was 5.13. Analyses of records of T. vaginalis suggest that even though there has been a drastic decline in prevalence, the infection still persist and requires efforts to ensure its absolute extinction. The monthly distributions did not reveal any particular month in which transmission of the infection remains constantly high. This suggests that the climatic seasons (rainy or dry/cold or warm) have no effect on the rate of transmission. More pragmatic measures are needed to ensure better records keeping of the infection at KATH. New and more efficient methods such as cultures and polymerase chain reaction (PCR) should be employed to increase efficiency in the detection of the organism in patients. Another study is being designed to ascertain the level of T. vaginalis from 2005 to date.

Key words: Sexually transmitted infection, Trichomonas vaginalis, Komfo Anokye Teaching Hospital (KATH).

INTRODUCTION

Human trichomoniasis is a common sexually transmitted infection (STI) caused by the flagellate protozoan parasite Trichomonas vaginalis. This infection is the most common non-viral STI worldwide (Cohen, 2000; Upcroft

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and Upcroft 2001). The protozoan infects the vagina and urethra of females and may lead to severe vaginitis. In males, it infects the prostate, seminal vesicles and urethra causing urethritis and prostatovesiculitis (Sylvia, 1997). It infects mostly humans and infections are more common in females. Males who are infected are usually asymptomatic.

Bowden and Garnet reported that the epidemiology of the disease is still poorly understood and some practitioners continue to question its relevance. However, there is increasing evidence that T. vaginalis is an important pathogen, both in its own right due to the “immediate” morbidity associated with infection (Petrin et al., 1998) for its role in the promotion of premature rupture of membranes, premature labour and low birth weight (Heine and McGregor, 1993; Cotch et al., 1997). The prevalence of this flagellate in developed countries is reported to be 5 to 20% in women and 2 to 10% in men (Murray et al., 2002). Worldwide, over 170 million cases of trichomoniaisis are reported each year, with 40 to 60% in Africa (Bowden et al., 1999; World Health Organization (WHO), 2001). Notably, research has shown that infection with T. vaginalis increases the risk of HIV transmission (Forna and Gülmezoglu, 2003; Wang et al., 2001). Trichomoniaisis is also associated with adverse pregnancy outcomes, infertility, postoperative infections and cervical neoplasia (Soper, 2004; Kaydos et al., 2002).

T. vaginalis infections can be diagnosed by observing the characteristic microscopic forms including the motile trophozoites (Koneman et al., 1992). The most practical method of diagnosis is the microscopic examination in a drop of saline (wet mount method) for motile trichomonads of the fresh vaginal discharge. Occasionally, cultures will reveal the organism when the microscopic examination is negative. Prostatic secretions following prostatic massage and urine of the male should be examined (Franklin and Harold, 1994; Lo et al., 2002). Other methods of T. vaginalis detection include Kupferberg liquid medium, Hirsch charcoal agar and the Papanicolaou smear. The latter is the least sensitive among the aforementioned methods (Thomason et al., 1988).

In Ghana, T. vaginalis cases is still a public health concern (Adu and Amankwaa, 2005) even though data available on the epidemiology in the country is scanty. This work aims to do a retrospective study of T. vaginalis and monitor the progress of the disease over the targeted year range. It will add to the body of knowledge available on T. vaginalis in Ghana.

MATERIALS AND METHODS

The high vaginal swab (HVS) and urine routine examination (R/E) records were obtained from the KATH microbiology and parasitology laboratories respectively, from which all cases diagnosed and recorded from January, 1994 to December, 2004 were listed. The attributes considered included age, sex, month and outcome of laboratory examination. From the records of the laboratories, the number of cases reported each month for the period of study was obtained and the following data derived: total number of cases diagnosed and confirmed as T. vaginalis infection, age and sex distribution, monthly distribution. Analyses were made covering the following areas: age and sex distribution of cases, monthly incidence. The observations made were compared for the years of study and the variations noted. An attempt was made to account for the observation. The information obtained was discussed and suggestions made on how to eliminate the infections.

Study design

This is a longitudinal study that employed retrospective data to determine T. vaginalis cases over eleven years period. The study population included all suspected cases referred for screening. Cases confirmed as T. vaginalis in the KATH laboratories were taken note of. All documented cases of the infection in the high vaginal swab, urine routine examination and urethral discharge examination records were included in the study. Suspected cases that have not been documented in the laboratory records were excluded.

Study area

KATH is located in Kumasi, the regional capital of Ashanti Region of Ghana, with a total projected population of 4,725,046 (2010). The geographical location of the over 1000-bed hospital, the road network of the country and commercial nature of Kumasi make the hospital accessible to all the areas that share boundaries with Ashanti region and others that are further away. As such, referrals are received from all northern regions (namely Northern, Upper East and Upper West Regions), Brong Ahafo, Central, Western, Eastern and parts of the Volta Regions.

Ethical statement

The study protocol was approved by the ethical committee of KATH in collaboration with committee on human research, publications and ethics, School of Medical Sciences, Kwame Nkrumah University of Science and Technology, Kumasi-Ghana.

Statistical analysis

The main statistical software used in this study was microsoft office excel 2007. All data recorded were entered into excel according to the age, sex, month, year and the outcome of the laboratory examination. The data were analysed to determine the monthly and annual trends of the infection. The age and sex distribution of the infection rate were also analysed.

