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RISK FACTORS OF CERVICAL INTRAEPITHELIAL LESION IN DOUALA-CAMEROON: IMPLICATIONS OF HERPES SIMPLEX VIRUS TYPE 2, CHLAMYDIA TRACHOMATIS AND TREPONEMA PALLIDUM.

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ABSTRACT
Infection with high risk oncogenic human papillomavirus (HPV) such as HPVs 16 and 18 is the main cause of cervical cancer. The objective of this study was to determine the impact of *Chlamydia trachomatis*, *Herpes simplex virus* 2 (HSV 2), *Treponema pallidum and* some sexual behaviour on malignant progression of cervical lesion in Douala, Cameroon. From July 2009 to January 2010, we performed routine cervical smears to 163 consenting women, who completed a questionnaire on risk factors of cervical cancer. Blood samples were obtained for each of these women and used for the detection of antibodies against *Chlamydia trachomatis*, HSV 2 and *Treponema pallidum*. Results obtained showed that 26/163 (17 LSIL and 9 HSIL) of women had abnormal cytology, 75.5% (123/163) had HSV 2 infection, 19% (31/163) infected by *Chlamydia trachomatis* and 4.3% (7/163) infected by *Treponema pallidum*. Among the LSIL-positive women 35.3% (6/17) and 94.1% (16/17) were infected with *Chlamydia trachomatis* and HSV 2 respectively. Among those with HSIL cytology, 22.2% (2/9), 66.7% (6/9) and 11.1% (1/9) respectively had *Chlamydia trachomatis*, HSV 2 and *Treponema pallidum*. High parity and pregnancy rate was observed among women with positive cytology. Our finding shown high rate of cervical abnormalities among women infected with HSV 2; and among those with a higher number of parities and pregnancies. These results suggest that further investigations should be made in Cameroon to access real burden of these risk factors in the progression and persistence of cervical lesion.

Key words: risk factors, cervical cancer, HSV 2, *Chlamydia trachomatis*, sexually transmitted infections.

INTRODUCTION

The genital infection by the human papillomavirus (HPV) is a sexually transmitted infection most common, known today as the main cause of cervical cancer in women (1). Epidemiological and molecular investigations have shown that more than 120 different genotypes of this virus have been identified and 40 were recognized as being able to infect the anogenital mucosa (2). Several study are demonstrated that only 18 of these type are considered as high risk oncogenic for the cervix, and 12 (HPVs 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) so as well established, with predominance of HPVs 16 and 18 (3,4,5). The study of genome organization, regulation of gene expression and protein characterization has allowed understanding the mechanisms of carcinogenesis associated with these viruses (6,7). Their involvement in the malignant transformation of cervix was recognized as serious problem of health in developing countries (8). Many authors' reports that, in most cases, particularly among women under 30 years, HPV infections are transient and only a small percentage of infection persist and may progress to neoplasic cervical lesion. Viral clearance of HPV is quite fast and frequent, on average 70% of infections regress in 12 months and 90% in 24 months (9).

The raison of variable period of latency observed in some women remain poorly understood, but it has been generally accepted that other factors contribute to the spread and persistence of precancerous cervical lesion. Among these factors, co-infection with multiple HPV types, viral load, immune deficiency, active smoking, multiparity, multiple sexual partners, early sexual intercourse, excessive use of hormonal contraceptive and other sexually transmitted infections (STI) have been investigated as potentially involved in the transformation of epithelial cells of the cervix. These factors greatly increase likelihood of developing cervical neoplasic lesion (10). Among, STI studied we have infection with *Herpes simplex virus* type 2 (HSV 2), *Chlamydia trachomatis* (*C. trachomatis*) and Treponema pallidum (*T. pallidum*). These pathogen agents are spread sexually, causing considerable morbidity and socioeconomic problems in several countries. HSV 2 infects the genital mucosa and establishes a life-long infection in sensory ganglia, and it is the most common cause of genital ulcers. Seroepidemiological data from worldwide studies performed during the last decade have estimated the HSV 2 seroprevalence to range from 0 % in children to more than 80 % in selected populations such as STI cohorts in some African countries

(11,12). Infection with *C. trachomatis* is the most prevalent bacterial STI causing symptomatic and, more commonly, asymptomatic genital infection. In women, *C. trachomatis* is an important cause of cervicitis and salpingitis as well as pelvic inflammatory disease (13). *T. pallidum* is the etiologic agent of syphilis. This microorganism is transmitted during sexual activity from a mucocutaneous lesion. In acquired infection, after an initial incubation period of 3 – 90 days, a solitary papule with spirochaetes, erupts at the site of inoculation, which is often found on the genitalia, and less frequently on the rectal mucosa. The fundamental histological changes in acquired syphilis are vasculitis and its consequences, necrosis and fibrosis (14).

The objectives of the present study were to determine the impact of *Chlamydia trachomatis*, *Treponema pallidum* and *Herpes simplex virus* 2 (HSV 2) on the malignant transformation of epithelial cells of cervix and also, to evaluate some behavioural factors involved in the natural history of cervical lesion in Douala, Cameroon.

MATERIALS AND METHODS

Study population

From July 2009 to January 2010, a total of 163 women were enrolled simultaneously in two health centre of Douala (Cameroon). Informed consent was obtained from all women and study was approved by national ethic committee. Women were eligible in the study if they were aged 17 and over, married or unmarried, were not pregnant and not had intercourse 48 hours before. All women were not menstruating and had not made a vaginal washing at time of diagnosis. They completed a structured questionnaire including number of sexual partners, parity, abortion, age of first sexual intercourse and the number of pregnancies.

Sample collection

During their visit to a gynaecologist, cervical smear was performed for early detection of cervical lesion. Results obtained were classified according to the Bethesda system; as negative, atypical squamous cell of undetermined significance (ASCUS), low squamous cell intraephithelial lesion (LSIL) and high squamous cell intraepithelial lesion (HSIL) (15). All pap smears were analyzed independently by two pathologists and the final result was based on a consensus between of both. Blood sample were obtained by venipuncture and centrifuged at 4000 rpm for 10 min and serum obtained was store at – 30 °C for detection of antibodies against *Chlamydia trachomatis*, HSV 2 and *Treponema pallidum*.

Detection of *Chlamydia trachomatis*, HSV 2 and *Treponema Pallidum*

C. trachomatis IgG Enzyme-linked immunosorbent assay (ELISA) from DRG Diagnostics laboratory was used for the detection of specific IgG antibodies against *C. trachomatis*. The assay was carried out as described previously by Piura *et al.,* 1985 (16). Immunoglobulin G antibodies to HSV 2 were determined by microtest-plate ELISA kits (Teco Diagnostics Laboratory, reference IGMH2G-96) using the general technical principles. Each sample was analyzed by indirect blocking ELISA protocol (17). The detection of antibodies to *T. Pallidum* was performed by the method of heamagglutination (18). Commercial Kit TPHA (*Treponema pallidum* heamagglutination assay) of Biomagrehb laboratory was used for this purpose. All manipulations were performed according to manufacturer's instructions.

Statistical analysis

Statistical analysis were performed by SPSS 16.0 software using chi squared test and T-student test. Statistical significance was accepted for $P < 0.05$.

RESULTS

This study investigated the impact of some sexually transmitted infection on cervical abnormalities, in 163 consenting women randomly recruited in two health centre of Douala (Cameroon). Table 1 summarize the incidence of some microbial infectious agents according to the cytology in the total population. The Pap smear results revealed that HSV 2 is the leading infection among women with 62 %, 9.8 % and 3.7 % for normal, LSIL and HSIL respectively while *Chlamydia* is the second with 14.1 %, 3.7 % and 1.2 %. *T. Pallidum* which count for 3.7 %, 0.0 % and 0.6 % for normal, LSIL and HSIL respectively in women.

TABLE 1: INCIDENCE OF *C. TRACHOMATIS*, *T. PALLIDUM* AND HSV 2 ACCORDING TO THE CYTOLOGY IN TOTAL POPULATION

Cytology	Normal		LSIL [a]		HSIL [b]		Total
	[n/163]	(%)	[n/163]	(%)	[n/163]	(%)	
HSV 2							
Positive	**101/163**	**(62)**	**16/163**	**(9.8)**	**6/163**	**(3.7)**	**123/163 (75.5)**
Négative	**36/163**	**(22.1)**	**1/163**	**(0.6)**	**3/163**	**(1.8)**	**40/163 (24.5)**
C. trachomatis							
Positive	**23/163**	**(14.1)**	**6/163**	**(3.7)**	**2/163**	**(1.2)**	**31/163 (19)**
Négative	**114/163**	**(69.9)**	**11/163**	**(6.7)**	**7/163**	**(4.3)**	**132/163 (81)**
T. pallidum							
Positive	**6/163**	**(3.7)**	**0/163**	**(0.0)**	**1/163**	**(0.6)**	**7/163 (4.3)**
Négative	**131/163**	**(80.4)**	**17/163**	**(10.4)**	**8/163**	**(4.9)**	**156/163 (95.7)**
Total	**137/163**	**(84.1)**	**17/163**	**(10.4)**	**9/163**	**(5.5)**	**163/163 (100)**

(%): frequency, [a]: Low squamous cell intraepithelial lesion [b]: High squamous cell intraepithelial lesion

Table 2 presents the frequencies of *C. trachomatis*, *T. pallidum* and HSV 2 in women with positive cytology. The data shown that cervical abnormalities are high in women infected with HSV 2: 94.1 % and 66.7 % for LSIL and HSIL respectively (vs 5.9 % and 33.3 % for LSIL and HSIL in women not infected). Significant difference was found between HSV 2 positive and negative cases compared with chi squared test (P = 0,018). *C. trachomatis* was diagnosed in fewer percentages among positive women: 35.3 and 22.2 for LSIL and HSIL respectively and *T. pallidum* was detected in very low percentage positive women. The difference between negative and positive women is not significant for these two infectious agents.

TABLE 2: FREQUENCY OF *C. TRACHOMATIS*, *T. PALLIDUM* AND HSV 2 IN WOMEN WITH POSITIVE CYTOLOGY

	LSIL [a] (N = 17)		HSIL [b] (N = 9)		P-value
	[n/17]	(%)	[n/9]	(%)	
Infections					
C. trachmatis					**0.134 [NS]**
Positive	**6/17**	**(35.3)**	**2/9**	**(22.2)**	
Négative	**11/17**	**(64.7)**	**7/9**	**(77.8)**	
HSV 2					**0.018 ***
Positive	**16/17**	**(94.1)**	**6/9**	**(66.7)**	
Négative	**1/17**	**(5.9)**	**3/9**	**(33.3)**	
T. pallidum					**0.161 [NS]**
Positive	**0/17**	**(0.0)**	**1/9**	**(11.1)**	
Négative	**17/17**	**(100)**	**8/9**	**(88.9)**	

(%): Frequency, *: statistical difference, [NS]: No significant difference, N: Total number. [a]: Low squamous cell intraepithelial lesion, [b]: High squamous cell intraepithelial lesion.

