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Mussel RL, De Sa Silva E, Costa AM, Mandarim-De-Lacerda CA (2003). Mast cells in tissue response to dentistry materials: an adhesive resin, a calcium hydroxide and a glass ionomer cement. *J. Cell. Mol. Med.* 7:171-178.

Booth M, Bundy DA, Albonico P, Chwaya M, Alawi K (1998). Associations among multiple geohelminth infections in school children from Pemba Island. *Parasitol.* 116: 85-93.0.

Fransiscus RG, Long JC (1991). Variation in human nasal height and breath, *Am. J. Phys. Anthropol.* 85(4):419-427.

Stanislawski L, Lefevre M, Bourd K, Soheili-Majd E, Goldberg M, Perianin A (2003). TEGDMA-induced toxicity in human fibroblasts is associated with early and drastic glutathione depletion with subsequent production of oxygen reactive species. *J. Biomed. Res.* 66:476-82.

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**ARTICLES**

**Review**

- Severe stress urinary incontinence: Pelvic floor muscle training program** 430  
Margarida Ferreira, Paula C. Santos and José A. Duarte

**Research Articles**

- The effect of cinnamon on glucose control in patients with type 2 diabetes mellitus in Pontianak, Indonesia** 434  
Suriadi, Atmiati, Hartono, Iswahyudi and Tuttur Kardiaturun

- HIV and malaria co-infection: Their combined effects on pregnancy outcomes in Anambra State, Southeast Nigeria** 438  
Uju M. E. Dibua, Lorina Badger-Emeka and Joseph A. Ugonabo

- The magnitude of tooth bud extraction in Uganda** 450  
J. F. Tirwomwe, E. Agwu and M. Ssamula

- Epidemiology and prevalence of urinary schistosomiasis in pre-school children in Lagos, Nigeria** 456  
Adewole, S. O and Fafure, T. H

- Prevalence and antibiotic susceptibility of uropathogens among patients attending University of Abuja Teaching Hospital, Gwagwalada, Abuja** 460  
N. J. Amaeze, A.U. Abah and I. N. Okoliegbe



Review

## Severe stress urinary incontinence: Pelvic floor muscle training program

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**International guidelines recommend a first line therapy in the treatment of female stress urinary incontinence (SUI), the pelvic floor muscle (PFM) training. This case report assesses the effects of the PFM training program in treating women with severe SUI. The urodynamic parameters allow diagnosed intrinsic sphincter deficiency and urethral hypermobility. The subjective and objective parameters were assessed at the beginning and after six-month of PFM training program. This case report confirms the efficiency of the intensive training program in severe SUI. The medical implications of the PFM training as first treatment option reflect favourable individual results and additionally contribute to the selection of the non-invasive treatment, the reduction of the incidence collateral effects, low costs and that does not prevent future treatment options.**

**Key words:** Pelvic floor muscle training exercises, stress incontinence urinary, urodynamic assessment.

### INTRODUCTION

The Portuguese Association of Urology and Neurourology and Urogynecology, has requested to the service of Hygiene and Epidemiology of the Medical Faculty of the University of Porto, a study on the "prevalence and treatment of the urinary incontinence on the non institutionalised Portuguese population". The study took place in the mainland Portugal, between 2007 and 2008, on women of age  $\geq 40$  years, and the prevalence of female urinary incontinence (UI) was estimated in 21.4 and 39.9% of females with predominance of stress urinary incontinence (SUI).

The International Continence Society defines SUI as a "complain of involuntary loss of urine during the increase of the intra-abdominal pressure such as coughing, sneezing, physical activities". Intra-abdominal pressure is transmitted to the bladder and, if the increase of the intravesical exceeds the intraurethral pressure, with absence of contraction of the detrusor muscle, the resulting urinary loss is designated as SUI (Nygaard and

Heit, 2004).

The Intrinsic Sphincter Deficiency (ISD) is a subtype of SUI and it occurs when the urethral sphincter is unable to maintain the closure of the bladder neck during the stress activities (Wilson et al., 2005). The urodynamic criteria of diagnosis for ISD have included the low pressure of the proximal urethral closure in absence of contraction of the detrusor muscle and the valsalva leak point pressure  $\leq 20$  cmH<sub>2</sub>O (Bump et al., 1997). The etiology of the intrinsic sphincter may be related to hypo-oestrogen, changes of the urethral cells (age/genetic) and obstetric causes (Heesakkers and Gerretsen, 2004).

SUI has implications in the quality of life of women in physical, social, sexual and psychic aspects (Moreno, 2004). The female restrains or decreases her social and physical activities progressing to emotional changes, including low self-esteem, depression, shame and confinement (Sanchez et al., 2004).

Several types of UI are treated, such as SUI, urge UI

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**Table 1.** Urodynamic assessment.

<b>Urodynamic</b>	<b>Assessment</b>
Urofluxometry	Vesical capacity of 456 ml Post-micturition residual volume 12 ml Continuous urinating pattern
Cystometry	1st urge to urinate, 222 ml Normal urge, 385 ml Strong urge, 462 ml Pressure of the initial detrusor of 6 cmH <sub>2</sub> O reaching a maximum of 14 cmH <sub>2</sub> O
Profilometry	<b>Static profilometry</b> Urethral pressure, 53 cmH <sub>2</sub> O Maximum urethral closure pressure, 37 cmH <sub>2</sub> O
	<b>Dynamic profilometry</b> Abdominal pressure transmission factor between 54 and 87%
VLPP	Urinary losses at low abdominal pressure, 20 cmH <sub>2</sub> O

and mixed UI and the treatment options may include behaviour changes and advice on daily hygiene, pelvic floor muscles (PFM) training program, electric stimulation, biofeedback, medication and surgery.

Re-education of the PFM, through a PFM training program is considered the first choice for treatment of women with SUI, who have the ability of voluntarily and correctly contracting their PFM (Wilson et al., 2005). The best results were shown in light and moderate SUI (Bø, 2004). The PFM training exercises include voluntary and repeated contraction/relaxation of these muscles (Wilson et al., 2005).

The purpose of this case study consisted of analysing the effects of the PFM's training program on severe female SUI.

## ANAMNESIS

A 58 year old female, Caucasian, married, educational assistant, and 6 years of school, with a body mass index of 25.2 kg/m<sup>2</sup>, obstetric history of 2 vaginal deliveries, hereditary history of UI (mother), 3 yearly episodes of constipation, 4 years after menopause. The urinary losses have lasted for 3 years approximately and are caused by cough, sneezes and stress. The urinary losses occur daily and in small quantity/flow (Sandvik Index/8/severe) (Sandvik et al., 2000), use a regular pad daily. She refers great discomfort while carrying heavy objects and she is afraid of odours.

## Physical examination

The initial evaluation through digital palpation was that the maximum voluntary contraction (MVC), the levator ani muscle was 3 (moderate contraction) and the resistance/

contraction maintained was of 5 s. She had a correct perception of the contraction (absence of contraction of the synergistic muscles, valsalva maneuver and inverse perineal command). Both the stop test and the stress test (300 ml/vesical) were positive. The frequency of urinary loss was 11 episodes (7 consecutive days), quantity of urinary loss of 10 g (Pad-test/1 h), perineometry (maximum voluntary contraction=15 cmH<sub>2</sub>O), 72 h urinary diary (7 daytime urinary frequencies and 1 night/ingestion of 1.3 ml of liquids) and in the scale of Ditrovie [classification of 1 (excellent QV) and 5 (bad QV)] it showed a classification of 2.7. In the self-esteem evaluation (Scale of Rosenberg/10 points (low self-esteem) and 60 (high self-esteem), the rate reached 32 points, with a possible direct connection between the emotional disorder and the UI.

## History/medical diagnosis

The medical record registered a grade II cystocele, atrophic vaginitis and reflex and sensitive integrity. The Q-tip test was >20° and the Bonney test were positive. The usual medication included Crestor (20 mg/daily) and Zolpidem Ratiopham (10 mg/daily).

The urodynamic assessment included urofluxometry, cystometry and profilometry (Table 1). The urodynamic diagnosis is severe SUI with ISD and urethral hypermobility.

## Treatment plan

Education is a key feature for any successful treatment. Clinical guidelines recommend that educational actions are included in the PFM training program, because an

**Table 2.** PFM training Program.

<b>Training</b>	<b>Program</b>
Educational conduct	-Teaching of the anatomy and physiology of the lower urinary tract;
	-Risk factors;
	-UI mechanism;
	-Locating and identifying the PFM;
Perception of the correct contraction	-Program for exercise at home;
	-Hygienic behaviour and lifestyle changes.
Supervised PFM training program	-Effective contraction of the PFM, suitable for increasing the pressure of the urethral closing, through digital palpation.
	-Intensity was verbally stimulated (isolated CVM of the PFM);
	-Resistance was defined by the contraction maintained, varied between 5 to 10 seconds,
	-Volume was registered by series (8-10) and repetitions (8-12),
	-Frequency was weekly with a 45' duration, for 20 weeks;
Training program at home	-Between series there were stretching, muscular strength and respiratory re-education exercises.
	-Progression of the program included the parameters: position (increasing resistance to gravity), duration of the contraction, number of repetitions in each series and increase of the abdominal pressure.
	-Voluntary contractions of the PFM during day-to-day activities (washing the dishes, seating on the sofa, at the bus stop...)
	- 3 series of 10 contractions in different positions during the day-to-day activities.

understanding of UI increases and promotes the motivation of women with SUI (Wilson et al., 2005) (Table 2).

The international continence society has concluded that a simple verbal or written direction does not represent a proper learning of the correct contraction in the exercises program for PFM. Observation of the correct contraction of the PFM was clinically demonstrated through magnetic resonance imaging (Bø et al., 2001). Kari Bø et al. (2001) have observed that during a voluntary contraction of the PFM, the coccyx showed a cranial movement towards the pubic symphysis, this voluntary contraction is a simultaneous contraction of all of the PFM and describes an elevation movement towards the head while closing the pelvic openings. It cannot involve any movement of the pelvis or contraction of other muscular groups in relation with the weak and untrained muscles of the pelvic floor (Table 2).

The supervised PFM training program has followed the Sports Medicine's strength training recommendations, based on the principles of physiology of exercise for the striated muscle (DiNubile, 1991). The strength training program for the PFM comprises the progressive training of the parameters: intensity, frequency, rest time, volume, duration and specificity (Kraemer et al., 2002) (Table 2). The PFM training program was based on scientific evidence as shown subsequently.

## RESULTS

After the 20 PFM training sessions the results of the perineometry were 18 cmH<sub>2</sub>O (increase of 3 cmH<sub>2</sub>O), the

quantity of urinary loss was reduced to 3 g and the frequency of the losses decreased to 4 weekly episodes.

For the subjective evaluation, the female refers having urinary losses one or several times a week and in drops (Sandvick Index/moderate/3), a day time daily pad, and the qualitative classification of the changes after the training of the PFM, she refers improving after the five months (the urinary losses have decreased) and is pleased with the results. Her quality of life has improved (scale of Ditrovie/2) and had repercussions in her day-to-day activities, that is, she participated more in social and leisure activities. Her self-esteem was influenced by the effective results of the training of the PFM, who reach from 32 to 54 points, expressing an attitude of value and self approval.

## DISCUSSION

The support structure (anterior vaginal wall, puborectal ligament, tendinous arch of pelvic fascia, endopelvic fascia and pelvic diaphragm) allows the maintaining of the urethra and the bladder in an intra pelvic position during the increase of the intra-abdominal pressure. The pressure transmitted to the urethra and bladder is neutralised by the balanced distribution of forces. The active concept of urinary continence comprises the reflex contraction of the intrinsic urethral sphincter and the resistance of the levator ani muscle "hammock", resulting in an increase of the urethral closure pressure (Heesakkers and Gerretsen, 2004).

On the case study, the urodynamic parameters of

pressure transmission rate are lower than 70 to 80% and the physical exam (Q-tip test) was higher than 20°, suggesting the presence of urethral hypermobility (Sand et al., 1990). The deficiency of the intrinsic sphincter was also confirmed with the valsalva leak point pressure (VLPP) lower than 60 cmH<sub>2</sub>O (McGuire et al., 1993). The pathophysiology of the SUI comprised the combination of urethral hypermobility and deficiency of the intrinsic sphincter.

Therefore, the principles for treatment of the SUI are (1) restore the anatomical position of the bladder and proximal urethra; (2) increase the urethral closure pressure. This supervised intensive training program has allowed a correct and effective teaching of the PFM contraction, feedback of the muscle control and awareness of the anticipated contraction of the PFM to stress. These features of the training program aim to increase the muscle strength, resistance, coordination and automatic contraction. The increase of the rest tone and contraction strength of the PFM, in association with a timely contraction to the increase of the intra-abdominal pressure may promote the stability of the support structures and increase the urethral closure pressure.

The results from this case study suggest that an intensive PFM training program may be effective on severe SUI. The results reflect a reduction of the quantity of urinary loss in 70%, decrease of the frequency of episodes of the urinary losses in 63.6 and 20% increase of the strength of the maximum voluntary contraction.

Hypo-oestrogen, age and neurological damage may contribute to the inefficiency if the muscles of the urethral sphincter (Heesakkers and Gerretsen, 2004). However, in the healthy striated muscle, a loss of the urethral closure pressure may be compensated with an increase of strength in 30% after intense and frequent exercise for 8 to 12 weeks (Skelton et al., 1995). This study, when assuming an increase of 30% from exercise, the initial rest closure pressure of 37 cmH<sub>2</sub>O increased to 48 cmH<sub>2</sub>O, an increase higher than a tenth of 100 cmH<sub>2</sub>O of the intravesical pressure during stress. So, it has been concluded that the training of the PFM may relieve the symptoms of severe SUI on sedentary women, that is, with activities of scarce effort.

Literature considers that the diagnosis of grade III/IV prolapsed and severe SUI, the training of the PFM should be excluded when following surgery. Some studies have also shown better results with light and moderate SUI (Pages et al., 2001; Bø et al., 1999). This study allows suggesting that, although training of the PFM has limited benefits for severe SUI, some women may respond favourably to treatment. Therefore, training of the PFM should not be an exclusion criterion when diagnosing severe SUI.

Future research should develop techniques and/or instruments to allow an evaluation of the tone of the intrinsic and extrinsic sphincter, morphologic changes of the PFM, coordination of the automatic contraction on

increase of intra abdominal pressure and anatomical position of the support structures.

## Conclusions

This case study confirms the efficiency of the intensive training program in severe SUI. The medical implications of the PFM training as first treatment option reflect favourable individual results and additionally contributes to the selection of the non invasive treatment, the reduction of the incidence collateral effects, low costs and that does not prevent future treatment options.