RESULTS

After the retrospective study the following analyses were made: These are shown in Table 1 and Figure 1. The prevalent age groups of the infection were 18 to 31 for both females and males. The mean age and standard deviation for females were 26.0 and 3.47, respectively and that of males were 28.8 and 15.53, respectively (Figure 2).
Table 1. Yearly prevalence of T. vaginalis infection.

<table>
<thead>
<tr>
<th>Year</th>
<th>Total no. of HVS patients (N)</th>
<th>No. of T.V positives recorded (n)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>3150</td>
<td>166</td>
<td>5.3</td>
</tr>
<tr>
<td>1995</td>
<td>3441</td>
<td>181</td>
<td>5.3</td>
</tr>
<tr>
<td>1996</td>
<td>3003</td>
<td>123</td>
<td>4.1</td>
</tr>
<tr>
<td>1997</td>
<td>2889</td>
<td>107</td>
<td>3.7</td>
</tr>
<tr>
<td>1998</td>
<td>2070</td>
<td>58</td>
<td>2.8</td>
</tr>
<tr>
<td>1999</td>
<td>2830</td>
<td>52</td>
<td>1.8</td>
</tr>
<tr>
<td>2000</td>
<td>2001</td>
<td>56</td>
<td>2.8</td>
</tr>
<tr>
<td>2001</td>
<td>1949</td>
<td>24</td>
<td>1.2</td>
</tr>
<tr>
<td>2002</td>
<td>1597</td>
<td>42</td>
<td>2.6</td>
</tr>
<tr>
<td>2003</td>
<td>2499</td>
<td>17</td>
<td>0.7</td>
</tr>
<tr>
<td>2004</td>
<td>2838</td>
<td>27</td>
<td>1.0</td>
</tr>
</tbody>
</table>

T.V = T. vaginalis.

DISCUSSION

This study was done to determine the trend that T. vaginalis infection has taken over eleven (11) years. An important finding in this study is that there has been a decline in the number of T. vaginalis cases recorded over the years from 1994 to 2004. This decline may be accounted for by the effective STI treatment regimen given to patients. Even though there is a general decline, it is evident that if proper measures are not put in place to find out the actual factors of this trend and steps not taken to check the transmission rate of T. vaginalis, its prevalence can go higher in the future. In 2003, a prevalence of 16.2% was found among a high school student of both female and male population by Kaydos et al. (2002) which exceeds any of the prevalence recorded over the years of this study (Figures 3 and 4).

In a study conducted by Lo et al. (2002) in Auckland, the mean age of females mostly infected was 26.5 years. The current study showed that the mean age of females was 26.0 and 28.8 years, respectively. These do not show any significant margin from that obtained in the study carried out by Lo et al. (2002). The age group predominantly infected with T. vaginalis is 18 to 31 for both males and females even though the mean ages of infection are 26.0 for females and 28.8 for males. This suggests that people who are mostly infected are the young sexually active group. This implies that unprotected sex may be a major factor in the transmission of T. vaginalis in the study area.
In a study carried out by Helms et al. (2008), the results showed that 4.6% of women had an incident infection of *T. vaginalis* while a study carried out by Sutton et al. (2007) showed 3.1% prevalence among women. This current study obtained a mean incidence of 2.8% which is lower than those obtained in the above studies. A study carried out in 2000 by Adu-Sarkodie et al. (2000) concluded that training in the syndromic management of STIs among pharmacists led to improvements in the treatment of urethral discharge. Between April, 1997 and June, 1998, health workers had attended training course which covered history taking and examination, therapy,
condom promotion, partner notification, health education and counseling and STI record keeping using the syndromic approach. It enables all trained first-line service providers to diagnose STI syndrome and treat patients ‘on the spot’. This helps prevent the further spread of STI, resulting in effective case management and decrease in the number of T. vaginalis cases. The reduction of T. vaginalis through the syndromic approach at KATH is consistent with previous research for the prevention of STI. In a landmark pilot study in Kwanza, Tanzania, use of the syndromic approach to STI treatment that AIDS CAP has advocated worldwide reduced HIV incidence and other STI by 42 percent. Again, recent research in Malawi produced strong Biological evidence that STI treatment can make HIV-positive men less infectious (AIDSCAP, 1991 to 1997).

The decrease in T. vaginalis and other STIs can also be attributed to the work of governmental organizations such as the Ghana AIDS Commission and non-governmental organizations. These organizations deliver innovative products and projects in support of HIV and AIDS prevention, fertility management, adolescent reproductive health and education. The combination of mass media and interpersonal activities has helped lower barriers for contraceptives and scaled-up family planning products and services in Ghana. This has increased people’s acceptability for condom use and improved their health seeking behaviors over the years. The organisations provided technical support and organised workshops for health workers on comprehensive STI case management including syndromic approach into the curricula of medical schools and training institutions of nurses and midwives. All these training are geared towards prevention and information to prevent STI acquisition, improve access to STI services at all levels and outlets of health care delivery, improve quality of STI care and promote early health care seeking behavior and to promote effective partner notification and management.

Conclusion

There has been a significant decrease in T. vaginalis infection reported to KATH over the eleven year period. Young people between the ages of 18 to 31 are those mostly infected and decline in infection rate could be attributed to increased reproductive health education, increased acceptability to condom use and improved health seeking behaviors in the study area.

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