Table 3 represents variations of type G immunoglobulin of HSV 2 and *Chlamydia* in patients with abnormal cytology. We note that this index believes with severity of disease *for C. trachomatis*: 0.6 ± 0.2 to 1.0 ± 0.2 respectively for LSIL and HSIL while it decreases for HSV 2 (4.4 ± 0.9 to 1.3 ± 0.2). The difference between LSIL and HSIL positive patients was statistically significant for HSV 2 compared with T-student test (P = 0.003).

TABLE 3: VARIATION OF IMMUNOGLOBULIN G ANTIBODIES AGAINST HSV 2 AND *C. TRACHOMATIS* AMONG WOMEN WITH CERVICAL ABNORMALITIES

	LSIL [a] (N = 17)	HSIL [b] (N = 9)	T-student test (P-value)
	Mean ± SE	Mean ± SE	
Infections			
Chlamydia T.	0.6 ± 0.2	1.0 ± 0.2	0.092 NS
HSV 2	4.4 ± 0.9	1.3 ± 0.2	0.003*

SE = Standard Error, NS: No statistical difference, *: Statistical difference, N: Total number, [a]: Low squamous cell intraepithelial lesion, [b]: High squamous cell intraepithelial lesion.

Table 4 examines some risk factors that might involve in development of cervical lesion. The results revealed the higher percentage of cervical lesion in women aged ≤ 17 years old at first vaginal sex intercourse (64.7 % and 55.6 % for LSIL and HSIL respectively) compared to women aged > 17 years old. No statistical difference was found for this factor between these two groups positive .

women. Regarding parity and pregnancy, we found a high rate of cervical abnormalities in women with a number of parity and pregnancy less than 3 and between 4 and 7: 82.4 % and 66.7 % for LSIL and HSIL for parity and 64.7 % and 66.7 % for pregnancy respectively. Significant statistical difference ($P < 0.05$) was observed in correlation with cervical abnormalities for these two factors

TABLE 4: INCIDENCE OF SOME RISK FACTORS IN ACCORDING TO THE CYTOLOGY IN WOMEN WITH CERVICAL ABNORMALITIES.

	LSIL [a] (N = 17)		HSIL [b] (N = 9)		χ^2 trend (P –value)
	[n/17]	(%)	[n/9]	(%)	
Factors					
AFVS [C] (years)					0.655 NS
≤17	11/17	(64.7)	5/9	(55.6)	
>17	6/17	(35.3)	4/9	(44.4)	
Parity					0.003 *
≤ 3	14/17	(82.4)	2/9	(22.2)	
4-7	3/17	(17.6)	6/9	(66.7)	
≥ 8	0/17	(0.0)	1/9	(11.1)	
Pregnancy					0.047 *
≤ 3	6/17	(35.3)	1/9	(11.1)	
4-7	11/17	(64.7)	6/9	(66.7)	
≥ 8	0/17	(0.0)	2/9	(22.2)	
Abortion					0.215 NS
0	3/17	17.6	4/9	44.4	
1-2	11/17	64.7	4/9	44.4	
>2	3/17	17.6	1/9	11.1	

(%): Frequency, NS: No statistical difference, *: Statistical difference, N: Total number, [a]: Low squamous cell intraepithelial lesion, [b]: High squamous cell intraepithelial lesion, [C]: Age at first vaginal sex.

DISCUSSION

The persistent infection with a high-risk HPV is recognized as the principal factor for malignant progression of cervical lesion. However in some women infected since several years, there are no complications while others develop cancer. This finding suggests that other factors play an important role in the transformation process. The aim of our study was to investigate the role played by some sexually transmitted infection on the development of cervical lesion. Our results showed that HSV 2 positive patients are a higher risk of developing cervical lesion. Positive variation of index of immunoglobulin G antibodies against HSV 2 was found among women with cervical abnormalities. Similar results were reported in studies carried out in others sites (19,20,21). This observation reveals the role played by HSV 2

infection in the persistence and the development of cervical neoplasia. Our knowledge about the specific role of this pathogen agent in the development of cervical lesion is limited. There are several mechanisms by which HSV 2 may act, such as direct genotoxicity. The most likely biologic mechanism is the induction of cervical inflammation leading genotoxic damage through oxidative metabolites (22). Several studies have reported that the carcinogenic molecules (nitric oxide for instance) produced during the inflammatory response induced by this infection, ulcerative lesions of the infection, decrease of local immunity could explain the persistence of cervical lesion related to HPV infection and therefore the loss of viral clearance in patients (23,24). HSV 2 infection increase susceptibility to HPV causing alterations of epithelial cells, thus facilitating the entry of HPV virions.

In our study, no significant correlation was found between cervical abnormalities, *C. trachomatis* and *T. pallidum*. The frequencies of antibodies against *C. trachomatis* and anti-*T. pallidum* are not high among women having cervical disease. Some previous epidemiological studies have produced the same results regarding association between cervical intraepithelial neoplasia and these two microbial infectious agents. However, others studies reported a positive association between *C. trachomatis*, *T. pallidum* and cervical lesion associated with HPV (25,26). Therefore, the effective role of these pathogens agents remain to be clarified.

In the same context, our data showed positive association between multiple parities, pregnancies and the increased risk of cervical intraepithelial lesion. These results agree with others epidemiological studies relating to association between these risk factors and malignant progression of cervical lesion. Biochemical mechanisms for demonstrating the association between these factors and natural history of cervical lesion is not documented and remain poorly understood today. But, some authors suggest that hormonal change and traumatic producing during pregnancy and childbirth may explain the involvement of these factors in the development of cervical abnormalities (27,28).

CONCLUSION

The results obtained of this study suggest that HSV 2 infection might increase risk of cervical intraepithelial lesion in our population. Multiple parities and pregnancies are also considerable factors involved in the development and progression of cervical abnormalities. Our data confirm and stress that the screening of cervical abnormalities are desirable for women who attending public and private health centre in Douala (Cameroon), to assess the future risks of cervical intraepithelial lesion and cervical cancer. More investigations should be made about of these risk factors to clarify their real impact in the progression of cervical lesion.

REFERENCES

1. Bosch FX, Nubia M. The viral etiology of cervical cancer. *Virus res* 2002; 89:183-189.
2. Barzon C, Giorgi C, Buonaguro FM, Palu' G. Guidelines of the Italian society for virology on HPV testing and vaccination for cervical cancer prevention. *Infectious Agents and cancer* 2008; 3:14.
3. Benjamin GE, Ma paz NB, Sonsoles R, Luis Fernando SS. *et al*. Genotype distribution of cervical human papillomavirus DNA in women with cervical lesions in bioko, Equatorial Guinea. *Diagnostic pathology* 2009; 4(31):1-8.
4. Iztel E, Macias C, Villa L, Prado JC. *et al*. Worldwide genomic diversity of the high-risk human papillomavirus type 31, 35, 52 and 58, four close relatives of human papillomavirus type 16. *Journal of virology* 2005; 79(21):13630-13640.
5. Olida M, Silvia de Sanjose, Quint W, Bosch XF. *et al*. Human Papillomavirus type distribution in invasive cervical cancer in Uganda. *BMC infectious diseases* 2008; 8:85.
6. Mougin CH, Bernard B, Lab M. Biology of papillomavirus infections: General caracteristics. *Annals of Clinical Biochemistry* 1997; 55(6):555-63.
7. Zur hausen H. Papillomaviruses Causing Cancer: Evasion from Host-Cell Control in Early Events in Carcinogenesis. *Journal of the national cancer institute* 2000; 92(9):690-695.
8. Ly A. Cancer of the cervix: News vaccines, News perspectives? *Afr J Cancer* 2009; 1:65-67.
9. Duport N. Epidemiological data on cervical cancer – the state of knowledge – updating 2008; 11-14.
10. Castellsague X, Mireia D, Silvia DS, Nubia M. *et al*. Worldwide Human Papillomavirus etiology of cervical adenocarcinoma and its cofactors: Implication for screening and prevention. *Journal of the National Cancer Institute* 2006; 98(5):303-313.
11. Smith JS, Robinson NJ. Age specific prevalence of infection with *herpes simplex virus* type 2 and 1: global review. *J infect dis* 2002; 186:3-28.
12. Gorander S, Mbwana J, Lyamnya E, Lagergard T. *et al*. Mature glycoprotein G presents high performance in diagnosing *herpes simplex virus* type 2 infection in sera of different Tanzania cohort. *Clin Vaccine Immunol* 2006; 13:633-639.
13. Gray-swain MR, Peipert JF. Pelvic inflammatory disease in adolescents. *Curr opin obster gynecol* 2006; 18:503-510.
14. Mehmet G, William JL. Syphilis in pregnancy. *Sex Transm Inf* 2000; 76:73-79.
15. Solomon D. The Bethesda system for reporting cervical/vaginal cytologic diagnosis: overview. *Int J Gynecol pathol* 1991; 10:323-325.
16. Piura B, Sarov B, Kleinman D, Chaim W. *et al*. Serum IgG and IgA antibodies specific for *Chlamydia trachomatis* in salpingitis patients as determinded by