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*Full Length Research Paper*

## The effect of cinnamon on glucose control in patients with type 2 diabetes mellitus in Pontianak, Indonesia

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The objective of this study was to examine pre- and post-glucose serum concentration levels in diabetes mellitus patients after the administration of cinnamon powder. A quasi-experimental design was done with 40 patients, with pre- and post-test non-equivalent control groups, without randomization. The first group was given a cinnamon powder extract solution to be taken 3 times per day and designated as the experimental group. The dose amounted to 3 g of cinnamon spread over the day, after breakfast, lunch, and dinner, respectively. The subjects were instructed to take the 3 g of cinnamon immediately following their meals. The patients with type 2 diabetes in the intervention group were aged  $54.4 \pm 8.7$  years, and in the control group were aged  $56.2 \pm 10.5$  years. There was a significant difference between the pre- and post-serum glucose concentration tests between the two groups. The measurements for the intervention group were  $311.4 \pm 96$  and  $185.8 \pm 21$ , respectively ( $p = 0.000$ ), while the control group pre-test was  $277.1 \pm 74$ , and the post-test was  $205.3 \pm 45$  ( $p = 0.003$ ). The results of this study demonstrated that intake of cinnamon reduces serum glucose in type 2 diabetes.

**Key words:** Cinnamon, diabetes mellitus, blood glucose.

### INTRODUCTION

Based on current epidemiological research, the number of diabetic patients worldwide has reached 200 million and is estimated to increase to over 330 million by 2025. In 2000, approximately 8.4 million people suffered from diabetes in Indonesia, and by 2030 this number is estimated to reach about 21.3 million (Mishra et al., 2007; Wild et al., 2004). In Indonesia, Mihardja et al. (2009) found that the prevalence of diabetes was 5.7%, almost similar to the estimate by the International Diabetes Federation in 2003. The prevalence of diabetes mellitus was 6.4% in women and 4.9% in men. Prevalence increases with age, with a sharp rise from middle age (35 to 54 years).

Due to the increasing incidence of diabetes mellitus type 2, new sustainable treatments are greatly needed. Although currently, a number of effective Western diabetes mellitus medications are available for treatment,

the management of diabetes mellitus using medications with fewer side effects at lower costs is still a big challenge (Wang et al., 2013). These medications frequently have side effects, such as weight gain, bone loss, and an increased risk of cardiovascular events (Prabhakar and Doble, 2011). These side effects can become more prevalent due to continued use. Furthermore, treatment is costly since diabetes mellitus is a chronic disease, and long-term medications are necessary. Herbal medications can be a good alternative to replace or at least supplement Western medications (Prabhakar and Doble, 2011; Li et al., 2004; Yang et al., 2011; Jia et al., 2003).

In Indonesia, several herbal medications have been proven to be clinically effective. Because herbal medicines are usually derived from natural plants, they are considered to be relatively safe and have fewer side

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**Table 1.** Characteristics of subjects.

Characteristic	Intervention group (n=20)	Control group (n=20)
Age (mean $\pm$ SD)	54.4 $\pm$ 8.7	56.2 $\pm$ 10.5
<b>Sex n(%)</b>		
Male	10(50)	7(35)
Female	10(50)	13(65)
<b>Education n(%)</b>		
Elementary	2(10)	10(50)
Junior High School	4(20)	3(15)
Senior High School	12(60)	4(20)
Higher Education	2(10)	3(15)

SD (standard deviation)

effects when compared to conventional drugs (Wang et al., 2013). Herbal medications treating diabetes mellitus can target multiple mechanisms, including the enhancement of insulin sensitivity, stimulation of insulin secretion, or a reduction of carbohydrate absorption (Prabhakar and Doble, 2011). Western drugs are typically more potent than herbal medicines in lowering blood glucose levels; however, herbal supplements have been shown to treat diabetic complications (Ceylan-Isik et al., 2008). Traditional plant remedies and herbal formulations have been in existence since ancient times and are still widely used, despite the controversy concerning their efficacy and safety (Fugh-Berman, 2000). Herbal medicine can also be used as supplementation, or in combination with Western medicine to improve therapeutic outcomes (Wang et al., 2013). In Indonesia, traditional medicine has long been used, over generations, for healing and disease prevention in the community.

In this study, we investigated one herbal remedy that is commonly used in Indonesia for controlling blood glucose, cinnamon, which is consumed with vegetables or as a beverage. Cinnamon has many pharmacological properties, such as antioxidants activity, antibacterial effects, natural insulin sensitizer and bioactive product that improve glucose and insulin metabolism (Hossein et al., 2012).

Diabetes mellitus patients require lifelong treatment and spend a large amount of money treating this disease. Cinnamon offers a promising solution as a traditional remedy and low-cost treatment. In Indonesia, no clear answer has been proven by several research studies, although some showed a relationship between cinnamon and blood sugar. The aim of this study was to examine pre- and post-glucose serum concentration levels in diabetes mellitus patients after the administration of cinnamon powder.

## MATERIALS AND METHODS

The design of this study was a quasi-experimental non-randomized use, quota sampling method, which was selected to assess 20 patients from each group with pre- and post-test non-equivalent groups. This study was conducted at the Wound Clinic of Kitamura, Pontianak in Indonesia, from December, 2012 until February, 2013. The study population included all diabetes mellitus type 2 patients admitted to the Wound Clinic, who were divided into two groups, an intervention group and a control group, with 20 subjects per group. Inclusion criteria included: (a) agreed to become a respondent, (b) diagnosed with diabetes mellitus, (c) routine out-patient, (d) age over 18 years, (e) not on insulin therapy, (f) not taking medicine for other health conditions, and (g) a fasting blood glucose level between 140 and 400 mg/dl. Selected subjects were given clear information regarding this study and asked to sign an informed consent after the explanation if they agreed to participate. The clinical research ethics committees of the Muhammadiyah Institute of Nursing, Pontianak, and Kitamura Clinic, Pontianak, Indonesia, approved the study protocol.

The first group was given a cinnamon powder extract solution to be taken 3 times per day and designated as the intervention group. The dose amounted to 3 g of cinnamon spread over the day, after breakfast, lunch, and dinner, respectively. The subjects were instructed to take the 3 g of cinnamon immediately following their meals. The serum glucose concentration was measured before and after cinnamon administration using the ACCU-CHEK Active (Roche Diagnostics, Germany, 2011). The cinnamon powder was administered to the patients for 2 weeks; however, the serum glucose levels were taken before the administration of cinnamon in the first week and after consuming the cinnamon in the second week.

### Extract preparation

The cinnamon powder extract solution processing was carried out at the Pharmaceutical Technology Laboratory, Department of Pharmacy, Faculty of Medicine, at the University of Tanjungpura. The cinnamon was finely ground into a powder. One preparation contained 3 g of cinnamon powder added to boiling water until reaching a full cup, and the cups were cooled and sealed with a cap to prevent essential oil evaporation. The subjects were asked to consume the clear upper part of the solution. Additionally, the subjects were advised to continue their normal diets and medications throughout the study.

### Statistical analysis

This study used univariate and bivariate analyses with 95% confidentiality ( $\alpha = 0.05$ ). The paired t-test was used to analyze the hypothesis with nominal and interval data. All data were analyzed using statistical package for social sciences (SPSS) version 17.0 (SPSS, Chicago, IL).

## RESULTS

The subjects in the intervention group were aged  $54.4 \pm 8.7$  years, and in the control group they were  $56.2 \pm 10.5$  years (Table 1). A serum glucose concentration measurement was performed before and after administration of the cinnamon powder extract solution. The bivariate analysis with t-test results can be seen in Table 2. From the analysis, it is shown that a significant difference

**Table 2.** Comparison of means of blood glucose test patients pre- and post-intervention in the two groups.

Variables	Blood glucose Pre-test (n=20)	Blood glucose Post test (n=20)	Differences	t-test	P value
Control group	277.1 ± 74	205.3 ± 45	71.8±93	3.470	0.003
Intervention group	311.4 ± 96	185.8 ± 21	125.5±90	5.507	0.000

between the pre- and post- serum glucose concentration tests exists between the two groups. The measurements for the intervention group were  $311.4 \pm 96$  and  $185.8 \pm 21$ , respectively ( $p = 0.000$ ), while the control group pre-test was  $277.1 \pm 74$ , and the post-test was  $205.3 \pm 45$  ( $p = 0.003$ ). The difference in mean of pre- and post-tests in the control group was  $71.8 \pm 93$ , and in the intervention group it was  $125.5 \pm 90$ .

## DISCUSSION

The aim of this study was to examine pre- and post-serum glucose concentration levels in diabetes mellitus patients after administration of cinnamon powder. This study found that there were significant decreases in the serum glucose tests between the two groups. The differences of pre- and post-intervention fasting glucose concentrations showed strong significance and a higher reduction in the cinnamon group than in the control group. The control group showed that blood glucose concentrations also decreased before and after administration of cinnamon. This condition may be caused by continuing their normal diets, activity, and medications. This study postulated that diabetes mellitus patients who consumed cinnamon powder decreased their serum glucose concentration, and the results confirmed a previous study which found that cinnamon reduced serum glucose levels in people with type 2 diabetes (Khan et al., 2003). Two studies reported that using 1 g cinnamon had no significant effect on the blood glucose of type 2 diabetics (Justin et al., 2007; Hasanzade et al., 2013), which were not similar to our study results.

In study of Hossein (2012), it was shown that 1.5 g of cinnamon supplementation for 8 weeks improve fasting blood glucose levels and lipid profiles in type 2 diabetic patients. One study reported that cinnamon consumption in doses of 1, 3, or 6 g daily for a period of 40 days led to a major reduction in fasting blood glucose, triglyceride, low-density lipoprotein (LDL), and total cholesterol levels (Khan et al., 2013). This study may conclude that a decrease in serum blood glucose will depend on the amount in grams given to patients with diabetes mellitus. The cinnamon does not contribute to caloric intake; those who have type 2 diabetes or those who have elevated glucose levels may benefit from the regular inclusion of cinnamon in their daily diets.

Khan et al. (2003) suggested that adding cinnamon to the daily diet of diabetes mellitus patients can reduce

cardiovascular disease risks. Similar to Mang et al. (2006), the absolute and percentage differences between pre- and post-intervention serum fasting glucose levels of the cinnamon-supplemented and control groups were impressively significant.

This study demonstrated positive effect of cinnamon supplementation on decreasing blood glucose levels. Therefore, we conclude that regular use of cinnamon can promote healthy glucose metabolism, and cinnamon supplementation in type 2 diabetic patients could provide evidence for the beneficial effects some of biochemical actions. Adequate amounts of cinnamon possibly represent an effective means to reduce the risk factors associated with type 2 diabetic patients. If diabetes patients apply cinnamon in their food preparations regularly, they may keep their fasting blood sugar levels and lipid profiles near to normal levels.

Mang et al. (2006) suggested that cinnamon extract seems to have a moderate effect in lowering serum fasting glucose concentrations in glycemically controlled diabetes mellitus patients. Cinnamon extracts have also been shown to improve insulin receptor function by activating insulin receptor kinase and inhibiting insulin receptor phosphatase, leading to increase insulin sensitivity (Mahpara et al., 2004). One limitation of this study was that the study did not compare the doses between patients in the intervention group. Additionally, cinnamon supplementation and its effect on hemoglobin A1C (HBA1C) in diabetes mellitus II were not investigated. Therefore, future studies will address both limitations.

## Conclusion

Given the discussion, we conclude that cinnamon powder extract solution may be consumed in diabetes mellitus patients to decrease serum glucose levels. However, we are not yet able to generalize the holistic conditions of the diabetes mellitus patients. We strongly suggest further research study into the proper cinnamon powder extract solution dose in patients with type II diabetes mellitus. It is also necessary to further explore these findings in all varieties of diabetes, including type I diabetes mellitus and gestational diabetes.

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*Full Length Research Paper*

# HIV and malaria co-infection: Their combined effects on pregnancy outcomes in Anambra State, Southeast Nigeria

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The daily increasing incidence of malaria and human immunodeficiency virus (HIV) co-infection and associated poor maternal and obstetrical outcomes among pregnant women in the malarious zone of Anambra east, southeast Nigeria, and the paucity of laboratory-based data on the deadly duo, necessitated this study on the prevalence of the co-infection in a case-cohort study of 450 women (15 to 45 years) from whom placental and peripheral blood samples were collected. Screening for HIV antibodies was by the DETERMINE and GENIE-II, confirmed at 95% confidence interval. Pregnancy screening was by human chorionic gonadotropin (HCG) one step pregnancy test strip. Placental malaria was determined by Pooled-biopsy; peripheral malaria, from maternal venous blood. Giemsa stain of thin and thick blood smears were assayed; results were confirmed by dipstick rapid test. Anaemia estimation was by packed cell volume. Population attributable fraction-associated co-infection was 62% (P-value, 0.019). Acute malaria (+++) was highest among dually infected multigravidas (53%) (P-value, 0.0672), and multiple infections highest in second trimester (mean parasite density,  $3,471.1 \pm 101.0$  parasites/ $\mu$ l), with preponderance in the 25 to 29 age bracket ( $4,720.51 \pm 110.3$  parasites/ $\mu$ l). Gravidity-associated co-infection was prevalent among the multigravidas, with mean parasite density of  $19,224.1 \pm 136.0$  parasites/ $\mu$ l. Placental malaria was a significant risk factor for mortality and morbidity (P < 0.01), and maternal anaemia which is the single most important adverse pregnancy outcome. Significance of abortion, pre-term delivery and low infant birth weights as serious adverse maternal outcomes were further established in the study.

**Key words:** Human immunodeficiency virus (HIV), placental malaria, gravidity-associated malaria, pregnancy outcomes.

## INTRODUCTION

Malaria remains an important public health concern, particularly in the malarious zones with regular transmission. Young children, pregnant women, and non-immune visitors to malarious areas are at greatest risk of severe or fatal illness (Desai et al., 2007; The Global

Fund, 2009). Malaria in pregnancy is associated with poor maternal, obstetrical and infant outcomes. Pregnant women in both high and low transmission areas fall victim of this scourge. However, in low transmission areas, primigravidas and multigravidas are both at increased

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risk, while in high transmission areas, primigravidas are the most affected, with more frequent bouts of severe malaria and associated febrile illnesses and adverse outcomes. Malaria commonly involves the placenta, where microscopic examination shows large intervillous accumulations of parasitized erythrocytes together with monocytes containing ingested pigment (Rennie and Robertson, 1999).

The increased high risk of malaria infection in pregnancy could be attributed to the ability of the *Plasmodium falciparum*, the major culprit, to adhere to the trophoblastic villi, extravillous trophoblasts, and syncytial bridges via its intracellular adhesion molecule-1, CD36, the chondroitin sulfate A (CSA) and hyaluronic acid which facilitate parasite attachment to placental cells, thereby interfering with oxygen and nutrient transport to the foetus and subsequently causing general hemorrhaging which contributes to the complications experienced by both mother and child (Steketee, 1996). CSA, to which the parasite binds, is present in pregnant women but not accessible to the parasite in non-pregnant tissue-beds. The putative ligand expressed by the parasite is PfCSA-L and this has been found to be antigenetically conserved among global cases of maternal malaria. Consequently, primigravidas (who have not had initial immunization or exposure to the CSA-binding parasite) are more highly susceptible to malaria infection. *P. falciparum* can be found in the cord blood of up to 16% of infants born to infected mothers (Diagne et al., 2000). Heavily parasitized placenta leads to neonatal malaria via vertical transmission (Rennie and Robertson, 1999). However, protection is observed to occur following successive pregnancies.

Pregnancy similarly elicits immunologic tolerance; a pregnant woman instinctively welcomes the implantation of the foetal allograft in her uterus (Suguitan et al., 2003). Type 2 cytokines and TGF- $\beta$  appear in the human placenta, to enhance implantation and inhibit inflammatory responses (Ouvray et al., 2000). Infections which require a Type 1 response for protection such as malaria, tuberculosis, toxoplasmosis, and leishmaniasis, which are more common in the developing countries are more severe during pregnancy; consequently, pregnancy allows Type 1 responses known to confer resistance to these infections. However, exposure to malaria elicits TNF- $\alpha$ , IFN- $\gamma$ , and IL-2 in the placenta, and these cytokine changes are associated with poor pregnancy outcomes (Adrian et al., 2000; Fried et al., 1998). Primigravidas are therefore highly susceptible to malaria infection, which culminates in high maternal and foetal mortality and morbidity and associated anaemia and low birth weight infants.

HIV infection intensifies the effect of malaria among pregnant women and infants, creating significant impairment and alteration in both cellular, humoral immunity

and resistance to *P. falciparum* infection. Acute malaria infection on the other hand increases viral load, and enhances transmission of HIV and more rapid disease progression with substantial public health implications (Ned et al., 2005). Malaria infection is more frequent and more severe in HIV-positive pregnant women in malaria-endemic settings. Multigravidas with HIV infection are similar to primigravidas without HIV infection in terms of susceptibility to, and negative consequences of malaria infection. Therefore, in the presence of HIV infection, the risk associated with placental malaria appears to be independent of the number of pregnancies. Pregnant women infected with both malaria and HIV are also at higher risk of developing anaemia, delivering a low birth weight infants, and delivering prematurely. Similarly, higher viral load increases the likelihood of maternal-to-child transmission (MTCT) of HIV (Briand et al., 2009; Inion et al., 2003).

Malaria infection during pregnancy increases risks of MTCT in the intrauterine and intrapartum as well as during the breastfeeding period, presumably by increasing HIV viral load. The prevalence of, and adverse consequences of malaria and HIV co-infection on mother-to-child transmission of HIV with subsequent high infant mortality and morbidity, including maternal anaemia, low birth-weight infants, preterm deliveries and/or abortions were therefore examined among pregnant women in some locations of Anambra East Local Government area of Anambra State, with the view to create the epidemic awareness and strengthen capacity building and governmental control strategies in these neglected and infrastructure-deprived areas of south eastern Nigeria.

## MATERIALS AND METHODS

### Study design

This study focused on establishing the prevalence of pregnancy-associated malaria and HIV co-infection in the malaria endemic rural communities of Nigeria, with case study of Anambra East, and raises the question on population prevalence rates of the dual infection, as well as the adverse outcomes or impact of, and association of the dual epidemic on pregnancy among the local populace. Relevance of this question was explored by investigation of the effect of HIV and malaria parasitemia through a case-cohort study of pregnant women from whom placental and peripheral blood samples were collected and subsequently analyzed.

### Study population

This is an economically disadvantaged area, where income per capita is below minimal wage of ₦500. Mortality and morbidity due to malaria and HIV co-infection is high, though not statistically estimated, nor epidemiologically investigated. However, the rate of pregnancy per year in the area is high; more than 3,000 women become pregnant each year, most of these deliver at home. Less than 30% of the pregnant women attend antenatal care visits during



their pregnancy terms, and there is neither intermittent presumptive treatment for malaria, insecticide-treated nets programs to specifically target the pregnant women, nor routine HIV testing. The significance of this study therefore centres on the following:

1. Poverty and related sexual promiscuity and attendant sexually transmitted infections.
2. Further spread of HIV infection as a result of increasing incidence of malaria co-infection in HIV-infected individuals.
3. Increased incidence of mortality and morbidity and loss of labour force and furthering of economic repression and associated poverty.
4. Unavailability of governmental attention in terms of infrastructure including hospitals, access roads and job opportunities.
5. Lack of surveillance programmes on improvement of living standards, and HIV/AIDS education.
6. Absence of antenatal care facilities or programmes on mother and child welfare or other pregnancy related education.
7. Call for establishment and strengthening of programmes for the prevention of malaria in the area

#### Data collection

Four hundred and fifty (450) women between the ages of 15 to 45 years from some local communities of Anambra East Local Government area were studied over a period of 24 months (November 3, 2009 to November 12, 2011). Due to the unreliability and unavailability of accurate procedure of gestational age in this area, women were enrolled based on the date of last menstrual period. Participants were administered a structured questionnaire with details of demographic, behavioral, pregnancy and other health related information. Informed consent was obtained from participants and promise of confidentiality duly declared. Women agreeing to voluntary counseling and testing (VCT) were screened for the HIV-1/2 antibodies in addition to receiving pre- and post-test counseling. Monthly visits were made, and at each visit, a finger-prick blood sample was collected from each woman for both thin and thick blood films for malaria parasites and hemoglobin level estimation, respectively. Maternal anemia was defined as having a packed cell volume (or haematocrit) less than 25%. However, those that presented with symptoms of acute malaria ranging from intermittent fever, headache, malaise, nausea and vomiting with joint pains were encouraged to make more regular visits. Patients were placed on monthly sulfadoxine-pyrimethamine (sulfadoxine, 1,500 mg and pyrimethamine, 75 mg), and daily multivitamin supplement consisting of 200 mg ferrous sulfate, and 5 mg folic acid until 33 weeks gestation. However, those that had intermittent fibril illnesses with PCV less than 30% were placed on 200 mg ferrous sulfate multivitamin twice daily and further advised to improve their feeding and/or diet with fresh fruits and vegetables. Those with positive malaria were then given repeat doses of sulfadoxine-pyrimethamine.

#### HIV screening

All the participants were screened for HIV antibodies. A finger-prick blood sample was collected from participants using sterile blood lancets after pre-test counseling and informed consent. Screening for HIV-1/2 antibodies was carried out according to manufacturers' instructions using two rapid *in vitro* test kits; the determine HIV-1 and HIV-2 (Abbot Laboratories Japan), an immunochromatographic test for the qualitative detection of antibodies to HIV-1 and HIV-2

and GENIE-II (Sanofi, Pasteur, France), which also detects HIV-1 and HIV. A 95% (95% confidence interval: 93.2 to 97.8%) between the two tests was established in the identification of HIV positive samples; HIV infection was thus defined as a positive result on the two rapid tests. HIV positive participants were categorized using the World Health Organization (WHO) guidelines on HIV staging into asymptomatic (stage-1) and symptomatic (stage-2) (WHO, 2007). Excluded from study were those in the stage-3 and 4 categories. The HIV positive women were then given post HIV counseling, assured of the safety of their babies, placed on highly active antiretroviral therapy (HAART), which was regularly supplied to them free of charge and encouraged to live normal lives with improved diet and nutritional supplement. They were further referred to nearby private hospitals for regular surveillance and privacy. The HIV status of each patient was not disclosed to any other to ensure none withdrawal out of shame or societal ostracization.

#### Pregnancy screening

Following oral interviews on their marital status, socio-economic and sexual behaviours, participants were screened for pregnancy by urine testing for human chorionic gonadotropin using the HCG one step pregnancy test strip (ACON Laboratories Inc, USA), a rapid chromatographic immunoassay for the qualitative detection of HCG in urine for early detection of pregnancy. It utilizes a combination of monoclonal and polyclonal antibodies to selectively detect elevated levels of HCG in urine. It has a sensitivity of 25 ml U/ml. The test strip was gently dipped into an early morning urine for 10 to 15 s to allow capillary movement of urine and subsequent reaction with the coloured conjugate, after which the strip was observed for appearance of coloured line on the test line region of the membrane. Two hundred and three (203) women: 112 primigravidas and 91 multigravidas, who tested positive to the pregnancy test were then screened for malaria parasitaemia, and thus considered eligible for the follow-up exercises.

#### Screening for malaria parasite

##### Sample collection for placental malaria

The pooled-biopsy method described by Suguitan et al. (2003) was adopted for investigation of placental malaria. A 5 × 5 × 5 (cm) piece of the placenta was excised from each placenta, allowing maternal intervillous blood to accumulate at the site. A 5 ml sample of heparinized maternal venous blood was then collected and used for both HIV screening and the placental malaria parasite examination.

##### Sample for analysis for peripheral and placental parasitemia

Maternal venous blood (peripheral) samples were collected from all pregnant women (with or without symptoms suggestive of malaria) as previously reported. Symptomatic malarial cases were defined as the presence of asexual forms of *Plasmodium* species on a blood smear, and associated with fever, headache, chills, and/or joint pain) for malaria parasite screening at enrollment and delivery. Screening was carried out following delivery using the "Gold Standard" by the microscopic examination of giemsa stained blood smears. Thin and thick blood films were prepared immediately in duplicate upon blood collection on the same slide. Thick films were

used to identify malaria parasites and determine parasite density, while thin smears were used for species identification. For thick films, 12 µl of blood was spread over a diameter of 15 mm, while 2 µl of blood was used for thin films. The slides were made in duplicates and labeled appropriately. The thin film was fixed in absolute methanol for 1 to 2 s and air dried. Slides were properly coded, and the blood films stained after 24 to 48 h with 3% giemsa stain solution at pH 7.2 and examined microscopically under the X 100 oil immersion microscope. Confirmation was then done using the dipstick rapid chromatographic immunoassay that detects *Plasmodium falciparum*-specific proteins and pan-*Plasmodium* aldolase antigens up to two weeks after the infection has cleared. This was carried out according to manufacturer's instructions. Two distinct coloured bands on the test strip were confirmatory for the presence of malaria parasite. Negative result was indicated by one line in the control region, and an additional line in the test region (T).

Placental blood smears were examined for the presence of both parasites and pigment. Presence of pigment within macrophages indicated parasite death; stages of infection were indicated by presence of parasites, or both; absence of infection was indicated by no parasites, no pigment; early infection, by presence of parasites, but no pigment; late infection, by presence of both parasites and pigment; cleared infection by presence of pigment without parasites.

#### Parasite counts

Malaria parasites for placental and peripheral parasite densities were quantified against 200 white blood cells (WBC) on giemsa-stained thick films. Slides were considered positive if the parasite load was > 200 parasites per 200 WBC; and negative following a review of 200 high-power fields. However, if parasites were identified from the smears, 100 to 200 fields of the thin smear were further examined. Quality control was ensured by further examination of 10% randomly selected slides. Parasitemia was defined as the presence of parasites in thick blood smears. Severity of malaria in women with placental malaria was defined as: mild parasitemia = < 20,000 parasites/µl; severe parasitemia = ≥ 20,000 parasites/µl. Malaria parasitemia at delivery was defined as peripheral and/or placental parasitemia following delivery.

#### Population attributable fraction (PAF)

##### Estimation of the impact and overall malaria-HIV prevalence using the population attributable fraction (PAF)

For more accurate analysis and better understanding of the magnitude of the HIV-malaria co-infection and the burden of the epidemic in the surveyed population, the population attributable fraction (PAF), which is an estimate of the proportion of overall malaria prevalence in the surveyed population attributable to HIV infection was evaluated. This was indicated as follows: G-All = all gravidae; G-1 = primigravidae; G-2= multigravidae; and then calculated as  $100 \times [p (RR - 1) / (1 + p (RR - 1))]$ . Where p is the HIV prevalence and RR, the overall risk ratio for the malaria parasitemia associated with HIV infection.

#### Pregnancy outcome

In establishing the impact of malaria and HIV infection in pregnan-

cy, associated adverse outcomes such as preterm deliveries and/or spontaneous abortion, low birth weight and maternal anaemia resulting to infant mortality were evaluated.

#### Birth weight, preterm deliveries and abortions

Infant weights and placentas (after births) were measured immediately following delivery using the bathroom scale. Infants were considered as having low birth weights at weights less than 2,500 g.

#### Preterm deliveries and/or spontaneous abortion

Cases of abortion were noted, while preterm delivery was measured within the 1st three days of delivery using the new ballard score for preterm delivery.

#### Maternal anaemia

**Estimation of packed cell volume (PCV) for investigation of anaemia:** Impact of *P. falciparum* infections on anaemia, considered a direct marker of maternal and foetal morbidity and mortality in the locality was assessed by estimation of the haemoglobin or packed cell volume (PCV). Heparinized haematocrit tubes were filled with maternal peripheral blood. A constant packing of the red blood cells was achieved with a centrifugation speed of 1200 × g for 3 min. The PCV (%) was measured and reported as a ratio of the whole blood volume.

**Levels of anaemia:** Anaemia was in this study categorized as: (1) Severe: PCV = 14 to 23%; (2) Mild: PCV = 22 to 29% and (3) Moderate: PCV = 24 to 25%. A woman was considered to be anaemic if the PCV was less than 25%.

#### Ethical consent

Approval for the study was obtained from consenting volunteers to whom the purpose of the study was explained following voluntary testing and counseling (VTC). Those that gave informed consent were therefore enrolled in the study prior to questionnaire distribution and laboratory studies. The approval was on the agreement that patient anonymity must be maintained, good laboratory practice/quality control ensured, and that every finding would be treated with utmost confidentiality and for the purpose of this research only. All work was performed according to the international guidelines for human experimentation in clinical research according to the Declaration of Helsinki (World Medical Association and Council for International Organizations of Medical Sciences (CIOMS).

#### Statistical analysis

Available data from questionnaire responses and laboratory results were entered into EpiData software, version 3.02 (EpiData), and the analysis done using the statistical package for social sciences (SPSS), version 18.0. The student's T-test and the percentile ratios were used for the normally distributed data, while the Pearson chi-square test with Fisher's exact test with odds ratios (ORs) and 95% confidence intervals (CIs) was used in making comparisons

**Table 1.** Baseline profile of participants.

Number of participants	Pregnancy positive volunteers (n=203)	%
<b>Mean age (years)±20</b>		
<b>Maternal age group</b>		
15-19	24	11
20-24	46	22
25-29	52	25
30-34	44	21
35-39	30	13
40>	7	3
Total	203	-
<b>Marital status</b>		
Single	72	35
Married	85	41
Divorced	46	22
Total	203	
<b>Gestational levels</b>		
1st Trimester	47	23
2nd Trimester	61	30
3rd Trimester	95	47
Total	203	
<b>Gravidity</b>		
Primigravidae	91	45
Multigravidae	112	55
Total	203	

between categorical data. The relationship between peripheral and placental malaria was compared using the Wilcoxon signed rank test. Estimation of the impact and overall malaria-HIV prevalence was carried out using the population attributable fraction (PAF); while multivariable log-Binomial regression models were used to determine adjusted ORs for risk factors for anaemia, including low birth weight, preterm deliveries/abortions. The Cox proportional regression models were then used to assess the relationship between anaemia and other variables after adjusting for the following important covariates: related factors, parity levels, gravidity and maternal age.

## RESULT

### Baseline profile

Baseline characteristics of the study population, which serves useful tool in the assessment of their health status and the investigated risk factors, are here presented. Descriptive characteristics of the population, including

maternal age, marital status, gravidity, and gestational levels are shown in Table 1. Among the 450 women of child bearing age enrolled in the study, 203 (15 to < 45 years; mean age 20 years) were considered eligible on the basis of their positive pregnancy results. Seventy-two (35%) of the participants were single, 85 (41%) married, and 46 (22%) were divorced; gestational level: 1st trimester (n = 47); 2nd trimester (n = 61); 3rd trimester (n = 95); gravidity: multigravidae (n = 112); primigravidae (n = 91).