the immunoperoxydase assay. *Eur. J. Epidemiol* 1985; 1:110-116.
17. Vertergaard BF, Grauballe PC. ELISA for *herpes simplex virus* (HSV) type specific antibodies in human sera using type heterologous rabbit antibodies. *Acta Pathol Microbiol Immunol Scand* 1979; 87:261-263.
18. Tomizawa T, Kasamaton S, Yamada S. The usefulness of the heamagglutination test using *T. pallidum* as antigen for serodignosis. *Jpn J Med sci bio* 1969; 22:341-345.
19. Clinton J. Cervical cancer: is *herpes simplex virus* type II a cofactor? *Clin Microbiol Rev* 1995; 8(4):549-556.
20. Finan RR, Musharrafieh U, Almawi. Detection of *Chlamydia trachomatis* and herpes simplex virus type 1 or 2 in samples in human papillomavirus (HPV) – positive and (HPV) – negative women. *Clinical Microbiology and infection* 2006; 12(9):918-940.
21. Smith JS, Herrero R, Bosetti C, Munoz N. *et al*. Herpes simplex virus-2 as a human papillomavirus cofactor in the etiology of invasive cervical cancer. *Journal of the national cancer institute* 2002; 94(21):1604-1613.
22. Szostek S, Zawilinska B, Kopec J, Kosz-vnenchak M. *Herpesviruses* as possible cofactors in HPV-16-related oncogenesis. *Actu Biochimica Polonica* 2009; 56(2):337-342.
23. Gorander S, Lagergard T, Malgorzata R, Viscidi RP. *et al*. Seroprevalence of herpes simplex virus type 2, five oncogenic human papillomavirus and *Chlamydia trachomatis* in Katowice, poland. *Clin Vaccine Immunol* 2008; 15(4):675-680.
24. Yu-yen Y, Lim-who K, Ju-hsin T, Chung-hung T. *et al*. Correlation of viral factors with cervical cancer in Taiwan. *J Microbial Immunol Infect* 2004; 37:282-287.
25. Gnaoui N, Benchakroun N, Benider A, Hassar M. *et al*. Typing of human papillomavirus and evaluation of risks factors associated with cervical cancer in morocco. *European journal of scientific research* 2009; 31(2):229-236.
26. Verteramo R, Pierangeli A, Mancini E, Calzolari E. *et al*. Human papillomaviruses and genital co-infection in gynaecological outpatients. *BMC infectious desease* 2009; 9:16.
27. Castellsague X, Nubia M. Cofactors in papillomvirus carcinogenesis-role of parity, oral contraceptives, and tobacco smoking. *Journal of National Cancer Institute* 2003; 31:20-28.
28. Moscicki AB, Hills N, Shiboski S, Powell K. *et al*. Risks for incident human papillomavirus infection and low grade squamous intraepithelial lesion development in young females. *JAMA* 2001; 285:2995-3002.

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WEIGHT GRADIENT AND PHYSIOLOGICAL RESPONSES TO CATION-TREATMENT BY SALMONELLA ENTERICA- INFECTED RABBITS

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Abstract
Interest in immunomodulators is increasing following the recognition that positive immunomodulators could be useful intervention tools in the control of diseases and infections. An attempt to determine the effects of some of the cations on body weight and physiological reactions was carried out. Thirty five female adult New Zealand white rabbits grouped into seven, 5 pairs per each of these cations (Zn^{2+}, Cu^{2+} and Mg^{2+}), and supplemented with 1ml/day of single and double strength concentrations of cation for 24 days; the control was not supplemented with any cation. During the study period the rabbits were fed with Guinea grower mash and water *ad libitum*. There was regular taking of body weight of the rabbits using a top- loading weighing balance, while feed consumption, rectal temperature, stool frequency, physical appearance and behavioural changes were noted. Weight gradient studies show gradual increase in body weight following cation treatment of rabbits, but after challenging the various groups with oral administration of 0.5 ml of 10^6 CFU / ml of saline suspension of *Salmonella. enterica* for three exposures on alternative days, there was a progressive decrease in body weight of rabbits. However, there was no significant difference in stool dropping, body weakness, dullness and rough furs in all the groups. Effects were more pronounced in control group than in cation supplemented groups. This result, therefore, provide evidence of the significance of zinc, copper and magnesium oral supplementation in mammals and, of course among these three cations, copper appears to be more effective in improving body weight gain, though the mechanism is not known.
Keyword: Trace elements, Body weight, Physiological responses, *Salmonella enterica*

INTRODUCTION

The significance of the biochemical and nutritional roles of trace elements is widely recognized, since trace elements are found as constituent components of many metalloproteins and metalloenzymes. Trace elements are substances (elements) found in trace amounts in food. They are essential for the normal functioning of the body system though in trace amounts. The definitive feature of a nutritionally significant trace element is either its essential interaction in physiological processes or its potential toxicity when present at above optimal concentration in tissues, food or drinking water. They are also important components of hormones and certain factors with special physiological functions (1). Arbitrarily, the term 'trace' has been applied to concentrations of elements not exceeding 250 g per gram of matrix (2).

An element is considered essential to an organism when reduction of its exposure below a certain limit results consistently in a reduction in physiologically important function or when the element is an integral part of an organic structure performing a vital function in that organism. However, not all essential elements are of equal practical importance for public health. Under-exposure, over-exposure or both is known to occur under certain environmental condition for selenium and zinc, whereas, fluorine and the heavy metals cadnium, mercury and lead are of great concern because of the concentration occurring in food chains (3).

At the tissue level, domestic animals require essentially the same amount of minerals per comparable unit of function performed. This does not imply, however, that the dietary intake should be equal. On the contrary, differences in the percentage concentration of the diet should be expected. Factors affecting dietary mineral requirement include the amount of food ingested per unit of weight, growth rate and species of the mammal, ambient temperature, kind of feed supplied and dietary balance of other nutrients, and level of antagonism in the ratio and age of the animal. It is evident that younger animals absorb higher and faster than the older ones.

Cations are immunomodulators from miscellaneous sources. Immunomodulators are biological response modifiers which specifically interfere in cellular and humoral immune mechanisms and are also involved in many biochemical processes supporting life such as metabolism and sequestration of free radicals. The relation between trace elements and human health has been noted (4). With respect to cardiovascular diseases and hypertension, attention has mostly been focused on manganese, zinc, arsenic, cadmium, lead, selenium, vanadium, copper, cobalt, chromium and fluorine (5). But the effects of cations on gross weight of individual remain unknown. Hence, the research on the physiological responses to cation treatment was designed with an aim to examine physiological reactions and changes in body weight, as well as pathological changes and specific organ weight which are not reported in this paper.

MATERIALS AND METHODS

Laboratory animals-
The experimental animals comprised of 35 female adult New Zealand white rabbits, obtained from the animal house of the Department of Microbiology, University of Benin, Benin City.

Organic salt - The salts (copper sulphate, zinc sulphate and magnesium sulphate) were obtained from the laboratory of the Department of Microbiology, University of Benin, Benin City. A doubling concentration of each cation was employed

in treatment of the rabbits, so that the effects observed if due to treatment, should also show multiplying effects. The treatment dosages were also chosen in relation to values obtained from human population studies carried out elsewhere (3). Treatment groups and supplementation given were group 1 (Mg x 1) 10.0 µg / ml, group 2 (Mg x 2) 20.0 µg / ml, group 3 (Zn x 1) 16.0 µg / ml, group 4 (Zn x 2) 32.0 µg / ml, group 5 (Cu x 1) 2.0 µg / ml, group 6 (Cu x 2) 4.0 µg / ml, and the control group which was not supplemented with any cation. The treatment groups were separately given oral supplements of zinc, magnesium and copper respectively for 24 days before being challenged with oral administration of saline suspension of pure culture of *Salmonella enterica* with a hypodermic syringe bearing 0.5 ml of 10^6 CFU / ml of the organism for three exposures on alternate days. During the entire study period, the rabbits were fed Guinea grower mash (Bendel Feed and Flour Mills, Ewu, Edo State) and water from University of Benin borehole, Benin City. Stool frequency was specifically noted and the body weight was taken regularly using a top-loading weighing balance (Five Goats, China) while their physical appearance, feed consumption, rectal temperature and behaviours were recorded daily.

RESULTS

Weight gradient - The physiological responses of the rabbit's treatments with cation and subsequent challenge with *Salmonella enterica* are shown in table 1 below. There was an initial increase in body weight of rabbits from a mean 1.70 kg to 2.00 kg for Mg x1, 2.10 kg to 2.45 kg for Mg x 2, 2.10 kg to 2.25 kg for Zn x 1, 1.80 kg to 2.00 kg for Zn x 2, 1.80 kg to 2.30 kg for Cu x 1, 1.70 kg to 2.05 kg for Cu x 2 for the treatment group and 1.85 kg to 2.00 kg for the control group. There was a significant increase in body weight following cation treatment of the rabbits, but after challenging the various groups with *Salmonella enterica,* there was a progressive decrease in body weight of rabbits from a mean 2.00 kg to 1.90 kg for Mg x1, 2.45 kg to 2.30 kg for Mg x 2, 2.20 kg to 2.00 kg for Zn x 1, 2.00 kg to 1.80 kg for Zn x 2, 2.30 kg to 2.23 kg for Cu x 1, 2.05 kg to 1.85 kg for Cu x 2 for the treatment groups and 2.00 kg to 1.75 kg for the control group. There was no statistically significant difference in the net weight loss in all the groups and the control at $P < 0.05$

Other observed physiological effects of exposure to the challenge were body weakness, dullness, rough fur and loss of appetite. These effects were more pronounced in the control rabbits than in the cation - supplemented groups. Despite the possible effects of toxicity on the groups treated with double strength of the cations, they however, survived the experimental period. There was no significant frequency in stool in the test period before and after challenge with *Salmonella enterica.* Seven days after challenge there were obvious signs of infection of the rabbits: body weakness, lack of appetite, generally rough furs in all the groups. The physiological effects were more on the control group than any other group.

Physiological signs and clinical scores

There was no difference in the rectal temperature before and after the challenge with *Salmonella enteric.* The physiological changes observed were scored positive: mild reactions as (+), Light reactions as (++), and heavy reaction as (+++) or more according to the degree of infection.

TABLE I: MEAN WEIGHTS (KG) OF RABBITS SUPPLEMENTED WITH CATIONS AND CONTROL

	GROUPS						
Period	**Mg x1**	**Mg x 2**	**Zn x 1**	**Zn x 2**	**Cu x 1**	**Cu x 2**	**Control**
Initial	**1.70**	**2.10**	**2.10**	**1.80**	**1.80**	**1.70**	**1.85**
1st week	**1.78**	**2.26**	**2.16**	**1.90**	**2.01**	**1.78**	**1.82**
2nd week	**1.88**	**2.32**	**2.20**	**1.94**	**2.14**	**1.81**	**1.85**
3rd week	**2.00**	**2.45**	**2.25**	**2.00**	**2.30**	**2.05**	**2.00**
4th week	**1.90**	**2.35**	**2.00**	**1.80**	**2.23**	**1.85**	**1.75**
Mean +SE	**1.85 ±0.1**	**2.30 ±0.1**	**2.14 ±0.1**	**1.89 ±0.0**	**2.10 ±0.1**	**1.84 ±0.1**	**1.85 ±0.1**

All values are means ± SEM of 5 values per group for each supplementation

TABLE II: PHYSIOLOGICAL CHANGES FOLLOWING *SALMONELLA ENTERICA* CHALLENGE

	GROUPS						
Parameters	**Mg x 1**	**Mg x 2**	**Zn x 1**	**Zn x 2**	**Cu x 1**	**Cu x 2**	**Control**
Rectal temperature	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Body weakness	**+**	**++**	**+**	**++**	**+**	**+**	**+++**
Lack of appetite	**++**	**++**	**++**	**++**	**++**	**++**	**++**
Rough furs	**+**	**++**	**+**	**++**	**+**	**+**	**+++**
Stool droppings	**N**	**N**	**N**	**N**	**N**	**N**	**N**
Weight (Kg) gained before challenge	**0.30**	**0.35**	**0.15**	**0.20**	**0.50**	**0.35**	**0.15**
Weight (Kg) loss after challenge	**0.10**	**0.10**	**0.25**	**0.20**	**0.70**	**0.20**	**0.25**

Key: + = mild signs, ++ = light signs, +++ = heavy signs, N= normal range 36 - 37°C

DISCUSSION

Attempt was made in this study to determine the physiological responses of rabbits to treatments with trace elements, the effects of the cations on body weight and their physiological reactions to the cations. It was observed that exposure to cations as oral supplements enhanced body weight gain. The body weight gained was concentration - dependent among those treated with zinc and magnesium, though not statistically significant at $P < 0.05$. The reverse was the case among those treated with copper, where there

was an appreciable increase in body weight of those treated with 2.0 µg of copper, than those of 4.0 µg, and the difference was not concentration -dependent. It is therefore implied that cations improved metabolic activities in the affected rabbits, an observation which is not at variance with the report of Camara and Amaro (6) who in their various studies indicated the importance of cations in mammalian metabolism.

Rabbits on cation supplement showed higher resistance to the damaging effects of *Salmonella enterica* as evidenced by the significant difference in the physiological responses of the treatment group of rabbits and the control. This finding may be related to the improved nutrition, which may have impacted on the immunity of the cation - supplemented groups. The relationship between nutritional status and immune functions has been reported by several authors (7, 8, 9 and 10). The result of the present study, therefore, provides evidence of the significance of zinc, copper and magnesium oral supplementation in mammals and of course among these three cations, copper appears to be most effective in improving body weight gain and physiological responses to the overwhelming effects of *Salmonella enterica*, though the mechanism is yet unknown. The potential effects of trace elements cannot be over emphasized during treatment of infections and convalescence. Nutritional supplements rich in essential trace elements should also be considered for recovery of weight loss due to infection, to improve metabolism and body activities. There is therefore, the need for further studies to explore the potential of trace elements on body weight in health and specifically as a tool to combat uncharacterized weight gain or loss in our society especially among children of school age and institutionalized adults living in the sub-sahara of Africa. We recommend that urgent action be taken by government at all levels to acquire more information not only on staple foods of developing countries but also to determine the effect of cultural, climate and environmental changes on the contents of these elements in diets derived from such foods.

Conclusion:

The physiological changes during the assessment indicate that the study cations impacted on the affected rabbits and improved metabolic activities which brought about progressive weight gain *ab nisu*. Consequently, higher resistance to the damaging effect of the *S. enterica* challenge was evident in physical parameters observed in the treatment group and not in the control group.

REFERENCES

1. Neilson FN (1997): Trace elements in Encyclopaedia of Human Biol. 2nd Ed. Academy Press, Philadiaphia **6:**4567-4572

2. Anonymous (1996): Trace elements in human nutrition and health, Report of a WHO Expert Committee, Geneva, World Health Organization, 1973. (WHO Technical Report series No 532

3. Park K (2007): Nutrition and Health in Preventive and Social Medicine. pp 494 – 495, 19th Ed. M/s Banarsidas Bhanot.

4. Prasad AS (2003): Zinc, a mineral complex of biological activities. *B. Med. J.* **326,** 409 – 410.

5. Reunanen A, Knekt P, Marniemi J, Maki J, Aromaa A (1996): Serum calcium, magnesium, copper and zinc and risk of cardiovascular death. *Am. J. Clin. Nutri.* **50(7),** 431 – 437.

6. Camara T and Amaro I (2003): Zinc a mineral complex of biological activities. *International J. Food Science and Nutrition* **54**, 143 151

7. Chevaliar P, Sevilla R, Zakes L, Segas E, Balmonte G, Parent G, Jambou B (1996): Immunonutritional recovery of children with severe malnutrition *Sante* **64,**201- 8

8. Bonham M, O'Conner JM, Hannigan BM, Strain JJ (2002): The immune system as a physiological indicator of marginal copper status. *Br. J. Nutrition.* **62**,412- 6.

9. Troost FJ, Saris HMW, Bull JV, Hoogewertt JA, Dainty JR, Brummer RM (2003): Iron supplement inhibit zinc but not copper absorption invivo in ileostromy subjects *Am. J. Clin. Nutr*. **78(4),** 1018-23.

10. Rahman MJ, Sarker P, Roy KS, Ahmed MS, Christi J, Azim T, Mattan M, Sack D, Anderson J, Raqib R (2005): Effect of zinc supplementation as adjunct therapy on the systemic immune responses in Shigel losis. *Am. J. Clin. Nutri* . **81(2),** 495 – 502.

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EXPERIENCE WITH HEPATITIS B VIRAL LOAD TESTING IN NIGERIA

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ABSTRACT

Background: Quantification of the viral burden is an important laboratory tool in the management of hepatitis B virus (HBV)-infected patients. However, widespread use of assays is still hampered by the high cost. Treatment reduces viral load to undetectable levels. HBV infected patients tend to have high HBV DNA levels, and severe liver disease.

Objectives: This study was carried out to determine the pattern of HBV viral load levels of patients assessing management in Nigeria.

Method: Variables included sociodemographics like age, sex, religion, income, educational background and residence. The COBAS Amplicor automated Analyzer (PCR based) was used to assay the virus quantitatively.

Results: 594 patients were tested from 2008 to 2009. Statistical analysis was done using Epi info version 2002 and test of significance by Kruskal-Wallis. Mean age of the patients was 36.8 (ranging from 9 to 69) years. HBV viral titre ranged between 4,145 and 68,011,800 DNA copies/ml.

Conclusion: There was a high occurrence of viral titre in the population studied. High viral load is a risk factor for hepatocellular carcinoma. A policy earmarked to combat this virus in Nigeria is hereby solicited.

Key words: HBV infection, HBV DNA, Nigeria

INTRODUCTION

Viral hepatitis is as old as the history of medicine. About a third of the world's population, more than 2 billion people, have been infected with the hepatitis B virus [1]. Hepatitis B virus (HBV) infection is a public health problem with 350 million chronic carriers worldwide. An estimated 500,000 to 1.2 million people die of HBV infection annually. The global disease burden is substantial due to its high related morbidity and mortality.

Prevalence varies greatly in different parts of the world, but is higher in tropical regions causing both acute and chronic liver disease [2]. In Nigeria, 11.6% prevalence rate has been reported from Maiduguri [3], 13.8% from Lagos [4], 4.3% from Port Harcourt [5], 5.7% from Ilorin [6], 8.3% from Zaria [7], 17.1% from female sex workers, 14.9% [8] from healthy blood donors [9] and 25.7% among surgeons [10]. Although HBV prevalence varies widely across the African continent, hepatitis B surface antigen (HBsAg) positivity is estimated at 8-20% [11]. About 15-40% of HBV infected patients would develop cirrhosis, liver failure, or hepatocellular carcinoma (HCC) [12].

Jatau *et al* while working on pregnant women showed that HBV infection increases with age to a level and decreases with higher age [13]. People with chronic hepatitis B virus infection who experience hepatitis B "e" antigen (HBeAg) seroconversion before age 30 have better long-term outcomes - including lower rates of cirrhosis and liver cancer - than those who seroconvert 10 years later [14]. The risk of primary liver cancer increases with age and alcohol consumption [15]. Influence of age on development of HBV carrier state has been studied and a consistent relation was found.

A study by Chen *et al* showed a persistent suppression of HBV DNA is important to prevent the development of HCC in known cirrhosis patients [16]. Treatment with the interferon group reduces viral load to undetectable levels with a 50-70% success rate. HBV is classified into 8 genotypes, A-H.

In Nigeria, centres where one can assess his or her hepatitis status are many, but for quantitative determinations, they are rare and expensive. Samples have previously been taken outside the country to foreign laboratories. This costly enterprise keeps the test out of reach of the average citizen. However, the HBV DNA test is essential to hepatitis B management.

The purpose of this study was to present the pattern of HBV viral load levels of people in terms of age/sex distribution and zonal distribution in Nigeria.

MATERIALS AND METHODS

Data, sample collection and processing: A biodata was raised for each of the 594 subjects (age range 9 - 69 years) containing details of their age, sex and residential address. 5 ml of blood was collected from each subject into EDTA

treated tubes and centrifuged for 10 minutes. The plasma was separated and stored at -20°C until analysed. Subjects had been previously confirmed HBsAg positive prior to assessing the HBV viral load test.

Study design: This is a descriptive retrospective study.

Sample population: This consists of five hundred and ninety four subjects, from all over Nigeria requesting HBV viral load test within 20 months. The inclusion criteria were those that tested positive for HBsAg. Only the first test carried out by a patient was included in this study.

Sample Analyses: This was carried out at the Human Virology unit of the Nigerian Institute of Medical Research, Lagos, Nigeria.

HBV DNA viral load assay: Samples were assayed for the quantity of hepatitis B virus according to the COBAS Amplicor HBV monitor test (Roche diagnostics GmbH, Mannheim, Germany) manual, Version 2.0, a PCR based technique, at the Human Virology Unit, Nigerian Institute of Medical Research, Lagos, Nigeria. The HBV viral load results were expressed in DNA copies/ml, after converting from the IU/ml value reported by the automated analyzer.

Statistical Analyses: This was done using Epi info version 2002 and test of significance using Kruskal-Wallis statistical packages. Differences of $p<0.05$ were taken to be statistically significant at 95% confidence interval.

RESULTS

The age and sex distribution of the HBV viral load values are shown in table 1. It revealed a hepatitis B DNA range of 4,145-68,011,800 DNA copies/ml. Male to female ratio was 2.9:1 respectively. The median HBV viral load for males and females were 10,941 and 10,257 DNA copies/ml respectively ($P>0.005$). The age bracket with the highest HBV prevalence was the 30–39 age group.

The steady increase in patient turn-out during the 20 month time period (ranging from 3-69 patients/month) is shown in figure 1.

Geographical distribution of subjects assayed shows the largest group coming from the south-west of the country, 51.3%, followed by the south-east 12.2%, north-central and south-south with 10.3% and 8.8% respectively, as shown in table 2.