### Prevalence of maternal malaria and HIV and co-infection

In consideration of the overall impact of HIV on the burden of maternal malaria (basically among the primigravidae and multigravidae), the magnitude of the epidemic was in this study expressed as the proportional increase in pregnancy-associated malaria in the HIV infected population, here referred to as the population attributable fraction (PAF), which is a function of the HIV-associated increased risk of acquiring malaria in pregnancy; also observed to rise with subsequent increase in HIV prevalence.

### PAF-associated malaria

Of the 91 (45%) primigravidas examined, the PAF-associated malaria and HIV infection was 57 (62%); 13 had only malaria and no HIV (single infection); while 9 were singly infected with HIV. Similarly, the PAF-associated malaria and HIV infection among the 112 multigravidas was 88 (79%); 21 (19%) had single malaria infection while 13 (12%) were singly infected with HIV. The pregnancy-attributable malaria due to HIV co-infection was therefore observed to rise in parallel with HIV prevalence. Overall parasite burden among the dually infected was 71% (Table 2).

### Acute malaria in pregnancy-associated coinfection

Acute malaria, here defined as high levels of parasitaemia (+++), with clinically defined signs and symptoms was nevertheless prevalent in 96 of the dually infected; with highest prevalence among the dually infected multigravidas, 51 (53%), compared with 45 (47%) among the dually infected primigravidas (Table 3).

### Gravidity-associated febrile illnesses

Febrile illnesses and malaria symptoms of fever,

**Table 2.** PAF associated HIV and malaria co-infections among dually infected patients.

Gravidity	Conditions investigated		
	Malaria and HIV	Malaria singly	HIV singly
Overall	145 (71.4%)	34 (16.7%)	22 (10.8%)
<b>Parity</b>			
Primigravidas (n=91)	57 (62%)	13 (14%)	9 (10%)
Multigravidas (n=112)	88 (79%)	21 (19%)	13 (12%)
X	6.426	0.718	0.153
P- value	0.019	0.453	0.822

**Table 3.** Acute malaria in pregnancy-associated co-infection.

Acute malaria parasitaemia	Number examined with Mal/HIV	Number infected	Prevalence (%)
Overall	145	96	66.2
<b>Parity</b>			
Primigravidae	57	45	47
Multigravidae	88	51	53
X		0.309	
P-value		0.672	

**Table 4.** Gravidity-associated febrile illnesses among dually infected volunteers.

Gravidity	No.	%
Primigravidae	91/203	44
Dually Infected with malaria and HIV	57/91	62
Symptomatic for malaria (febrile illness)	41/57	72
Asymptomatic	16/57	28
Multigravidae	112/203	55
Infected with malaria and HIV	88/112	78
Symptomatic for malaria (febrile illness)	63/88	71
Asymptomatic	25/88	28

headaches, sweating, tiredness, abdominal pain, diarrhea, loss of appetite, nausea and vomiting were observed in 41 (72%) of the dually infected Primigravidas; 16 (28%) were asymptomatic, while 63 (71%) of the multigravidas were symptomatic for malaria febrile illnesses while 25 (28%) were asymptomatic (Table 4). Investigation of the association of gestational levels with malaria/HIV co-infection indicated that the preponderance of the multiple infections is higher in the second trimester of pregnancy: out of the 61 (42%)

persons screened, 46 (75%) were dually infected, with malaria parasite density (MPD) of  $3,471.1 \pm 101.0$  parasites/ $\mu$ l; 35 (57%) had febrile illness, symptomatic for malaria parasitaemia, while 11 (18%) were asymptomatic. However, there was no statistical difference in the gestational parasitaemia of 1st and 3rd trimester of pregnancy of the study population ( $1,501.0 \pm 215.0$  and  $1,696.0 \pm 117.1$  parasites/ $\mu$ l), hence not a significant risk factor for the dual infections ( $P < 0.05$ ) (Table 5).

**Table 5.** Gestation-related parasitaemia among dually infected volunteers.

Trimester parasitemia level	No infected	%	Mean parasite density ± SEM (parasites/μl)
<b>1st</b>			
No Screened	47/145	32	
Infected with malaria and HIV	32/47	68	1,501.0±215.0 <sup>b</sup>
Symptomatic	24/47	51	
Asymptomatic	8/47	17	
<b>2nd</b>			
No Screened	61/145	42	
Infected with malaria and HIV	46/61	75	3,471.1±101.0 <sup>a</sup>
Symptomatic	35/61	57	
Asymptomatic	11/61	18	
<b>3rd</b>			
No Screened	95/145	65	
Infected with malaria and HIV	67/95	71	1,696.0±117.1 <sup>b</sup>
Symptomatic	45/95	47	
Asymptomatic	22/95	23	

**Table 6.** Age-associated mean parasite density of multiple infection.

Participants characteristics		Number infected (n =145)	%	Mean parasite density±SD (parasite/μl)
Number of participants	203			
Mean age (years)	20			
<b>Maternal age group</b>				
15-19	24	13	54	1650.1±214.0 <sup>d</sup>
20-24	46	34	77	2065.7±320.3 <sup>b</sup>
25-29	52	49	94	4720.51±110.3 <sup>a</sup>
30-34	44	30	68	1950.08±100.0 <sup>c</sup>
35-39	30	17	57	1602.2±211.0 <sup>d</sup>
40>	7	2	22	820.0±216.2 <sup>e</sup>
X		31.597		
P value		>0.0001		

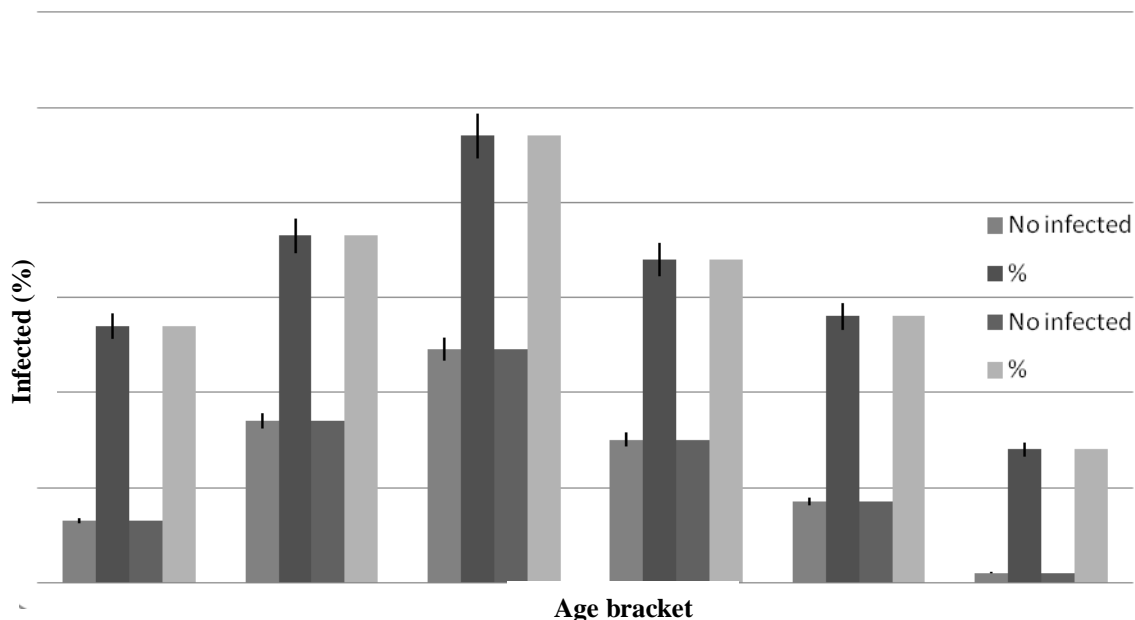
Homogeneous subsets of values are represented by superscript of the same letter.

### Maternal age as risk factor

In consideration of maternal age as constituting an increased risk of morbidity and mortality for both mother and neonate, age as risk factor was therefore evaluated at varying age brackets. The age related parasite density (mean and SD) of the dually infected pregnant women was highest among those aged 25 to 29 years: out of the 52 women screened, 49 (94%) had multiple infections, with mean parasite density (MPD, and ± SD of 4,720.51 ± 110.3 parasites/μl), followed by those aged 20 to 24

years: multiple infectivity, 73%; MPD, 2,065.7 ± 320.1 parasites/μl. However, there was no statistical difference in both the percentage multiple infection as well as the mean parasite density between the age groups; 15 to 19 (54%, MPD, 1,650.1 ± 214.0) and those aged 35 to 39 years (54% percentage multiple infection and 1,602.2 ± 211.0 parasites/μl), respectively. Nonetheless, using univariate analysis, multiple infections occurred irrespective of age; hence, age-associated risk factor in multiple infections was not statistically significant (P < 0.01) (Table 6 and Figure 1).





**Figure 1.** Maternal age and associated co-infection.

To establish the association of gravidity/parity as an important risk factor for the HIV/malaria co-infection, the primigravidae and multigravidae co-infectivity were compared in relation to their mean parasite density. From available results, highest prevalence of the multiple co-infections, 88 (96%), occurred among the multigravidas with mean parasite density  $\pm$  standard error of mean (SEM) of  $19,224.1 \pm 136.0$  parasites/ $\mu$ l, compared with the primigravidas, 57 (51%), mean parasite density  $\pm$  SEM of  $18,689.0 \pm 620.1$  parasites/ $\mu$ l. The difference was however not statistically significant ( $P < 0.01$ ), indicative that pregnancy-associated co-infection with both malaria and HIV occurs irrespective of gravidity, thereby invalidating gravidity as a significant risk factor for the multiple HIV/malaria infection. There was significant difference in the prevalence of co-infection among pregnant women but the difference in mean parasite density was not significant.

**Peripheral versus placental malaria**

Since placental malaria (PM) is one of the major features of malaria during pregnancy and has been globally used as a standard indicator to characterize malaria infection in epidemiologic investigations, a comparison of the placental and peripheral blood smear positive cases was evaluated to establish the level of significance and implications of the available results. Malaria parasites were detected in both placental and peripheral blood

smears of the study population. In spite of the paucity of placental parasites however, more than 48% parasitized erythrocytes were detected. Among all the malaria positive cases obtained, 104 (58%) were placental malaria while 75 (42%) were peripheral blood malaria positive cases. There was therefore a significant positive correlation between parasitaemia levels determined by placental blood ( $P < 0.01$ ), consequently, establishing placental malaria a significant risk factor for mortality and morbidity in pregnancy.

**Adverse pregnancy outcomes**

Following the observation that HIV and malaria co-infections have serious adverse complications, the associated pregnancy outcomes of the deadly duo were assessed to establish their relationship with maternal anemia and associated hypoxia and perinatal mortality, low birth weight, abortion/preterm deliveries.

**Anaemia as risk factor**

Result of the study reports malaria as the commonest medical condition associated with pregnancy especially among those dually infected with HIV and malaria. The incidence of maternal anaemia, herein defined as PCV  $<$  25%, was high among the dually infected women irrespective of their age and gestational levels. Overall

**Table 7.** Pregnancy associated risk factors: burden of HIV/malaria co-infection.

Parameter	No.	%
No of dually Infected with malaria and HIV	145	71
<b>Anaemia</b>	137	94
Severe or Acute anaemia (PCV $\leq$ 18%)	96	71
Sub-acute or Moderate anaemia (PCV = 19-26%)	30	21
Mild anaemia (PCV = 27-29%)	11	8
<b>Gravidity associated anaemia</b>		
Primigravidas	71	52
Multigravidas	66	48
<b>Delivery outcome</b>		
Abortion	103	
Primigravidae	71	69
Multigravidae	32	31
Low birth weights	69	
Primigravidae	44	64
Multigravidae	25	36
Pre-term delivery	40	
Primigravidae	23	58
Multigravidae	17	42

prevalence of anaemia among the dually infected was 137 (94%) (Table 7).

### Severity of anaemia

Severe or acute anaemia, with PCV range of  $\leq$  18% occurred in 96 (71%) of participants; sub-acute or moderate anaemia, PCV level of 19 to 26%, in 21%; while mild anaemia, PCV range of 27 to 29%, was observed in 11(8%) participants. There was no significant statistical association between anaemia among the primigravidas (48.8%) and the multigravidas (52%) as well as the different gestational levels; 28% in the 1st trimester, 31% in the 2nd trimester and 40% in the 3rd trimester, respectively. The prevalence occurred irrespective of gravidity (Table 7).

### Precipitating factors to the high incidence of anaemia

In addition to pregnancy-associated malaria parasitaemia and HIV-associated immunosuppression with resultant high rates of haemorrhage and consequently inadequate hematopoiesis among the surveyed pregnant women, other reported precipitating factors to the high incidence of anaemia (from questionnaire responses of this study)

included poverty (51%), older maternal age (15%), poor nutrition and prenatal care (34%).

### Pregnancy-related adverse birth outcomes

The prevalence of and impact of pregnancy-related adverse birth outcomes: low birth-weight infants, and pre-term deliveries and/or abortions among the dually infected gravid women, studied to ascertain their association with the deadly duo, malaria parasitaemia and HIV infection is here presented. Available results indicated the prevalence of several adverse birth outcomes among the co-infected, with highest prevalence in the primigravidas. The study observed a total of 103 cases of abortions out of the 145 multiply infected persons screened: 71 (69%) among the primigravidas and 32 (31%) in the multigravidas, respectively. Mean infant birth weights were very low; the percentage of low birth weight (LBW) babies were significantly higher among the primigravidas, 44 (64%), compared with the multigravidas 25 (36%). Pre-term delivery (PTD) was similarly higher among the primigravidas 23 (58%) than the multigravidas 17 (42%) (Table 7). Using multivariable log-Binomial regression models, a significant relationship was established between the multiple infections and increased risk of anaemia, abortion, low birth-weight and

pre-term delivery (adjusted OR, 2.0; 95% CI, 1.1 to 2.6;  $P < 0.001$ ). Juxtaposing the high incidence of anaemia and the established adverse birth outcomes, the study therefore indicates a direct relationship between these risk factors and the multiple co-infections, malaria and HIV. While establishing anaemia as a significant risk factor of the multiple infections (from reported high prevalence rates), its association with or impact on other risk factors and birth outcomes is also here underscored, further implicating anaemia as a single most important adverse pregnancy outcome.

## DISCUSSION

The study examined the relationship of HIV and malaria in mother to child transmission (MTCT), and their combined effects on pregnancy outcomes. The observed preponderance of, and risk of young single women of child bearing age (mean age, 20 years) to pregnancy; reasons, consequences and the health implications which questions their economic status, social and or/sexual life style were evaluated. Though high incidence of pregnancy was observed among both the married and single women, however, the reported single parent pregnancy (out of wedlock pregnancy) was reckoned and has remarkable societal problem with serious economic and public health implications. This questionable phenomenon could be attributable to prevailing lifestyle in the locality, which is synonymous with sexual promiscuity, a common occurrence in economic repressed areas. The observed effect of poverty among the natives in the rural settings is consistent with reports from Dibua (2010), suggesting that the underlying cause of promiscuity and associated exposure to sexually transmitted diseases including HIV/AIDS is a combined effect of poverty, lack of job opportunities and improved life condition as well as adverse socio-cultural norms, ignorance and illiteracy, parental neglect, low self-esteem, influence of media, widowhood, divorce or separation.