The test-turnaround-times (TAT) from first assay until December 2009 are shown in figure 2. Initial TAT was 55 days (requiring the lab to call patients for result pick-up), but it gradually improved to 10 days as sample size increased. Of the 594 subjects tested, 2 results were sent by email in 2008 and 36 in 2009. Number of unclaimed results was 3 in 2008 and 10 in 2009. Number of repeat testing was 11 in 2008 and 12 in 2009.

TABLE 1
AGE AND SEX DISTRIBUTION OF OF PATIENTS AND THEIR HBV VIRAL LOAD VALUES (DNA COPIES/ML)

Age group (years)	N (%)	Median HBVL (DNA copies/mL)	P value
0-9	1 (0.23)	68,011,800.0	0.0536
10-19	19 (3.21)	4,145.0	
20-29	126 (24.28)	18,463.0	
30-39	223 (36.04)	8,100.0	
40-49	152 (24.11)	8,363.0	
50-59	53 (9.55)	17,331.5	
60-69	10 (1.91)	46,998.0	
Sex			
Male	441 (74.2)	10,941.0	0.3636
Female	153 (25.8)	10,257.0	
Total	**594 (100)**	**10,625.0**	

TABLE 2
ZONAL DISTRIBUTION OF SUBJECTS ASSAYED FOR HEPATITIS B VIRAL LOAD IN NIGERIA

Geographical Zones	HBV viral load tests N (%)
NE	**1 (0.2%)**
NW	**3 (0.5%)**
NC	**61 (10.3%)**
SS	**52 (8.8%)**
SE	**72 (12.2%)**
SW	**305 (51.3%)**
Unknown	**100 (16.9%)**
Total	**594 (100%)**

DISCUSSION

Infection with HBV is under control in developed countries [1], but it is still a serious public health problem in developing countries like Nigeria. In this study we examined the viral load pattern of those assessing laboratory services at our site, being the only laboratory at present where HBV

viral load is carried out commercially within the country. As such samples were received from a wide range of localities across the country (Table 2). There was a wide age range observed by the patients, with the highest prevalence being between 30-39 years. This compares to what was observed by Jatau and colleagues [13], and Belo [10].

In this study also, more males requested the test than the female counterparts. This is in consonance with those observed in previous studies [4]. This pattern could be because of the higher purchasing power of men, as the test is expensive. There was a wide disparity in the viral titre amongst the age groups, but it was of no statistical significance (p>0.0536).

FIGURE 1
PROFILE OF HBV VIRAL LOAD ASSAYS CARRIED OUT FROM MAY 2008 TO DECEMBER 2009

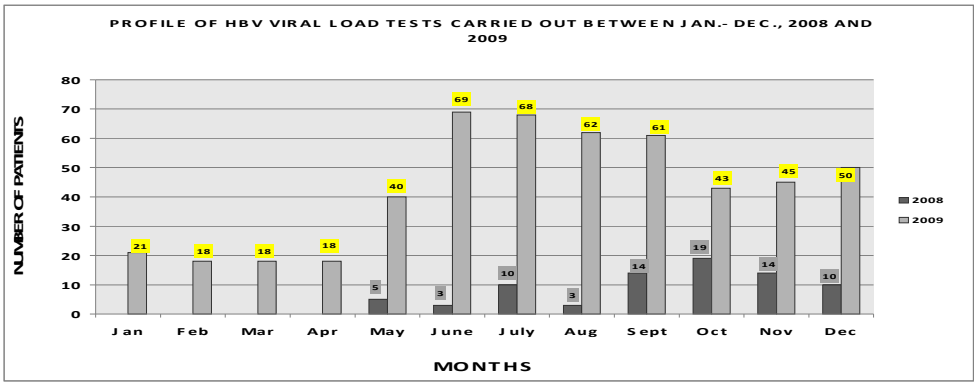
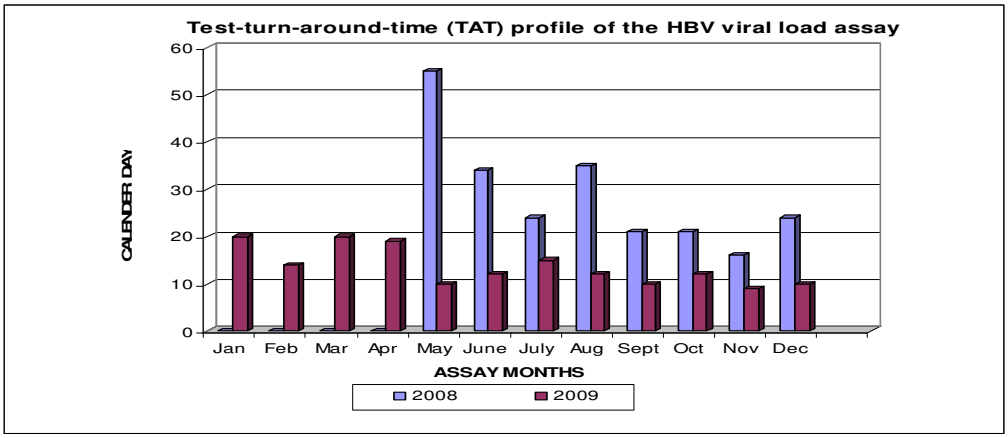


FIGURE 2
TEST-TURN-AROUND-TIME (TAT) PROFILE OF THE HBV VIRAL LOAD ASSAY



**TAT is the average number of calendar days from sample collection to lab assay*

Since HBV viral load is an independent predictor of liver cancer risk, this points to the importance of both close clinical monitoring and antiviral treatment for individuals with high HBV viral load (>10, 000 copies/ml). The lower and upper limit of detection of the COBAS Hepatitis B monitor assay used was 316 and 199,880 DNA copies/ml respectively. HBV viral load may be seen as a good surrogate marker for slower clinical progression. The risk of HCC is closely associated with HBV, according to a Taiwanese study [17]. This association was shown to be independent of other risk factors, and to have a dose-response relationship. That study also showed that HBV DNA levels were persistently elevated in patients at highest risk of liver cancer [12]. There is therefore a high a risk of hepatocellular carcinoma among this population studied because of the high viral load observed. However this could be averted if regular/routine screening of the populace is in place. This risk could be minimized if the National programme on immunization (NPI) scheme for HBV vaccination is implemented. This study, again confirms the previously reported high prevalence of HBsAg in this part of the world [5]. Patients

infected with HBV, treated with peg-interferon2alfa and lamivudine are more likely to The challenges of carrying out the assay in our laboratory include, clients travelling from far to Lagos, limited shelf life of the reagents in the kits, delay in kit supply from Germany, electronic payment of the test by some clients amongst others. It is therefore suggested that there be put in place a national programme to mitigate the scourge of the virus which is a hundred times more infectious than HIV. Taiwan and Saudi Arabia have seen the tremendous impact of a mass programme of HBV immunization and the integration of HBV vaccine in the Expanded Programme of Immunization (EPI) in controlling HBV infectivity in their general population [17]. This is a route which Nigeria might well consider.

have a sustained virologic response [19].

CONCLUSION

In conclusion, given the high viral load observed in this study group and the risk of HCC involved, it is recommended that there be health education for public and health care providers. Also screening and vaccination of all special risk groups especially surgeons, laboratory workers, dentists, emergency workers and law enforcement agents. A comprehensive effort is needed to promote hepatitis B testing in the Nigerian population, with community outreach, increased access to testing and physician education.

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REFERENCES

1. Marjolis, H.S., Alter, M.J. and Hadler, S.C. (1991). Hepatitis B: Evolving epidemiology and implications for control. *Sem. Liver Dis.* 1991; **11:** 84–92.
2. Szmuness, W., Dienstag, J.L, Purcell, R.H., Steven, C.E., Wong, D.C. and Ikram, H. (1977). The prevalence of antibody to hepatitis B antigen in various parts of the world: a pilot study. *Amer. J. Epidem.* 1977; 106**:** 392-8.
3. Harry, T.O., Bajani, M.D., and Moses, A.E. (1994). *Hepatitis B virus* infection among blood donors and pregnant women in Maiduguri, Nigeria. *East Africa Medical Journal* 70: 596-597.
4. Nasidi, A.T.O., Vyazor, S.O., Numumbe, G.M.R. Azzan, B.B., and Ancinlev, V. A. (1983). Prevalence of Hepatitis B Infection Marker in two different geographical areas of Nigeria. *Preceedings of the first international conference, 12-15 December, 1983, Lagos,* Nigeria.
5. Akani, C.I., Ojule, A.C., Opurum, H.C. and Ejilemele, A.A. (2005). Seroprevalence of HBs Ag in pregnant women in Port Harcourt, Nigeria. *Post graduate Medical Journal* 12(4): 266-270.
6. Agbede, O.O., Iseniyi, J.O., Kolewale, M.O. and Ojuowa, A. (2007). Risk Factors and seroprevalence of *hepatitis B* antigenemia in mothers and their preschool children in Ilorin, Nigeria. *Therapy,* 4(1): 67-72.
7. Luka, S.A., Ibrahim, M.B. and Iliya, S.N. (2008). Seroprevalence of Hepatitis B surface antigen among pregnant women attending Ahmadu Bello University Teaching Hospital Zaria, *Nigerian Journal of Parasitology* 29 (1):38-41.
8. Forbi, J.C., Onyemauwa, N., Gyar, S.D. Oyeleye, A.O., Entonu P. and Agwale S.M. (2008) High Prevalence of Hepatitis B Virus among Female Sex Workers in Nigeria. Rev. Inst. Med. trop. S. Paulo 50(4):219-221.
9. Ejele, O., Nwauche C. and Erhabor O. (2004). The prevalence of hepatitis B surface antigen in HIV-positive patients in the Niger Delta Nigeria. Niger J Med 2004, 13: 175-9.
10. Belo, A.C. (2000). Prevalence of hepatitis B virus markers in Surgeons in Lagos, Nigeria. East African Medical Journal Vol. 77 No. 5 May 2000; 283-285.