This study observed endemicity of malaria, as well as a high prevalence of both HIV and malaria co-infection (a superimposed condition): HIV infection almost doubling the risk of malaria parasitemia and clinical malaria episode; overall parasite density of 71%; expressed as population attributable risk (PAF), an indication of a direct or synergistic interaction between the deadly duo; one increasing the susceptibility of the other and thus accelerating the progression of the HIV disease, AIDS. The increasing incidence and magnitude are multifaceted, and could thus be traced to an overlap between the geographic and transmission intensity in the studied rural malarious zone where malaria and HIV advocacy have neither attention nor impact. Previous reports had

indicated that areas of such overlap are characterized by heavy infestation of chloroquine-resistant *P. falciparum* (TerKuile et al., 2004). Result of this study thus suggests that individuals living in areas with high *P. falciparum* parasitemia therefore have a higher risk of being infected with HIV, and that severe malaria in HIV-co-infected patients presents with higher parasite burden as previously reported elsewhere in Africa (Whitworth et al., 2000). This research further showed that pregnant women living with HIV/AIDS in the rural and peri-urban malarious areas of Nigeria (irrespective of their gravidity levels) have higher incidence of both placental and peripheral malaria with higher parasite densities and associated higher parasite burden, in conformity with reports on the risk of HIV-associated malaria, attributable to modifications in systemic and placental immunologic factors following gross impairment of the gravidity-dependent acquired immunity to malaria resulting from HIV infection and associated high viral load (UNAIDS/WHO, 2007; vanEjik et al., 2003).

Previous reports on the cellular consequences of the co-infections on pregnancy, attributed the increased susceptibility to excessive destruction of the IL-12-mediated IFN- $\gamma$  pathway due to HIV infection: intervillous blood mononuclear cells (IVBMCs) which produce interferon- $\gamma$  (IFN- $\gamma$ ), usually accords protection against placental malaria in the absence of HIV (Moore et al., 1999); nevertheless, this protection is lost in HIV-infected pregnant women with *P. falciparum* malaria, in response to malarial antigen stimulations, result of severe destruction of the interleukin-12 (IL-12); (IL-18-mediated IFN- $\gamma$  pathway is however not affected), with resultant low CD4 counts, thus indicating a shift in the cytokine responses with HIV progresses (Chaisavaneeyakorn et al., 2002).

The observed prevalence of placental malaria among the dually infected women is in this study, considered as a major determinant of congenital malaria, with serious consequences for mother to child transmission, suggestive of trans-placental transmission of *P. falciparum* in neonates during malaria-exposed pregnancies as indicated by previous reports (Uneke et al., 2007; Mwapasa et al., 2004). Mounting evidence also suggests that malaria increases HIV viral load in pregnant women and increases CCR5 expression on intervillous maternal and fetal villous macrophages, which may increase risk for fetal infection, posing an increased risk for mother-to-child transfer of HIV-1, and buttressing that high density of population-associated *P. falciparum* malaria is intrinsically linked to the geographical location of the area, (riverrine area); characterized by numerous waterbodies and/or waterlogs, several of which are stagnant and grossly contaminated with human and domestic wastes, which thus provide favourable breeding grounds for infestation by *Anopheles* mosquitoes, the implicated

species involved in *P. falciparum* parasitaemia. Next to this dilemma is the lack of health education and/or governmental intervention programmes in the area.

The study further observed prevalence of placenta-associated anaemia and other adverse pregnancy outcomes: birth weight, (ILBW babies), and preterm deliveries (PTD) and/or abortions, and aptly indicates that placental malaria significantly increased the prevalence of adverse obstetric outcomes in the multiply infected group irrespective of age or gravidity, underscoring the severity of malaria infection in HIV-positive pregnant women particularly in malaria-endemic setting; further implicating pregnancy-associated malaria as enhanced risk factor for MTCT in the intrauterine and intra-partum periods through lateral transfer of HIV infection by increased HIV viral load, as well as establishing that malaria, anaemia and HIV infections are the indirect causes of poor maternal health outcomes and serious public health problem in the local settings and poor resource areas of Nigeria.

This study reported an approximate 40 cases of preterm delivery and 103 cases of abortions (basically spontaneous), reflecting a breakdown in normal mechanisms responsible for maintenance of uterine quiescence, attributed to formation of an early gap junction and abnormal rise in oxytocin receptor concentration as earlier reported (Nahlen et al., 1996). Though these abnormalities may be reckoned as common adverse pregnancy outcomes, nevertheless, the overtly amplified condition suggests a subtle interplay between the already compromised placental integrity due to the combined effects of malaria parasitaemia and high viral load associated with HIV disease progression, resulting in further down-regulation and/or dysfunction of the uterine musculature, as well as dys-balance of the oxytocin and prostaglandin receptors which could have triggered the observed anomalous physiologic and pathological changes in the placenta. Therefore, the study generally observed and concluded that, dual infections with HIV and malaria have serious complications for both mother and child as a result of the increased susceptibility of pregnant women infected with HIV to malaria; preponderance of clinical malaria, expressed as fever or a history of fever in the presence of microscopically detected malaria parasitemia among dually infected pregnant women, culminates in high maternal and foetal mortality and morbidity and associated anaemia, preterm delivery/spontaneous abortions and low birth weight infants; acute malaria triggers increased HIV replication (high viral load), and subsequently, increases transmission of HIV and more rapid disease progression to advanced disease, AIDS, with significant public health implications; higher viral load resulting from increased HIV replication and malaria parasitemia, in addition to placental inadequacies, increases the likelihood of MTCT

of HIV in the intrauterine and intrapartum periods. Increased maternal anemia resulting from multiple interactions of HIV and malaria infections is a complication for and serious risk factor for other adverse pregnancy outcomes including preterm deliveries and spontaneous abortions.

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*Full Length Research Paper*

## The magnitude of tooth bud extraction in Uganda

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Uganda traditional healers consider that extraction of un-erupted deciduous canines cures childhood diarrhea, vomiting and fever. This study was therefore designed to establish tooth bud extraction (TBE) practice and determine the prevalence of missing deciduous teeth due to TBE in Uganda. Purposive multistage sampling was used to get a total of 1,121 children (<36 months), who were clinically examined. Interviews of identified parents, traditional healers, elders and health workers were conducted using questionnaires. Results were analyzed using epi-info version 5. TBE was found in 29.3% of the children. This was the highest among Nilotics (45.5%) and the lowest among Bantus (22.3%). It differed significantly across the six districts ( $p$ -value < 0.01). The mean number of affected teeth per child was 3.8, and 99.4% of these were canines. TBE was higher in children staying with guardians as compared to those staying with parents ( $p$ <0.01). Children from rural areas were more affected (33.8%) than those in the urban areas (23.9%). The operation was carried out by traditional healers using crude and unsterile instruments without anesthesia. TBE was the highest in the north and the lowest in the south, supporting the suggestion that it spread from Northern Uganda Southwards. TBE in Uganda is a real health problem that needs further investigation and appropriate intervention.

**Key words:** Tooth, bud, extraction, traditional healer.

### INTRODUCTION

Tooth bud extraction (TBE) by traditional healers has been reported in different parts of Uganda and Tanzania for over forty years (Pindborg, 1966; Tirwomwe and Ekoku, 1987; Raikes, 1988; Mosha, 1983; Hiza and Kikwilu, 1992). Traditional healers, in their attempts to find the causes of diarrhoeal diseases and fevers in child population, found prominencies in the areas corresponding to unerupted deciduous canines. Underneath these prominencies are developing tooth buds. The traditional healers consider these prominences abnormal

and were incriminated as the causes of diarrhea, vomiting and fever in children. They therefore, thought that extraction of these tooth buds would cure these childhood diseases.

Mothers in Uganda, have commonly noticed that soon after birth or at the earliest sign of ill-health, there is a swellings on the gums, commonly seen at the canine sites in both jaws. The baby is thus taken to "Traditional Healers", for treatment which in most cases involves the crude extraction of the tooth bud. In Uganda, the

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extracted tooth buds are commonly referred to as “false teeth”, “ebinyo”, “telek”, etc., depending on the ethnic locality.

The magnitude of the problem is not precisely known, but isolated and scanty information available shows its existence in both Uganda and Tanzania. The study in the then four Uganda districts (Pindborg, 1969) revealed that 16.1% of the Acholi tribe had abnormal dental conditions related to the ritual extraction of the primary canines. The Uganda National Oral Health Survey results (Tirwomwe and Ekoku, 1987) showed that some teeth in children aged 6 years had been lost due to “ethnic reasons” which could be attributed to TBE. The survey on “Social Aspects of Oral Health in context of Primary Health Care in Uganda (Raikes, 1988) revealed that 95.1% of the respondents had heard of “false teeth” but 89.1% did not know what caused this condition. In Tanzania, reports about cases of TBE in Arusha (Moshia, 1983) have been recorded. Reports of missing teeth due to “nylon teeth” extraction in Tanzanian Children (Matee and Van Palenstein, 1991) revealed prevalence with a weighted mean of 9.5%. Canines were the most involved teeth, accounting for 95% of all the missing teeth. It is clear that the magnitude of this harmful belief and practice of TBE in Ugandan children is not well known. This study was therefore designed to determine the prevalence of missing deciduous teeth due to TBE practice by district, ethnicity, sex, age, jaw and tooth bud type; identify and describe the TBE practice itself and establish the consequences of the practice on the dental health of the victim.

## METHODOLOGY

The sampling methodology differed according to the specific objectives: for purposes of achieving objective one, and a purposive multi-stage sampling technique was used to select households with children under the age of 36 months.

### Sample size

Using the formula for descriptive studies, the estimated minimum sample size was 186 children aged 36 months and below for each district. A total of 1,121 children were identified and included in the 6 study districts of Arua (200), Gulu (69), Kabale (205) Kampala (204), Masindi (202) and Mbale (241).

### Sample selection

In the first stage, purposive sampling was done to identify six districts out of the then thirty eight districts of Uganda. Based on the 1987 National Oral Health Survey report which revealed TBE practices, and ensuring that each of the social-cultural and geographic regions in Uganda were represented by at least one district, Kampala, Kabale, Mbale, Masindi, Gulu and Arua were included in the study.

In the second stage, four sub-counties were randomly selected

from each of the five districts except Kampala where four parishes were selected. The deviation from sub-county to parish choice was due to high density of the population in Kampala. A parish is smaller than a sub-county in area but the population size in a Kampala parish is estimated to be close to the population size in a sub-county in other districts.

In the third stage, specific villages were selected on the basis of verbal reports of TBE in the area by the civic and political leaders, health workers and traditional healers in each district. In Kampala, a list of households and their family members was obtained from the Chairman of a Local Council 1 (LC1) in the study parish. The LC1 chairman’s residence was used as starting point. The households were numbered from one to infinite. A coin was used to determine a factor which was added to the numerical list of house hold to obtain the final house hold for inclusion in the study.

In the other five districts, the use of comprehensive household lists was not possible due to large surface areas to be covered. Using the LC1 chairman’s residence as a starting point, the direction with the highest concentration of homesteads was selected. Children of age 36 months and below were identified among all selected. Children included in this study were diagnosed with diarrhoeal diseases, fevers in the child population, and with unerupted deciduous canines.

### Mouth examination

If the visited house hold had a child below 36 months, consent to have the child clinically examined was sought from the parent/guardian. The clinical examination was by direct inspection of the oral cavity using natural light and a mouth mirror to retract the lips and cheeks. These clinical examinations were carried out by two previously trained and calibrated dental surgeons. The clinical diagnostic criteria used were: Score 0 = Normal; Score 1 = Missing deciduous tooth due to TBE; (clinically observed on the position where a given tooth is supposedly to have erupted or is erupting in a child younger than twenty months; and/or tooth which is not seen in oral cavity in a child older than twenty months. In both cases, this was accompanied by confirmation from parent of the child that the child was a victim of the practice); Score 2 = As in score 1 but not confirmed by the parent/guardian; Score 3 = Tooth malformed/malpositioned as a consequence of TBE; Score 4 = incising/scratching of gum; Score 5 = incising/scratching of gum and rubbing in herbs.

The interviews were conducted by previously trained student interviewers from the Department of Social Sciences at Makerere University, Kampala Uganda. Consent to interview of parent/guardian whose child was found affected was obtained. The interview was done using the questionnaire for parents. The parents interviewed were mostly mothers because the fathers were not available at the household site. The local councilors, health workers and local leaders were consulted in identifying the traditional healers in the area. The residences of the identified traditional healers were then visited. Consent to interview them was obtained and those that consenting were interviewed using the structured and pre-tested questionnaire for traditional healers. Local councilors were consulted to identify the village leaders and those identified were then visited. Consent to interview them was obtained and those consenting were interviewed using questionnaire. The health workers were found in government and private health units, clinics and drug shops. Their consent to be interviewed was obtained and those that consented were given a self-administered questionnaire which was left with them for completion. The duly completed questionnaire was collected later.

Clinical data and responses from health workers, elders, traditional healers, and parents were recorded. The developed data



**Table 1.** General characteristics of the examined children

Variable	Number examined	Sex	
		Male	Female
<b>District</b>			
Arua	200	118	82
Gulu	68	32	36
Kabale	104	96	108
Kampala	204	111	93
Masindi	202	91	111
Mbale	239	122	117
<b>Ethnicity</b>			
Bantu	751	367	384
Nilotics	134	82	52
Nilo-Hamites	201	113	88
<b>Locality</b>			
Rural	523	290	233
Urban	590	276	314
<b>Age (Months)</b>			
1-10	334	162	172
11-20	279	154	125
21+	491	249	242

**Table 2.** Common diseases among children by category of respondent

Disease	Category of respondent			
	Health worker (%)	Traditional healer (%)	Elders (%)	Parents (%)
Diarrhoea	88.4	78.8	58.2	60.4
Measles	47.0	40.0	33.4	24.3
Whooping Cough	19.0	31.8	45.5	48.6
Malaria	89.9	74.1	82.7	76.4
False teeth	28.4	83.5	43.9	27.5

capture screens in dbase III+ were used to enter the data. Frequencies and cross-tabulations of key variables were done using epiinfo version 5.

## RESULTS

A total of 1,121 children drawn from the districts of Arua (200), Gulu (69), Kabale (205), Kampala (204), Masindi (202) and Mbale (241) were examined (Table 1). A total of 354 parents, 440 elders, 270 health workers, and 85 traditional healers were interviewed (Table 2). Experience of TBE was found in 328 (29.3%) of the children. From Table 3, the prevalence of TBE in children was the highest in districts Gulu (55.1%), Arua (41.0%), and Masindi (36.1%). In the other study districts, the preva-

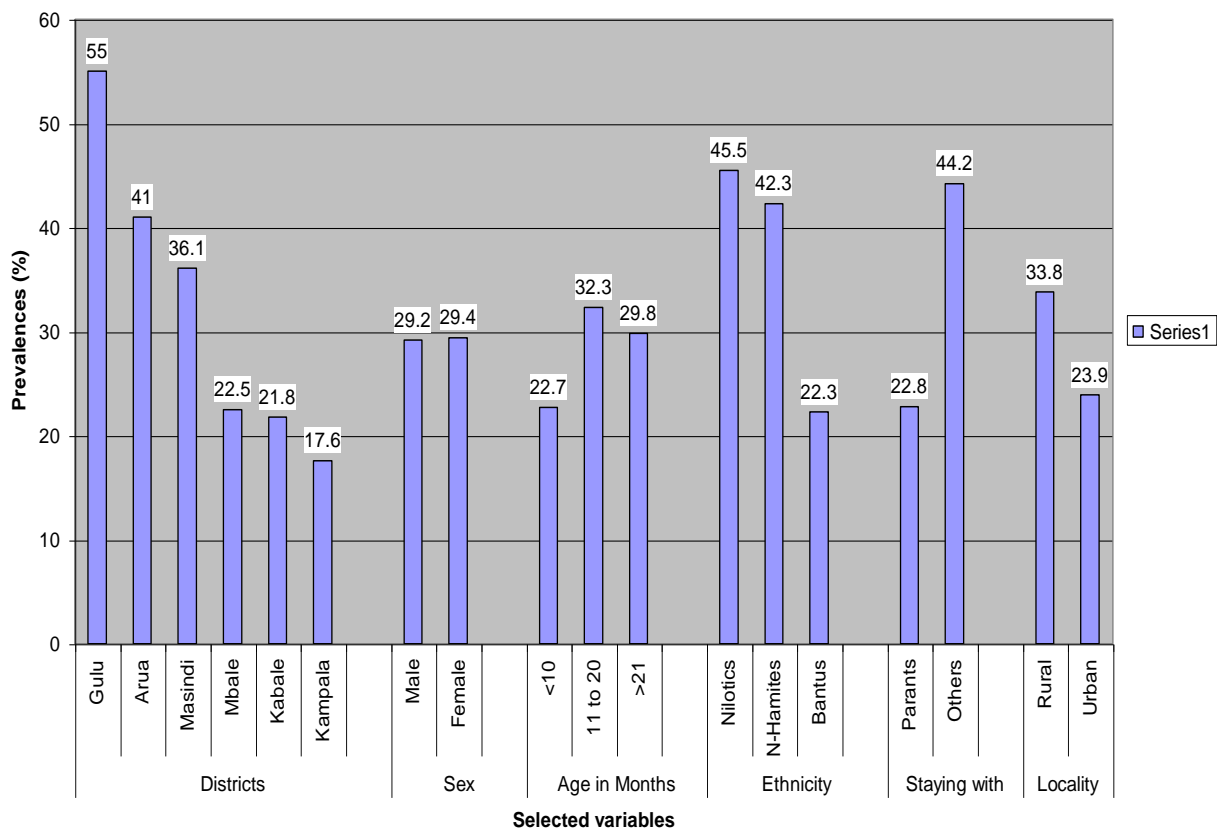
lence of TBE practice was 22.5% in Mbale, 21.8% in Kabale and 17.6% in Kampala (Figure 1). This shows that the prevalence of the practice of TBE differed significantly across the six districts ( $p$ -value < 0.01).

The number of affected teeth per child with TBE experience ranged from 2 to 6 with a mean of 3.84. Over 90% of the cases had at least four affected teeth due to TBE practice. Overall, the canine was found affected by the TBE practice in 99.4% of the affected children. Both jaws were equally affected by the TBE practice and there was no difference between the sides of the jaws.

The prevalence of TBE practice was 45.5% among Nilotics, 42.3% among Nilo-Hamites and 22.3% among Bantus (Figure 1). Further analysis shows that the practice of TBE is more than twice more prevalent among

**Table 3.** Summary of study subjects by district.

District	Category of respondent				
	Children	Parents	Elders	Health workers	Traditional healers
Arua	200	89	63	56	11
Gulu	69	43	3	2	45
Kabale	206	50	102	58	4
Kampala	204	49	42	42	15
Masindi	204	82	98	56	3
Mbale	240	81	132	56	7
Total	1121	354	440	270	85



**Figure 1.** Pravalence of tooth bud extraction practices by selected variables

the non-Bantu than among the Bantus (Odds ratio = 0.38,  $p < 0.01$ ). TBE practice was found in 44.2% of the children staying with guardians and 28.7% of the children staying with at least one of the parents. The prevalence of TBE practice was higher in children staying with guardians than in the other children ( $p < 0.01$ ).

The TBE experience was found in 35.7% of the children whose parents were peasants. This prevalence was higher than in children whose parents were engaged in other occupations (22.8%). Children staying in the rural

areas (33.8%) were more affected by the TBE practice than those in the urban areas (23.9%). Affected children in the rural were also found to have more teeth affected by the TBE practice than their urban counterparts.

The practice involves the crude and brutal extraction of mainly the canine tooth buds. The operation was carried out by known traditional healers in the villages. The parent identifies the false teeth by the presence of the associated conditions of diarrhea, fever, and vomiting in the child. The parent who highly believes that these false

teeth cannot be treated by health workers takes the affected child to the traditional healer.

Crude extraction of the tooth bud was done by 95.7% of the traditional healers at their residence and 28.6% used sharp pointed chisels, 26.2% used sharp bicycle spokes, 19.0% used razor blades, and sharp pointed nails, respectively while 4.8% used locally made clippers/tongs. During TBE, the traditional healers were assisted by 75.0% of the patient's parents in holding the affected child. Only 29.8% of the TBE practitioners mentioned having sterilized their instruments by boiling prior to use. Of these traditional healers, 11.9% admitted having had complications such as excessive bleeding, infection of the gum and jaw bone following false teeth extraction. About 67.9% of the traditional healers claim to have ever referred false teeth patients to health units. About 81.0% of them claimed that they followed up false teeth cases after treatment.

## DISCUSSION

Overall, the prevalence of TBE practice was high at 29.3% among the studied population in this region. The prevalence of TBE practice differed significantly across the six districts ( $p < 0.01$ ). The 29.3% prevalence is higher than the 9.5% reported (Matee and Van Palenstein, 1991) in Tanzania, lower than 87% reported in rural Kenya (Hassanali et al., 1995). The TBE prevalence of 55.1% in Gulu district is higher than the old study report of 16.1% by Pindborg (1969). It is similar to another 31.4% reported by Kikwilu and Hiza (1997) in Tanzania. The significant (intra-country) variation in TBE practice underscores the strength of socio-demographic, socio-economic and cultural dynamics of TBE practice in East-African region. This observation needs effective intervention to be quickly developed and implemented to optimize the uptake of healthcare delivery services available at various health centers and hospitals nationwide.

The TBE practice was found more prevalent among the Nilotics (45.5%) and Nilo-Hamites (42.5%) than among the Bantus (22.3%). This trend across the ethnicity variable is similar to that reported in by Pindborg (1969). These findings tend to support the suggestion that the TBE practice originated from Southern Sudan and spread southwards to the northern districts of Uganda and eventually to southern districts of Uganda. This is further supported by the fact that 72.9% of the traditional healers were of Nilotics and Nilo-Hamite ethnicity.

The mean number of affected tooth buds was higher ( $3.93 \pm 0.48$ ) in the affected rural children than in the affected urban children. The prevalence of the TBE practice was higher in children staying with guardians (44.2%) than among those staying with at least one of the parents (28.2%). The number of teeth affected by the

practice per child is high ranging from 2 to 6 teeth with a mean of  $3.84 \pm 0.61$ . Over 90% of the cases had at least four of their teeth affected by the TBE practice. Overall, the canine was found affected in 99.4% of the cases. This figure is similar to 95.0% affected canine reported in Tanzanian children (Matee and van Palenstein, 1991), and higher than 72% reported in rural Kenya (Hassanali et al., 1995; Paul et al., 2008). Risks for children who undergo these procedures are extensive, including septicaemia, potential for HIV transmission, numerous dental complications and death (Johnston and Riordan, 2005).

Almost everybody interviewed had ever heard about false teeth: health workers (98.5%) and elders (97.3%). Of the health workers, 74.4% had ever had at least a case of false teeth in their practice. Almost all the parents (97.2%) claimed to have noticed improvement in the health status of the child after treatment by the traditional healer. This is higher than the 65.0% previously reported (Welbury et al., 1993) among Sudanese mothers.

The traditional healer uses crude and unsterilised instruments to extract false teeth. This may lead to the TBE complications and it is not surprising that the health worker receives patients with these complications such as infection of the gum/bone, excessive bleeding, fever and tetanus-like conditions.

Conclusively, the TBE practice is generally high, being the highest in the north and the lowest in the southern part of Uganda. The TBE in Uganda is a real health problem that needs further investigation and appropriate intervention that may bring about changes in people's attitudes and behaviour regarding TBE.

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Full Length Research Paper

## Epidemiology and prevalence of urinary schistosomiasis in pre-school children in Lagos, Nigeria

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The prevalence of urinary schistosomiasis in pre-school children below the age of 5 years in five settlements was investigated. Of the 1,402 children examined and screened for schistosomiasis, 573 children representing 40.8% were infected. Females between the ages of 3 to 4 years had the highest infection of 37% while the least percentage prevalence of 6.4% was recorded in females below one year. Similarly, males between the ages of 3 to 4 years had the highest percentage of 28.9% while 7.1% prevalence was recorded in age cohort below one year. The prevalence of infection was higher in the male children representing 22% while the female recorded 18.8%. The highest prevalence of haematuria (43.5%) was recorded between 4 to 5 years, followed by 3 to 4 years age cohort with 31.4 and 0.0% in the ages between 1 to 3 years. However, there was no significant difference ( $t = 1.51$ ;  $P > 0.05$ ) in the level of infection between male and female subjects. Similarly, there was no significant difference ( $t = 1.67$ ;  $P > 0.05$ ) in the prevalence of infection at the study locations. The intensity and prevalence of the infection increased significantly ( $P < 0.05$ ) with age. There was a positive correlation ( $r = 0.37$ ;  $P < 0.05$ ) between individuals infected in the different locations. In these settlements, schistosomiasis was caused by unsanitary habits, contact with stagnant water which accumulate due to poor drainage and fishing activities by the communities.

**Key words:** Prevalence, transmission, schistosomiasis, pre-school children, snail and parasite.

### INTRODUCTION

Urinary schistosomiasis is one of the main occupational diseases acquired by man through activities associated with fresh water such as washing, bathing, fishing and recreation (Mafiana et al., 2003). Schistosomiasis, being mainly an occupational disease, principally affects people engaged in agricultural activities or fishing, but in many areas, a population of children are infected (Okoli and Odaibo, 1999).

In Nigeria, schistosomiasis due to *Schistosoma haematobium* is wide spread and constituting a public health problem, particularly in children (Akogun, 2008). The snail host of *S. haematobium* are species of the

*Bulinus* and *Planorbidae* which act as the intermediate host where the miracidium develops (Ezeamama et al., 2005). In an investigation carried out by Mafiana et al. (2003) on surface water use and its potential for the transmission of parasitic diseases, it was observed that pre-school children in communities near the Oyan reservoir constituted an unexpectedly high proportion of children with water contact. The infection and transmission in pre-school children would occur through early exposure to infected water bodies when they accompany their mothers to fishing, bathing and washing activities. Also, at about three years of age, exposure

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**Table 1.** Prevalence and intensity of infection with *Schistosoma haematobium* by age.

Age group (years)	Number examined	Number infected (%)	Intensity of infection (%)		
			Negative	Light	Heavy
Under 1	92	39 (42.4)	57.6	42.4	0.0
1 – 2	213	94 (44.1)	55.9	44.1	0.0
2 – 3	273	101 (37.0)	63.0	32.3	4.7
3 – 4	396	187 (47.2)	52.8	40.6	6.6
4 – 5	428	152 (35.5)	64.5	29.3	6.2
Total	1,402	573 (40.8)	59.2	37.3	3.5

to infection was unaided as most children had acquired the ability to swim, bath and wash on their own (Mafiana et al., 2003).

The social aspects of the parasite infection has a lot of stigma associated with it, some of which include the challenges of semi immorality, depression as well as mental ill-health due to embarrassment (Akogun, 2008). The higher prevalence of the disease in male children is due to higher water contact since their fathers engage every male in their household profession usually fishing, while females are usually more restricted from swimming and bathing in rivers due to religious and socio-cultural backgrounds but still perform other activities such as washing (Ndvomugenyi and Minjas, 2001).

In view of the paucity of information on the distribution of schistosomiasis in these settlements in Lagos State and the general concern that the disease may be increasing in prevalence, distribution and importance, this study was undertaken to establish the occurrence, prevalence and intensity of *S. haematobium* infection among pre-school children in the area of Lagos State, Nigeria.

## MATERIALS AND METHODS

### Study area

The study was carried out in five selected communities in Lagos State. These communities are Ajegunle (Pako estate), Zion (Sari Iganmu), Orile Iganmu, Badir and Epe, respectively. These places are known for lack of proper drainage systems and poor sanitary habits. The roads are very deplorable, thereby becoming flooded with water both during the rainy and dry season, and quite inaccessible. The stagnant water becomes filled with human wastes due to indiscriminate urination and defaecation. Fishing activities predominate at Epe.

### Ethical clearance

Before the commencement of the study, we intimated the village heads on the objective of the study and obtained their consent. The communities were then later mobilized to participate in the study.

### Parasitological investigation

A house-to-house census was conducted with the assistance of medical personnel during which children below 5 years of age in the five communities were listed. Children were registered using an

epidemiological field form which documented their age, sex, household head and community (Mafiana et al., 2003). Urine samples were collected from 1,402 children and tested for *S. haematobium* using the sedimentation of urine by gravity method (Asaolu and Ofoezie, 1990). Urine samples were collected between 10.00 a.m. and 2.00 p.m. and once from each child. Mothers were given specimen bottles with the instruction to collect any voided urine between the specified hours from children below 3 years. The intensity of infection was expressed as negative (0 egg/10 ml urine), light (< 50 eggs/10 ml urine) and heavy ( $\geq$  50 eggs/10 ml urine). In addition, haematuria (visible blood) in urine was scored visually.

### Statistical analysis

Descriptive statistics were used to calculate the percentage(s) infection and significant differences between males and females susceptibility to infections. Also, correlation coefficient was used to test the level of correlation between individual(s) infected in locations.

## RESULTS

Of the 1,402 children examined and screened for *S. haematobium*, 573 children representing 40.8% were infected. Children between the ages of 3 to 4 years had the highest infection representing 47.2% while the least percentage prevalence of 37.0% was recorded in 2 to 3 years age cohort (Table 1). The highest prevalence of haematuria (43.5%) was recorded between 4 to 5 years, followed by 3 to 4 years age cohort with 31.4 and 0.0% in the ages between 1 to 3 years.