12. Apurva A.M. and Jordan J.F. (2007). Viral hepatitis and HIV in Africa. AIDS Reviews 2007; 9: 25-39.
13. **Aboulhab J. (2006). HBV viral load is an independent predictor of progression to hepatocellular carcinoma in chronic HBV patients. Euro Surveill. 2006; 11(1):** E060126.6. **Available online:** http://www.eurosurveillance.org
14. Jatau, E. D. and Yabaya, A. (2009). Sero Prevalence of Hepatitis B Virus In Pregnant Women Attending A Clinic In Zaria, Nigeria. *Science World Journal Vol 4 (No 2) 2009, 7-9.*
15. Yi-Cheng C., Chia-Ming C. and Yun-Fan L. (2010). Age-specific prognosis following spontaneous hepatitis B e antigen seroconversion in chronic hepatitis B. Hepatology, Vol 51, Issue 2, pages 435–444.
16. Orholm M., Aldershvile J., Tage-Jensen U., Schlicting P., Nielson J.O., Hardt F., and Christoffersen P. (1981). Prevalence of hepatitis B virus infection among alcoholic patients with liver disease. J. Clin. Pathol. 1981; 34:1378-1380.
17. Chen C.J., Yang H.I., Su J., Jen C.L., You S.L., Lu S.N., Huang G.T. and Iloeje U.H. (2006). Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; **295**(1): 65-73.
18. Lin D.B., Wang H.M., Lee Y.L., Ling U.P., Changlai S.P., and Chen C.J. (1988). Immune status in preschool children born after mass hepatitis B vaccination program in Taiwan. Vaccine 1998; 16: 1683-7.
19. Lau G.K., Piratvisuth T. and Luo K.X. (2005). Peginterferon alfa2a, Lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med 2005; 352: 2682-95.
20. Al-Faleh F.Z., Al-Jeffri M., Ramia S., Al-Rashed R., Arif M., and Rezeig M. (1999). Seroepidemiology of hepatitis B virus infection in Saudi children 8 years after a mass hepatitis B vaccination programme. *J Infect*. 1999; 39: 167-70.

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MOLECULAR SCREENING FOR *PLASMODIUM FALCIPARUM* RESISTANCE MARKERS FOR ARTEMISININS IN MBITA, KENYA

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ABSTRACT

Artemisinins-based combination therapies (ACTs) are being recommended against uncomplicated malaria in endemic areas of Africa. However, in these areas data on their long term usefulness is limited. It has been demonstrated that ACTs resistance may be due to single nucleotide polymorphisms (SNPs) in the chemotherapeutic target, the SERCA-type ATPase protein (PfATPase6). This study analyzed PfATPase6 mutations in asymptomatic infections from samples collected from Mbita, a malaria endemic region in Kenya. Mutations in A623E and S769N residues were screened with gene specific primers followed by sequencing. The study demonstrates that there is no mutation in Mbita, Kenya because neither A623E nor S769N *Pf*ATPase6 mutations were detected. Resurgence of infections in this area could be due to re-infections and not drug failure. The study recommends that other sites be assessed for PfATPase 6 mutations to verify the long-term usefulness of ACT and monitor any emergency of resistance.

Keywords: ACT (Artemisinins-based Combination Therapy), Molecular, Mutations, PfATPase 6

INTRODUCTION

Resistance to anti-malarials is a major drawback in effective management and control of malaria in sub-Saharan Africa. *Plasmodium falciparum* has developed high levels of resistance to the available, cheap and safe drugs such as, chloroquine (CQ) and sulfadoxine pyrimethamine (SP) [1, 2], and this has led to the recommendation of artemisinins-based combination therapy (ACT) as a first-line drug in various sub-Saharan countries [2]. Following increased SP resistance in Kenya, the country revised its malaria treatment policy to adopt artermether+lumefantrine [3, 4].

The artemisinins compounds act by the haem-catalysed intra-parasitic production of highly reactive carbon-centred free radicals [5, 6]. Certain haemo-globinopathies have been associated with reduced artemisinins activity, possibly because of reduced intra-erythrocytic availability of iron to catalyse opening of the peroxide bridge [6]. Recent study suggested that a sarco-endoplasmic reticulum Ca^{2+} ATPase (SERCA)-type protein encoded by a gene denoted PfATPase6 might be the major chemotherapeutic target of artemisinins drugs [7]. Artemisinins has been found to interact and selectively inhibit PfATPase6, the only SERCA-type Ca^{2+}-ATPase6 in the *P. falciparum* genome [7, 8].
Artemisinins have the broadest anti-malarial activity against parasites, from the ring stage to early schizonts, and cause the fastest decline in parasite numbers of all the anti-malarials.

Their short half-lives limit the possibility of selection for resistance [6]. Heavy use of artemisinins in mono-therapy has been associated with selective pressure in parasites isolated from French Guyana and in *in-vitro* study in Senegal [9]. Recently, partial artemisinins resistant *P. falciparum* malaria has emerged on the Cambodia–Thailand border.

Exposure of the parasite population to artemisinins monotherapies in sub-therapeutic doses for over 30 years, and the availability of substandard artemisinins, have probably been the main driving force in the selection of the resistant phenotype in the region [10]. Studies with murine malaria model demonstrated increased resistance to artemisinins [11].

A subsequent *in-vitro* study in French Guyana showed that *P. falciparum* with elevated IC_{50} values for artemisinins shared specific point mutations at codon S769N of the *ATPase6* locus. In addition, ATPase6 A623E and E431K mutations were also associated with reduced *P. falciparum* susceptibility to artemisinins [9].

Strong evidence shows that resistance to artemisinins may depend on SNPs in the drug's putative chemotherapeutic target, the SERCA-type

ATPase protein (PfATP6) but epidemiological evaluation of gene copy numbers in natural parasite populations has not been carried out [7]. Residues S769N, L263E, E431K and A623E are associated with resistance [9, 13]. The S769N mutation differs from the engineered L263E replacement, which abolished artemisinins inhibition of the *PfATPase6* activity [13], while the residue S769N is located within the cytoplasmic N (nucleotide binding) domain close to a conserved hinge, which in other species is essential in the structural transitions crucial in the progress of the ATPase cycle, calcium binding and release [8].

To date no relevant clinical resistance to artemisinins has been reported in Kenya. However, the long-term usefulness of ACT in endemic areas remains unclear [12]. Therefore, as ACT becomes widely used in sub-Saharan Africa, regular and comprehensive surveillance of resistance is of great importance. Predicting the emergence and spread of resistance to current anti-malarials and newly introduced compounds is necessary for planning malaria control and instituting strategies that might delay the emergence of resistance.
The present study analysed PfATPase6 mutations in asymptomatic infections from samples collected from Mbita, a malaria endemic region in Kenya. Mutations were screened at A623E and S769N residues. The study recommends that other sites be assessed for PfATPase 6 mutations to verify the long-term usefulness of ACT and monitor any emergency of resistance.

MATERIALS AND METHODS
STUDY SITE AND SAMPLE COLLECTION
The study protocol was approved by the scientific steering committee and the ethical review committee of the Kenya Medical Research Institute (KEMRI), Nairobi, Kenya. Written and informed consent forms presented in native language and translated to the patients were obtained from the study participants. Blood samples were collected as dry blood spots on Whatman 3M filter papers from thirty patients presenting with asymptomatic malaria. The filters were air dried and packed in sealed plastic bags and stored at 4 ^{o}C until further analysis.

DNA EXTRACTION AND AMPLIFICATION
DNA from filter papers was extracted according to Warhust *et al* method, [14], with slight modifications. Briefly, 4mm^2 piece of filter with blood spot was cut with a sterile scalpel blade and incubated in 0.5% saponins in 1× PBS overnight at 4 ^{o}C. The brown solution was removed and replaced with 1× PBS and then incubated for 20 minutes. The solution was removed and 100µl of DNAse free water was added followed by 50µl of 20% Chelex. The tubes were placed into a heated block and vortexed every two minutes. This was repeated up to 5 times. The solution was centrifuged and the supernatant carefully separated. The supernatant contains DNA and 30 µl aliquots were taken into eppendorf tubes and stored at -20 ^{o}C for PCR analysis.

The amplification of a 696 base pair fragment of the PfATPase6 gene was carried out on an MJ Thermocycler™ using the following as forward and reverse primers;
7F-AATCACCAAGGGGTATCAAC and
8R-ACGTATACCAGCCATATGG,
and these targeted a 1771–2467bp region (S769N and A623E) codons on PfATP*ase6* gene.

Amplification was carried out in 30 µl reaction volumes which comprised of 3 µl of the template DNA, 30nM of each primer, 200 µM of dNTP mix and 0.3 units of Taq Polymerase. For each run, a positive control (molecular weight marker) and a negative control (all reagents minus DNA template) were included. The cycling profile consisted of initial denaturation at 94 ^{o}C for 3 minutes followed by 35 cycles at 94 ^{o}C for 1 minute, 55 ^{o}C for 1 minute and 72 ^{o}C for 1 minute and a final extension at 72 ^{o}C for 10 minutes. The PCR products were visualized on a 1.5 % ethidium bromide stained agarose gel in Tris Acetate EDTA (TAE) buffer. A PCR product of 696bp was obtained as shown in figure 1.

PCR PRODUCTS CLEANING AND SEQUENCING
Five microlitres of each PCR product for the target fragment were cleaned using 2 µl of ExoSAP-IT® (Affymetrix, USA) following manufacturer's instructions. This was carried out to check for mutations in the 623 and 769 residues. Each product was sequenced using Bigdye® Technology using the 3700 X Genetic Analyzer (Applied Biosystems) with the primers designed for amplification.

SEQUENCE ANALYSIS
The sequences were subjected to BLAST analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi)
to determine similarity with other sequences. The confirmed sequences were then analyzed using the SeqScape software version 2.5 (Applied Biosystems) that aligns and compares the sequences with the reference sequence.

RESULTS
DNA extracted from 30 samples collected from Mbita, was amplified with primers specific for PfATPase6 gene. A fragment of 696 base pair was found in all samples as shown in figure 1.

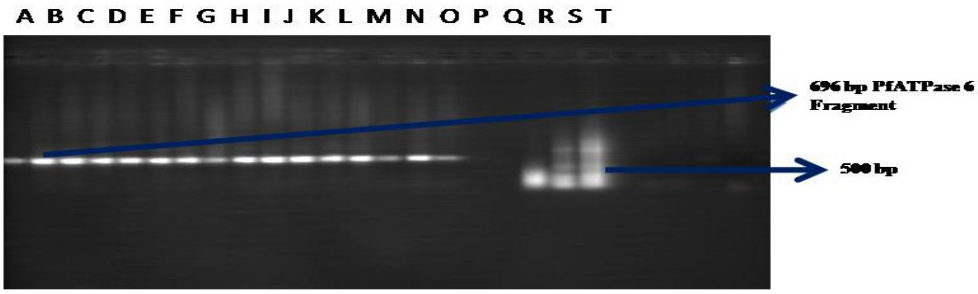

Figure 1: PCR product of PfATPase gene from P. falciparum samples collected from Mbita. The amplicons are 696 base pairs in samples labeled A up to P. Q and R represents negative control while, S and T is 1000 molecular weight marker.

The fragment was cleaned from the gel and sequenced to check for mutation in the residue S769N and A623S. It was found that there was no PfATPase6 S769N or A623E mutations in the samples sequenced. The sequences obtained were aligned with reference sequences for the purposes of comparison using BLAST, and then annotated in Genbank.