The overall prevalence of infection was higher in the male children with 18.8%. There is no significant difference ( $t = 1.67$ ;  $P > 0.05$ ) in the level of infection between male and female subjects. In a similar development, there is no significant difference ( $t = 1.67$ ;  $P > 0.05$ ) in the prevalence of infection at the study locations. However, there is a positive correlation ( $r = 0.37$ ;  $P < 0.05$ ) between individuals(s) infected in the different locations. Table 1 shows that 37.3% of the children had light infection while 3.5% had heavy infection. The levels of infections in the five selected communities are shown in Figure 1. It is evident from Figure 1 that Epe community had the highest infection of 32.2% while the least infection of 7.5% was recorded in Orile Iganmu.

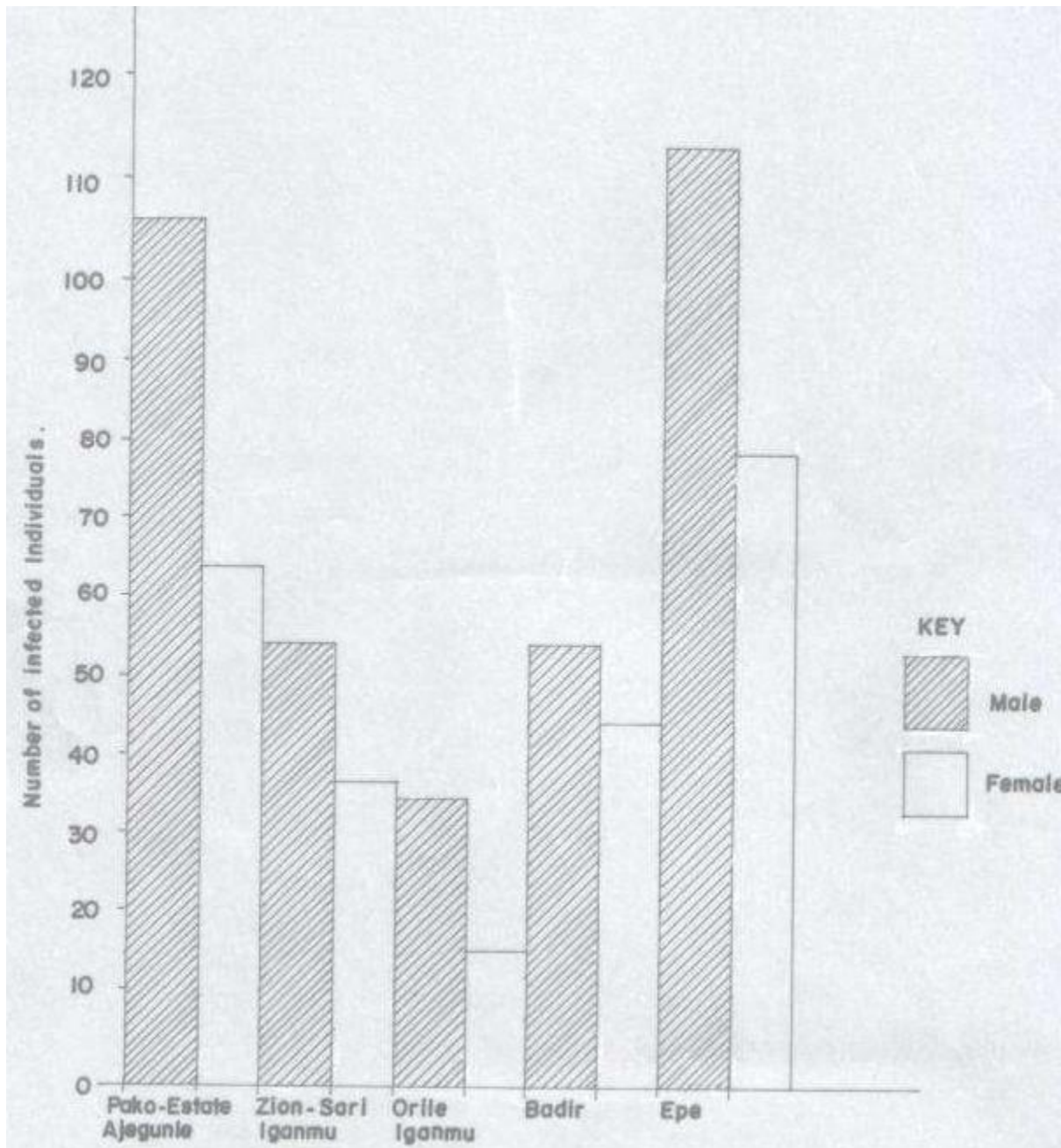


Figure 1. Infected individuals in different location.

## DISCUSSION

This study shows that schistosomiasis is prevalent among the pre-school children in selected communities in Lagos State. In Nigeria, helminthic infections are still a disease of poverty, as there exist a strong correlation between socio-economic status and parasitosis in children (Mafiana et al., 2002b). In this investigation, a higher prevalence (47.2%) was found in children between 3 to 4 years which is very high compared with Egypt: 14.5% noted by Nour et al. (1990), and none in that age group

seen by El Katsha and Watts (1997). Also, Mafiana et al. (2003) recorded 71.8% prevalence of schistosomiasis in pre-school children in Oyan, Nigeria which is much higher than 40.8% prevalence recorded in this study. The differences may be attributed to the fact that collection of urine samples was extended to 2.00 pm in some cases because of the difficulty in obtaining samples from these younger age cohort.

However, Akogun and Akogun (1996) recorded a prevalence of 47% similar to ours in children aged 3 to 5 years in a settlement near Lake Geriyo near Yola, Nigeria.



Of particular interest in our study is the pro-portion of infants (42.4%) who had acquired the infection before the age of 1. It is therefore likely that infection is acquired very early in life through water contact in these and other communities that live by Lakes or reservoirs where no other safe source of water supply is available (Mafiana et al., 2003). Infection and transmission in pre-school children occurred through early exposure to infected water bodies when they accompany their mothers to fishing, bathing and washing (Adewole et al., 2001; Okoli et al., 2006).

The non-significant difference ( $P > 0.05$ ) between males and females susceptibility to infection may be due to the fact that both sexes received their initial infection at about the same age (Mafiana et al., 2003). The higher prevalence in ages 3-5 years old may be attributed to the fact that they are allowed to roam about the streets bare footed, and males play games such as hide and seek, soccer inside the water. Most residents are aware of the health risks of wading in the polluted water yet they lack the capacity to change their behaviour (Adewole and Olofintoye, 2004).

The recognition of schistosomiasis as a major health problem is still limited in these communities as previously observed by Ofoezie et al. (1998). Any control measure that is to succeed here must involve the communities (El Katsha and Watts, 1997), such as developing participatory health education programmes with community members to effect behavioural change by mothers who expose their young children to *S. haematobium* infection. Mothers should wear protective shoes and gloves while in contact with water as well as providing a community crèche where mothers could leave their young children when going for fishing or other water contact activities in the study area.

Children in the study area should be restrained from bathing or swimming in the stream, stagnant water and pools of water on surface ground. Parents in the study area should be encouraged to boil drinking water before use. In areas where the disease is endemic, as a result of water source, projects and fishing activities predominate. There is urgent need to incorporate active schistosomiasis control strategy to prevent the possible spread of the disease to other parts of Lagos State. Therefore, this study demonstrates that urinary schistosomiasis is still being actively transmitted among the pre-school children in Lagos State. It is therefore very indispensable to extend the already incorporated control programmes in both primary and post primary schools to pre-school children.

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Full Length Research Paper

## Prevalence and antibiotic susceptibility of uropathogens among patients attending University of Abuja Teaching Hospital, Gwagwalada, Abuja

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A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract become infected by the presence and growth of microorganisms that overcome the structures strong natural defenses. This study reports the examination of bacterial organisms implicated in urinary tract infections among 180 patients suspected to have UTI that attended University of Abuja Teaching Hospital (UATH), Gwagwalada, Abuja for a period of 3 months and the antibiotic susceptibility patterns of the bacterial isolates. First morning mid stream urine sample cultures were done by the calibrated loop technique delivering 0.002 ml of urine plated on Cystine Lactose Electrolyte Deficient (CLED) agar and blood agar medium for isolation of uropathogens which were further analyzed for drug susceptibility by disk diffusion method. Result showed that 104 (57.78%) patients had urinary tract infection. The uropathogens isolated were *Escherichia coli* as the most abundant (43.27%) followed by *Staphylococcus aureus* (35.57%), *Klebsiella* species (11.54%), *Proteus* species (5.77%) and *Pseudomonas* species (3.85%) as the least occurring. *In vitro* antibiotic susceptibility test revealed that the Gram negative bacteria were most sensitive to quinolones (ofloxacin and levofloxacin), gentamycin and nalixidic acid while the Gram positive isolates were sensitive to erythromycin, chloramphenicol and quinolones (ofloxacin and levofloxacin). The most effective drug in both cases was the quinolones—ofloxacin (78.32%) and levofloxacin (76.98%), while the least sensitivity pattern was observed with ampicillin (14.29%).

**Key words:** Urinary tract, uropathogens, antibiotics susceptibility.

### INTRODUCTION

The urinary tract includes the organs that collect and store urine and release it from the body which include kidneys, ureters, bladder and urethra. A urinary tract infection (UTI) is a condition where one or more structures in the urinary tract (that is, kidneys, ureters, bladder and urethra) become infected by the presence and growth of microorganisms that overcome the structures strong natural defenses. UTI have become the most common hospital-acquired infections, accounting for as many as 35% of nosocomial infections, and are the second most common cause of bacteria in hospitalized

patients (Stamm, 2002; Kolawole et al., 2009).

Even though several different microorganisms, that is, protozoan, parasites, fungi and viruses can cause UTI, bacteria are the major causative organisms and are accountable for more than 95% of UTI cases (Bonadio et al., 2001). Common pathogens that have been implicated in UTIs are primarily Gram negative organisms with *Escherichia coli* having a higher prevalence than other Gram negative pathogens. Other pathogens include *Klebsiella* species, *Enterobacter* species, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Citrobacter*

species (McLaughlin and Carson, 2004; Blair, 2007). These bacteria usually enter the system from external sources. Bacteria that live in the digestive tract, vagina or around the urethra, which is at the entrance to the urinary tract have also been implicated in UTI. The bacteria usually multiply at the opening of the urethra and travel up to the bladder (known as ascending route) much less often, as reported by Simon (2002).

There are several factors and abnormalities of UTI that interfere with its natural resistance to infections. These factors include sex and age diseases, hospitalization and obstruction (Kolawole et al., 2009). Numerous reports have also suggested that UTI can occur in both males and females of any age, with bacterial counts as low as 100 colony forming units (CFU) per milliliter in urine (Ebie et al., 2001). Females are however believed to be more affected than males. This is due to the short and wider female urethra and its proximity to the anus. The female urethra is also adjacent to the genital and intestinal tracts. Bacteria from the rectum can easily travel up the urethra and quickly gain access to the bladder and cause infections (Ebie et al., 2001; Kolawole et al., 2009).

The traditional guideline that urine containing more than 100,000 bacteria per milliliter is an indication of a urinary tract infection has been modified to counts as low as 100 ml<sup>-1</sup> of any single bacteria type or as few as 100 ml<sup>-1</sup> of coliform (intestinal bacteria such as *E. coli*) are now considered as an indication of significant infection especially if leukocytes, appear in the urine. Before therapy is initiated, urine bacteria are usually cultured to antibiotic sensitivity (Tortor and Funke, 2004).

The aim of this research is to study the prevalence rate of urinary tract infections among symptomatic patients attending University of Abuja Teaching Hospital (UATH) in terms of sex, to determine the causative agents and their susceptibility patterns to commonly used antibiotics in the treatment of UTIs.

## MATERIALS AND METHODS

### Study population

The study population was drawn from patients attending University of Abuja Teaching Hospital Gwagwalada, Abuja. One hundred and eighty patients (between 18 and 45 years) with suspected urinary tract infection consisting of 85 males and 95 females were screened in the study. Those excluded from the study were patients already on antibiotic therapy.

### Specimen

With the help of trained nursing staff, the first morning urine samples were collected from one 180 patients and were transported to the laboratory in an ice pack and analyzed within 6 h of collection.

### Collection of samples

Early morning, clean catch, mid-stream urine samples were collec-

ted in sterile universal containers as described by Karlowsky et al. (2006) and Solberg et al. (2006) into sterile, wide mouthed bottles with screw cap tops. The following details were indicated on the urine sample bottles as per standard laboratory procedures; name, sex and time of collection.

## Sample processing

### Macroscopy

The samples were examined, observed and recorded for colour and cloudiness on arrival at the laboratory (Anochie et al., 2001).

### Culture

Culture was done by the calibrated loop technique as described by Cheesbrough (2000). A loop full of the well mixed urine sample was inoculated and plated on Cystine Lactose Electrolyte Deficient (CLED) and blood agar using streaking technique. The loop used transfers 0.002 ml of urine sample. All plates were then incubated at 37°C aerobically for 24 and 48 h in negative cases. The plates were further examined for bacterial growth. Observations were made on plates for growth, size of colony, morphology, consistency, aeration, color and odor (Mbata, 2007).

A ten-fold serial dilution was also carried out by transferring 1.0 ml of the urine sample into 9.0 ml of sterile distilled water. Using a sterile Pasteur pipette, 0.02 ml of the third dilution was inoculated onto the already prepared CLED agar plates in duplicates and spread with a sterile hockey stick-shaped glass rod; inoculated plates were incubated at 37°C for 24 h.

A significant bacteriuria count was taken as any count equal to or in excess of 100,000 cfu/ml (Smith et al., 2006). Colonies isolated were subcultured and identified by macroscopy, microscopy and biochemical tests.

### Dipstick test

Urine test strips were used to detect the presence of protein, nitrite and leukocyte in the urine. The urine samples were properly mixed and transferred into test tubes. The strip was subsequently inserted into it. The development of an immediate colour change was noted and compared with the colour chart on the urine strip container (Smith et al., 2006; Bachur and Harper, 2001).

### Antibiotic susceptibility testing

Antimicrobial susceptibility of isolates was tested by the Kirby Bauer disk diffusion method (Bauer et al., 1966), using commercially available disc. Antimicrobial agents tested were levofloxacin. Antimicrobial susceptibility of isolates was tested by the Kirby Bauer nitroflurantoin (30 µg), nalixidic acid (30 µg), ampicillin (30 µg), augmentine (30 µg), streptomycin (30 µg), chloramphenicol (15 µg), tetracycline (30 µg), gentamycin (10 µg), erythromycin (15 µg), cotrimoxazole (10 µg), cloxacillin (30 µg) and floxacilin (10 µg). With the use of a sterile wire loop, colonies of the test organism were emulsified in 2 ml of sterile physiological saline. The turbidity of the suspension was matched with the turbidity standard (0.5 McFarland Solution); this was viewed against a sheet of paper for easier comparison. Using a sterile syringe, 2 drops of the suspension was placed on the surface of a nutrient agar plate, with the use of a sterile glass rod; the suspension was evenly spread on the surface of the medium, rotating the plate approximately 60° to ensure even distribution. With the Petri dish lid in place, it was allowed to stand for about 5 min for the surface of the agar to dry. Using a sterile

**Table 1.** Bacterial load of urine samples.

Number of sample	Colony forming unit (cfu)/ml
82	0 - $9.5 \times 10^5$
8	$1.0 \times 10^6$ - $1.95 \times 10^6$
5	$2.0 \times 10^6$ - $2.95 \times 10^6$
3	$3.0 \times 10^6$ - $3.95 \times 10^6$
2	$4.0 \times 10^6$ - $4.95 \times 10^6$
2	$5.0 \times 10^6$ - $5.95 \times 10^6$
12	$6.0 \times 10^6$ - $6.95 \times 10^6$
21	$7.0 \times 10^6$ - $7.95 \times 10^6$
16	$8.0 \times 10^6$ - $8.95 \times 10^6$
14	$9.0 \times 10^6$ - $9.95 \times 10^6$
9	$1.0 \times 10^7$ - $1.095 \times 10^7$
2	$1.1 \times 10^7$ - $1.195 \times 10^7$
4	$1.2 \times 10^7$ - $1.295 \times 10^7$
Total	180

forceps, the antibiotic disc was placed on the surface of the plate. Within 30 min of applying the disc, the plate was inverted and incubated aerobically at 35°C for 18 h. After overnight incubation, a ruler was used to measure the zone of inhibition in mm underside of the plate (Cheesbrough, 2000).