The sequences were then aligned with reference sequences using SeqScape software version 2.5 (Applied Biosystems). The software determines regions which have mutated in the corresponding sequence. It was found that the query and reference sequences matched perfectly, an indication that there were no mutations in the residues S769N and A623E. Sample alignment output from SeqScape software is shown in figure 2.

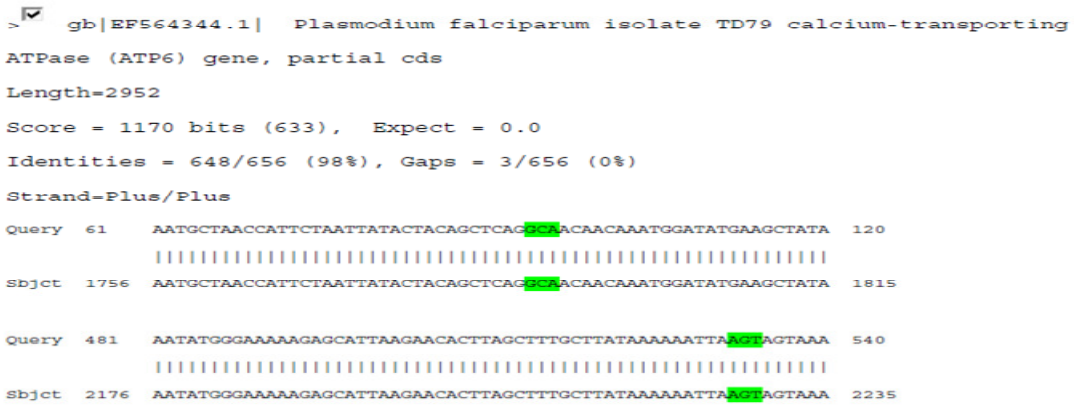

Figure 2: The query comprises of the sequence sent to the database and the subject (sbjct) comprises of the reference sequence in the database.The green alignment shows the base pairs of codon 769 and 623 on both the query and the subject. It also shows the percentage similarities between the query and reference sequences.

The sequences were submitted to GenBank for annotation and are available online as follows; GenBank: FJ384391, GenBank: FJ384392, GenBank: FJ384393, GenBank: FJ384394, GenBank: FJ384395, GenBank: FJ384396, GenBank: FJ384397, GenBank: FJ384398, GenBank: FJ384399, GenBank: FJ384400, GenBank: FJ384401, GenBank: FJ384402, GenBank: FJ384403, GenBank: FJ384404, GenBank: FJ384405, GenBank: FJ38406,
GenBank: FJ384407, GenBank: FJ384408, GenBank: FJ384409, GenBank: FJ384410, GenBank: FJ384411, GenBank: FJ384412, GenBank: FJ384413, GenBank: FJ384414, GenBank: FJ384415, GenBank: FJ384416, GenBank: FJ384417, GenBank: FJ384418,
GenBank: FJ384419.

DISCUSSION

Malaria poses a risk to half of the world's population and more than a million people die of the disease each year. To this end, the disease has defied eradication in areas of intense transmission. Kenya adopted the artemisinins ACT in 2006 as the first line treatment for all uncomplicated malaria

Artemisinin and its derivatives are the most potent and rapidly acting anti-malarials. Strong evidence has shown that resistance to artemisinins may depend on single nucleotide polymorphisms in the drug's putative chemotherapeutic target known as SERCA-type ATPase protein [13]. It has been shown that residues S769N, L263E, E431K and A623E are associated with resistance to artemisinins [13]. Residue S769N is located within the cytoplasmic N (nucleotide binding) domain close to a conserved hinge, which in other species is essential for the structural transitions needed for the progress of the ATPase cycle and calcium binding and release [8].

Epidemiological evaluation of gene copy numbers in natural parasite populations has not been conclusively carried out in malaria endemic parts of Kenya. This study found that there was neither S769N nor A623E PfATPase6 mutations in *P. falciparum* sequences sampled from Mbita, Kenya. A previous study by Bousema and colleagues reported that ACT administration in Mbita did not reduce the proportions of infected children [3]. It is clear to conclude from this study that the observed drug failure in the previous study [3] could have been due to re-infections and not resistance to ACT. This shows that the studied codons are in their wild type and the drug is effective in this area for the management of malaria. This study is consistent with findings from Kefas *et al* [13], that there were no S769N or A623E in 1205 subjects studied in Tanzania [13].

CONCLUSION

Though the current study showed that there is no *P. falciparum* resistance to artemisinins because the screened codons were in their wild forms, there is a need to investigate factors that could aggravate infections despite use of ACTS. Screening for other genes which could confer resistance to ACTs is urgently needed. Further screening of clinical samples from endemic areas in the region using L263E and E431K codons, and active surveillance of clinical response to ACT therapy will help establish the levels of efficacy of ACTs in malaria endemic regions in Kenya.

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REFERENCES

1. The East African Network for Monitoring Antimalarial Treatment (EANMAT): The efficacy of antimalarial monotherapies, sulfadoxine-pyrimethamine and amodiaquine in East Africa: Implication for sub-regional policy. *Trop Med Int Health* 2003, 8:860-867
2. World Health Organization 2006. Guidelines for malaria treatment (http://www.who.int/malaria/docs/Treatment guidelines2006.pdf)
3. Bousema T. J, Petra S, Louis C, Gouagna C. J, Drakeley A. T, Rein H, Githure J. I, Ord R, Colin J. S, Sabah A. O, Robert W. S. (2006). Moderate Effect of Artemisinin-Based Combination Therapy on Transmission of *Plasmodium falciparum*. *J of Inf Dis* 2006, 193: 1151–9
4. **Bousema T. J, Louis C. G, Chris J. D, Annemiek M. M , Bernard A. O, Ikupa N. J, John C. B, John I. G, Robert W. S.** Treatment failure of pyrimethamine-sulphadoxine and induction of *Plasmodium falciparum* gametocytaemia in children in western Kenya. *Trop Med Int Health* 2003, 8: 427–430
5. Meshnick S. R, Taylor T. E, Kamchonwongpaisan S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microb Rev*. 1996: 60, 301-315
6. Kamchonwongpaisan S and Meshnick S. R. The mode of action of the antimalarial artemisinin and its derivatives. *Gen Pharmacol*, 1996: **27**, 587-592
7. Eckstein-Ludwig U, Webb R. J, Van-Goethem I. D. A, East J. M, Lee A. G, Kimura M, O'Neill P. M, Bray P. G, Ward S. A, Krishna S. Artemisinins target the SERCA of *Plasmodium falciparum*. *Nat* 2003, 424: 957–961
8. Uhlemann A. C, Cameron A, Eckstein-Ludwig U, Fischbarg J, Iserovich P, Zuniga F. A, East M, Lee A, Brady L, Haynes R. K, and Krishna S. A single amino acid residue can determine the sensitivity of SERCAs to artemisinins. *Nat Struct Mol Biol*, 2005, 12: 628-629

9. Jambou R, Legrand E, Niang M, Khim N, Esterre P, Fandeur T, Mercereau-Puijalon O. Resistance of *Plasmodium falciparum* field isolates to in-vitro artemether and point mutations of the SERCA-type PfATPase6. *Lanc* 2005, 366: 1960-1963
10. Noedl H, Se Y, Schaecher K, Bryan L, Smith M. D, Socheat D, Fukuda M. M. Evidence of Artemisinin-Resistant Malaria
12. Duffy P E and Sibley C H: Are we losing artemisinin combination therapy already? *Lanc* 2005, 366: 1960-1963
13. Kefas M, Blaise G, Hassan M, Hans P. B. (2006). Molecular monitoring of *Plasmodium falciparum* resistance to artemisinin in Tanzania. *Mal* 2006, 5:126

Lim P, Volney B, Ekala M.T, Bouchier C, in Western Cambodia. *N Engl J Med* 2008:2619–262
11. Ferrer-Rodiriguez I, Perez-Rosado J, Gervais G. W, Peters W, Robinson B. L. *Plasmodium yoelii*: Identification and partial characterization of an mdr1 gene in an artemisinin-resistant line. *J Parasitol 2004*, 90: 152–160
14. Warhust D. C, Kariem F. M. A, Miles M. Simplified preparation of malarial blood samples for polymerase chain reaction *Lanc* 1991, 337: 303-304

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PREVALENCE OF URINARY SCHISTOSOMIASIS IN COMBONI AND TOKLOKPO JUNIOR HIGH SCHOOLS (JHS) AT SOGAKOPE

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ABSTRACT

Schistosomiasis remains an important parasitic disease worldwide. The level of prevalence of schistosomiasis in the Sogakope district is essentially due to the frequent exposure of the inhabitants to water bodies such as the Lake Volta and other lakes. This research investigated the prevalence of schistosomiasis in Toklokpo (about two kilometres from the bank of the Volta River) and Comboni (situated along the bank of the river Volta) in the Sogakope district. A total of 150 samples of urine were taken: Seventy-five from Toklokpo JHS and 75 from Comboni JHS. Each of the 75 samples was selected randomly from boys and girls. The urine samples were processed by the centrifugal sedimentation technique. The total prevalence of urinary schistosomiasis in the study area was 26%. The prevalence rates of urinary schistosomiasis in the two schools were 12.6% and 13.3% for Comboni JHS and Toklokpo JHS respectively. The highest prevalence occurred in the males between 10-11 years age group. For both sexes, Toklokpo Junior High School recorded higher prevalence of the disease numerically .It is recommended that urgent measure is taken to curb the menace.