Interpretation of results was done using the zone sizes. Zone of inhibition of greater than and equal to 18 mm was considered sensitive, 13 to 17 mm intermediate and less than 13 mm resistant (NCCLS, 2000).

## RESULTS

Over a period of three months (June to August), 180 mid-stream urine samples from patients attending University of Abuja Teaching Hospital (UATH) were analyzed, of which 104/180 were positive, while 76/180 were negative. The prevalence was found to be higher in females with a percentage rate of 62.50%, while the males had a percentage rate of 37.50%.

The colony forming unit (CFU) as was determined is shown on Table 1. Counts less than 100000 ( $10^5 \text{ ml}^{-1}$ ) were regarded as insignificant while counts greater and equal to 100000 ( $10^5 \text{ ml}^{-1}$ ) were indicated as significant bacteriuria.

Dipstick test of the uncentrifuged urine revealed that 46/180 of the specimen were positive for protein, while 134/180 were negative. Of the 46 positive protein samples, 22 yielded significant bacterial growth, 15/46 yielded no bacterial growth and 9/46 had an insignificant bacterial growth. For nitrite 28/180 of the specimens were nitrite positive while 152/180 showed negative. Of the 28 nitrite positive samples, 21 yielded significant bacterial growth, 3/28 yielded no bacterial growth while 4/28 had an insignificant bacterial growth. Dipstick test was also used to detect the presence of leukocyte (white blood cells) in the sample. Of the 180 samples examined, 63 were positive for leukocyte, while 117/180 were negative.

Of the 63 positive samples, 51 showed significant bacterial growth, while 5/63 showed no bacterial growth, whereas 7/63 showed an insignificant growth (Table 3).

Of the 104 isolates obtained, Gram negative bacteria had a higher frequency of occurrence than Gram positive with *E. coli* having the highest frequency rate of 43.27%. Other Gram negative organisms isolated include *Klebsiella* spp. 12 (11.54%), *Proteus* species 6 (5.77%) and *Pseudomonas* species 4 (3.85%). The only Gram positive organisms isolated were *S. aureus*, which had a prevalence rate of 35.57%. It was found that the rate of occurrence of *E. coli*, *Klebsiella* spp. and *Proteus* spp. were higher in females than males. However, *Staphylococcus aureus* was found to be slightly higher in males than females (Table 2).

*In vitro* antibiotic susceptibility patterns of isolates to common antimicrobial agent are shown in Table 4. It was found that *E. coli* were most sensitive to levofloxacin (75.56%), ofloxacin (71.11%), gentamycin (62.22%), chloramphenicol (57.78%) and nitrofurantoin (53.33%). It however showed a high resistant rate to cloxacillin (86.67%), cotrimoxazole (88.89%), ampicillin (80.00%), erythromycin (62.22%) tetracycline (77.78%) and nalixidic acid (53.33%). *S. aureus* were found to be highly sensitive to erythromycin (86.49%), ofloxacin (62.16%), streptomycin (54.1%), chloramphenicol (70.27%) and levofloxacin (67.68%). They showed resistance to ampicillin (81.08%), nitrofurantoin, (51.35%) nalixidic acid (51.35%) and augmentin (54.1%). *Klebsiella* spp. were found to be highly sensitive to levofloxacin (83.33%), ofloxacin (75%), nalixidic acid (58.33%), chloramphenicol (58.33%), gentamycin (66.67%) and erythromycin (50.00%). They showed resistance to cloxacillin (58.33%), augmentine (50.00%), streptomycin (66.67%), ampicillin (66.67%) and nitrofurantoin (50.00%). *Proteus* spp. were found to be sensitive to levofloxacin (83.33%), ofloxacin (83.33%), gentamycin (66.67%), erythromycin (50.00%) and nalixidic acid (50.00%). They showed high resistance to cloxacillin (66.67%), cotrimoxazole (100%), augmentine (100%), chloramphenicol (66.67%), and ampicillin (66.67%). The susceptibility pattern of *Pseudomonas* spp. showed a high sensitivity rate to levofloxacin (75%), gentamycin (50%), chloramphenicol (50%), ofloxacin (100%) and nalixidic acid (50%) and a resistant rate of 100% to cloxacillin, cotrimoxazole, augmentine, streptomycin, and ampicillin. It was also resistant to tetracycline by 75% and nitrofurantoin by 50%.

## DISCUSSION

Urine is one of the sterile fluids in the body, but when it is colonized with bacteria, all the structures of the urinary tract are at risk of being invaded. Infections of the urinary tract are one of the most common infectious diseases and they affect all age groups and people (including men, women and children) worldwide (Llenerrosos, 2004;

**Table 2.** Prevalence of isolates in relation to sex of patients.

Isolate	Isolates from males	Isolates from females	Percentage
<i>Escherichia coli</i>	12	33	45 (43.27)
<i>Staphylococcus</i> spp.	19	18	37 (35.57)
<i>Klebsiella</i> spp.	3	9	12 (11.54)
<i>Proteus</i> spp.	1	5	6 (5.77)
<i>Pseudomonas</i> spp.	4	0	4 (3.85)
Others	-	-	-
Total	39	65	104 (100)

**Table 3.** Relationship between significant bacteriuria and protein, nitrite and leucocytes in urine samples of patients.

Parameter	Protein	Nitrites (%)	Leukocytes
Positive	25.56	15.56	35.00
Negative	74.44	84.44	65.00
Positive with growth	47.83	75.00	80.95
Positive without growth	32.61	10.71	7.94
Positive with insignificant growth	19.57	14.29	11.11

Blair, 2007). The antibiotic sensitivity patterns of organisms change rapidly over a short period. It is especially true for developing countries where antibiotics are not sold by only the medical practitioners but are also purchased directly from the pharmacists without prescription (Hooton and Stamm, 1997). Periodic evaluation of sensitivity pattern is therefore essential for rational and appropriate use of antibiotics (Karlowsky et al., 2006).

In this study of 180 urine samples collected, only 104 gave significant growth and thus had a bacterial urinary tract infection, while 76/180 showed no growth and no significant bacteria growth. This figure is higher than the prevalence rate of 11.9% reported by Aiyegoro et al. (2007) among children and adolescents in Ile-Ife and 35.5% rate recorded by Ebie et al. (2001) among children and adults in Rukuiba Military Cantonment Jos. It is slightly lower than the 77.9% recorded by Mbata (2007) among prison inmates in Nigeria. It however, agrees with the 52.83% reported by Maripandi et al. (2010) and 58% recorded by Onifade et al. (2005) among pregnant women. The high prevalence may be due to genuine population susceptibility, because factors like sexual intercourse, peer group influence, pregnancy, low socio-economic status are common among Nigerian men and female (Mbata, 2007).

Protein result from dipstick test showed that 46/180 of the samples were positive for protein out of which 22 yielded bacteria growth. This corresponded with the works of Sandberg et al. (2005) and Zhang and Bailey (1995) which showed 8 (53.33%) and 24 (42%) positive protein samples with bacteria growth, respectively. This does not correspond with the result obtained by Sultana

et al. (2001) which showed a relatively low number of 87 (21.75%) of protein positive samples with bacteria growth.

Dipstick test showed that 28/180 of the samples were positive for nitrite; of this few, 21 yielded bacteria growth. This is similar to the report of Russell et al. (2007) where 29 (85.7%) of the positive samples yielded bacteria growth.

Similarly, 65/180 of the samples were positive for leukocyte, while 51 of the positive samples yielded bacteria growth. This is relative to the result obtained by Kolawole et al. (2009) where 125 out of 130 samples positive for leukocyte yielded bacteria growth.

The most common organisms isolated were *E. coli* (43.27%), *S. aureus* (35.57%), *Klebsiella* spp. (11.54%), *Proteus* spp. (5.77%) and *Pseudomonas* spp. (3.85%). This finding is similar to other reports which indicate that a Gram negative bacterium, particularly *E. coli* is the commonest pathogen isolated in patients with UTI (Ebie et al., 2001; Njoku et al., 2001). In other similar studies, the commonest isolates reported were also *E. coli*, *S. aureus* and *Klebsiella pneumoniae* (Kolawole et al., 2009). However, the 35.5% incidence rate of *S. aureus* in this study brings to light the fact that *Staphylococcus* species are becoming more predominant as aetiological agents of UTI than previously reported in reports by Amin et al. (2009) where it had a prevalence rate of 2.2%. Relative high incidences of 27.3 and 28.9% have also been reported by Nwanze et al. (2007) and Okonkwo et al. (2009). The high incidence of *S. aureus* recorded in this study could be due to virulent nature of the organism, which gives it the ability to overcome body defence mechanism and resistance to antibiotics (Ojiegbe and

**Table 4.** Antibiotic sensitivity patterns of isolates.

Isolate	Levo (%)	Clox (%)	Cotr (%)	Eryt (%)	Gent (%)	Augm (%)	Strep (%)	Tetra (%)	Chlo (%)	Ampi (%)	Oflo (%)	Nitro (%)	Nalix (%)
<b><i>Escherichia coli</i></b>													
S	75.56	11.11	8.89	31.11	62.22	22.22	15.6	17.78	57.78	13.33	71.11	53.33	28.89
I	6.67	2.22	2.22	6.67	15.56	11.11	26.67	44.44	13.33	6.67	11.11	15.56	17.78
R	17.78	86.67	88.89	62.22	22.22	66.67	57.78	77.78	28.89	80.00	17.78	28.89	53.33
<b><i>Staphylococcus aureus</i></b>													
S	67.68	35.14	37.84	86.49	48.65	40.54	54.1	43.24	70.27	8.11	62.16	27.03	33.43
I	8.11	45.95	24.32	8.11	18.82	5.41	29.73	24.32	10.81	10.81	13.51	21.62	16.22
R	24.32	18.92	37.84	5.41	32.43	54.1	16.22	32.43	18.92	81.08	24.32	51.35	51.35
<b><i>Klebsiella species</i></b>													
S	83.33	25.00	33.33	50.00	66.67	16.67	33.33	41.67	58.33	33.33	75.00	25.00	58.33
I	-	16.67	25.00	8.33	-	33.33	-	41.67	16.67	-	18.33	25.00	16.67
R	16.67	58.33	41.67	61.67	33.33	50.00	66.67	16.67	25.00	16.67	66.67	50.00	25.00
<b><i>Proteus species</i></b>													
S	83.33	16.67	-	50.00	-	66.67	33.33	50.00	-	83.33	16.67	33.33	50.00
I	-	16.67	-	33.33	-	-	16.67	16.67	33.33	16.67	16.67	50.00	16.67
R	16.67	66.67	100.00	16.67	33.33	100.00	33.33	33.33	66.67	66.67	-	16.67	33.33
<b><i>Pseudomonas species</i></b>													
S	75.00	-	-	25.00	50.00	-	-	25.00	50.00	-	100.00	25.00	50.00
I	25.00	-	-	50.00	50.00	-	-	-	-	-	-	25.00	25.00
R	-	100.00	100.00	25.00	-	100.00	16.22	75.00	50.00	100.00	-	50.00	25.00

Levo, Levofloxacin; Tetra, Tetracycline; Clox, Cloxacillin; Chlo, Chloramphenicol; Cotr, Cotrimoxazole; Amp, Ampicillin; Eryt, Erythromycin; Oflo, Ofloxacin; Gent, Gentamycin; Nitro, Nitrofurantoin; Augm, Augmentin; Nalix, Nalixidic Acid; Strep, Streptomycin S=Sensitive I=Intermediate R=Resistant.

Nworie, 2000).

The prevalence of UTI occurred more often in females than in males. Of the 104 isolates obtained, 65 were from females, while 39 were from males. These results also agree with other reports, which showed that UTIs are more frequent in females, than males during adoles-

cence and adulthood (Ibeawuchi and Mbata, 2002; Mbata, 2007). This high prevalence rate in females has been reported to be due to the shorter and wider urethra of females than those of males. Also, the anatomical relationship of the female urethra and the vagina makes it susceptible to trauma during sexual intercourse as well

as bacteria being massaged up the urethra into the bladder during pregnancy and child birth (Arthur et al., 2005).

The most useful antibiotics in this study were quinolones (ofloxacin and levofloxacin), gentamycin, nalixidic acid, erythromycin and chloramphenicol (in Gram positive), because they

inhibited most commonly isolated UTI pathogens. This concurred to other reports where quinolones are the most effective (Ebie et al., 2001; Mbata, 2007).

Nitroflurantoin, ampicillin and cotrimoxazole (septrin) which are commonly used antibiotics were poorly effective against majority of the organisms isolated in this study. The resistance observed with these drugs used may be due to antibiotics being used for a long period and must have been abused and as a result the organisms must have developed mechanisms of circumventing their mode of action (Kolawole et al., 2009). In the evaluation of the efficiency of the quinolone drugs used, these drugs now appear as promising therapeutic agents for the treatment of acute urinary tract infection (Mbata, 2007).

## Conclusion

This study shows that there is a high prevalence rate of UTI among symptomatic patients attending University of Abuja Teaching Hospital, especially among the females. It also shows the necessity of obtaining sensitivity pattern reports before the start of antibiotics treatment in case of suspected urinary tract infection. However, the decision to use a particular antibiotic depends on its toxicity, cost and attainable level. Even though the susceptibility pattern shown in this study buttressed the need for *in vitro* sensitivity reports before antibiotics therapy initiation, it should be borne in mind that *in vitro* antimicrobial sensitivity is only a guide and that conditions *in vivo* may be quite different (Ibeawuchi and Mbata, 2002).

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## *UPCOMING CONFERENCES*

**13th Congress of the Asia-Pacific Federation for Clinical Biochemistry and Laboratory Medicine (APCCB 2013), Bali, Indonesia, 27 Oct 2013**



**7th International Conference on Communication in Veterinary Medicine (ICCVM), St. Louis, USA, 4 Nov 2013**



## Conferences and Advert

### **September 2013**

13th Asian Federation of Sports Medicine Conference 2013, Kuala Lumpur, Malaysia, 25 Sep 2013

### **December 2013**

20th World congress on Parkinson's Disease and Related Disorders, Geneva, Switzerland, 8 Dec 2013



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