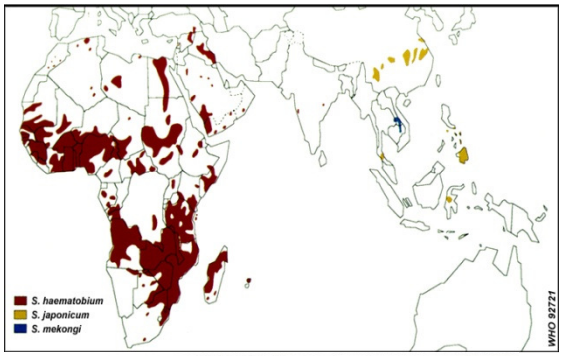
INTRODUCTION

Schistosomiasis is water borne disease, caused by a parasitic trematode worm. Infection with S. *mansoni,* S. *haematobium* and S *japonicum* cause illness in humans. Although schistosomiasis is not found in the United States, over 200 million people are infected worldwide (1)

Schistosomes belong to the kingdom Animalia, Phylum Platyhelminthes, Class Trematoda, Subclass Digenea, Order Strigeata, Family Schistosomatidae and Genus *S*chistosoma (2) .Members of this family are dioecious and parasitic in the blood vascular system of vertebrates. A general feature for the family is that, the mature female is more slender than the male and it is normally carried within a ventral groove called the gynaecophoric canal which is formed by ventrally flexed, lateral out growth of the male body (3)

Schistosomiasis has been recognized since the Egyptian Pharaohs. The worms responsible for the disease were eventually discovered in 1851 by Theodore Bilharz, working at the Kasrel-Aini hospital in Cairo. In a letter to Prof. Th. Von Siebold he describes his new discovery made during a post – mortem examination (4)

Fig 1 Distribution of schistosomiasis worldwide



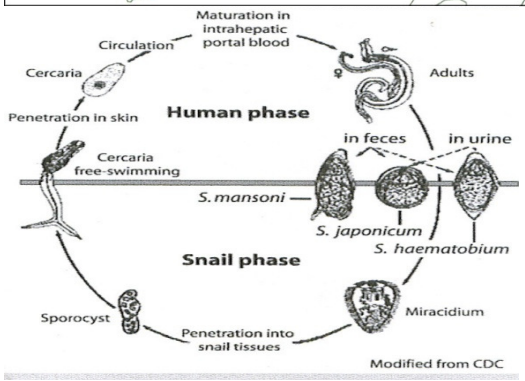


Fig 2: Life cycle of schistosomiasis (7)

Fi g 3 Snail intermediate host of S. haematobium, *Bulinus* species

Schistosome eggs have been recovered from both Chinese and Egyptian mummies showing that the infection was present in early civilizations of mankind. This was first noted as early as 1910 by Sir Armand Ruffer (4) who found calcified eggs in the kidneys of two mummies of the twentieth dynasty. It is estimated that about 200 million schistosomiasis cases occur in tropical countries every year (1) .Urinary schistosomiasis affects 66 million children throughout 54 countries. In some villages around Lake Volta in Ghana over 90% of the children are infected by the disease (4).

The main forms of human schistosomiasis are caused by five species of the flat worm or blood flukes, namely *S. mansoni*, *S. japonicum, S.mekongi* and *S.intercalatum* cause intestinal schistosomiasis *and* S. *haematobium* which causes urinary Schistosomiasis. The disease is transmitted by specific aquatic or amphibious snails (Family, Planorbidae) in a wide variety of fresh water habitats (5)
Schistosomes undergo an alternation of generations with sexual reproduction taking place in the The life cycle of this parasite involves many steps. Adult worms in humans reside in mesenteric venules in various locations which at times seem to be specific for each species. For instance, *S. mansoni* occurs more often in the superior mesenteric veins and *S. japonicum* more frequently in the inferior mesenteric veins so it is not possible to state equivocally that one species only occurs in one location. *Schistosoma haematobium* most often occurs in the venal plexus of the bladder, but it can also be found in the rectal venules. The females (size 7-20mm; males slightly smaller) deposit eggs in the small venules of the portal and perivesical system. The eggs are moved progressively toward the lumen of the intestine (*S. mansoni* and *S. japanicum*) and of the bladder and ureters (*S. haematobium*), and are eliminated with faeces and urine, respectively. Under optimal conditions the eggs hatch and release *cercariae,* which

It was noted by Sir Patrick Manson, Physician to the Seamen's Hospital in Greenwich, that the eggs passed in the urine from schistosomiasis patients all had a terminal spine, while only those found in the faeces had a lateral spine. He then went on to speculate that "possibly" there are two species of bilharzias, one with lateral spine ova and the other with a terminal spine (1)
Schistosoma *haematobium* is endemic in 54 countries, mainly in Africa and in the Eastern Mediterranean (1). It is also found in several Indian Ocean islands and small islands off the coast of East and West Africa (Fig. 1). In some areas the distribution of S. *haematobium* overlaps with S. *mansoni* causing double infections (1) *S. mansoni* is found in parts of South America and the Caribbean, Africa and the Middle East. *S. japonicum* is found in the far East. S. *mekongi* and S. *intercalatun* are found mainly in South East Asia and Central West Africa (1)

Life cycle
definitive host (man and other mammals) and asexual reproduction in the intermediate snail host (Fig. 2). swim and penetrate specific snail intermediate host. The stages in the snail include two generations of *sporocys*ts and the production of *cercariae.* Upon release from the snail, the infective cercariae swim, penetrate the skin of the human host, and migrate through several tissues and stages to their residence in the veins. Human contact with water is thus necessary for infection by schistosomiasis. Various animals serve as reservoirs for *S. japanicum*.

The snail host (Fig. 3) of *S. haematobium* belongs to the genus *Bulinus* species (8). . Schistosomiasis ranks among the major public health problems in the tropics and sub-tropics. The disease is caused by the parasite *Schistosoma*, a fluke with a lifecycle including man as the definitive host and a fresh water snail as the intermediate host. Two

hundred million people are infected worldwide (1) The main forms of human schistosomiasis are caused by five species of the flat worm or blood fluke which is transmitted by specific aquatic or amphibious snails (Family, Planorbidae) in a wide variety of fresh water habitats (5).

In some endemic areas the rate of symptomatic infections are lower in females than in males (4). These may reflect lifestyle differences between male and female regarding the likelihood of bathing or drinking from contaminated streams or irrigation ditches (4)

In Ghana, previous studies show that people living along the banks of the Volta Lake are prone to schistosomiasis infection, since they depend on the water for their subsistence (4). Sogakope is a busy and important commercial town along the Volta Lake in the South Tongu District of the Volta Region. Toklokpo and Comboni are two suburbs both of Sogakope and are settlements along the Volta Lake. The aim of the study is to determine the prevalence of schistosomiasis infection in male and female students The specific objectives of the study were to:

1 Estimate the prevalence of *S. haematobium* in school children at Comboni and Toklokpo JHS.

MATERIALS AND METHODS

Study Area

A field survey was carried out in two communities (Toklokpo and Comboni) both of Sogakope in the south Tongu district in the Volta region of Ghana. Both are settlements along the Volta Lake. Toklokpo, however, is about three kilometres eastward from the lake. The Volta lake supplies piped-borne water and supports irrigation and fishing activities of the two communities. The economic activities of both communities are mainly farming and fishing. The communities comprised mostly of Ewes. See fig 4

Fig 4 Study Communities

Collection and Analysis of Urine Specimen

Seventy-five boys and 75 girls between the ages of ten and nineteen years were randomly selected from each of the two schools. Urine samples were collected from each child by means of 600ml well labelled specimen bottles. Each urine specimen was collected between the hours of 11.00 and 14.00 GMT and taken to laboratory in Winchesters for analysis (10). To avoid

The prevalence and severity of schistosoma infection vary with age. Children and adolescents are affected most often and are infected most heavily. Infections peak in individuals aged 10-19 years (4) In some areas, the prevalence in this group may approach 100 per cent. In a person older than 19 years, the prevalence of active larvae and egg counts slowly decline in populations living in endemic areas (2). These declines in active infection may reflect that individuals have an increasing host immune response or decreasing exposure to contaminated water as they age.

Toklokpo, is about three kilometres east of Comboni. Toklokpo has other sources of water such as stagnant water and waters from Lake Aka. Comboni however depends only the Volta lake. Though it is known that those living along the lake have a higher prevalence rate of the worm, no study has been done in the Sogakope District to ascertain the level of prevalence, this therefore calls for the study.

in Comboni and Toklokpo Junior High School at (JHS) Sogakope.

2 Determine differences in the prevalence rate between the schools with reference to their ages and sexes.

the miracidia of schistosoma hatching from the eggs, the samples were kept in a dark container (7).

For each urine sample, reagent strip analysis (urine chemistry) was first performed to detect microhaematuria. An Ames urine test strip was dipped in each urine sample. Excess urine was drained off and the strip was left for 60 seconds after which colour reaction was compared with the standard. Results were recorded for glucose, blood, pH and protein.

After the urine chemistry, microscopy as described by (9) was carried out. The rest of the urine in the centrifuge tubes were taken through the process of sedimentation. The centrifuge tube was inverted to allow for the uniform mixing of the urine. Urine samples of the same volume were then centrifuged at 250rpm for 5minutes. After the centrifugation, urine deposits at the bottom of centrifuge tube were examined microscopically using the x10 objective for identification and x40 objective for detail view of ova objectives for the characteristic eggs. Infected students were marked as positive.

The following materials are used for Collection and Analyses of Urine Specimen for Schistosoma haematobium ova

- A clean dry container of about 600 ml to collect urine sample from the school children
- Wax pencil and water proof black markers for labelling.
- A bag to carry empty container to the field.
- Winchesters to transport urine samples from the field to the laboratory
- Disposable latex gloves for protection against infection
- Laboratory coat also for protection against infection and other external agents
- Centrifuge, D-78532 (Heltich Tultlingen) for centrifuging and concentration of schistosoma ova
- Microscope (Olympus CH_3O) with 10X and 40X objectives for observation of schistosoma ova
- Pasteur Pipette for pipetting samples
- Work Sheets for record keeping
- Microscope slide (76mmX26mm) for sample preparation and observation
- Microscope Cover slips (22mmX22mm) for observation
- Test tube racks for holding test tubes.

Prevalence of infection

Analysis of results obtained from laboratory, diagnosis involved the determination of prevalence and intensity of *S. haematobium* infection. The prevalence of infection refers to the proportion of subjects who are infected at a point in time and this is usually expressed as a percentage. In calculating the prevalence of infection among the students, the formula below was used according (1); $\Pr evalance = \frac{Number\ of\ subjects\ testing\ positive}{Number\ of\ subjects\ investigated} \times 100$

Table 1 Prevalence of urinary schistosomiasis by age and sex in Comboni JHS

	NUMBER EXAMINED			NUMBER WITH OVA			%PREVALENCE		
Age/yr	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
10-11	0	0	0	0	0	0	0	0	0
12-13	8	12	20	2	1	3	25	8.3	15.0
14-15	16	16	32	1	2	3	6.3	12.5	9.4
16-17	9	6	15	1	1	2	11.1	16.7	13.3
18-19	5	3	8	0	0	0	0	0	0.0
Total	38	37	75	4	4	8	10.5	10.8	10.7

RESULTS

Total Prevalence

The total prevalence of urinary schistosomiasis in the study area was 26%. Figure 5 below represents the percentage prevalence of schistosomiasis infection by ages and sexes in Comboni and Toklokpo Junior Secondary Schools. The prevalence rates of urinary schistosomiasis in the two schools were 12.6% and 13.3% for Comboni JHS and Toklokpo JHS respectively. The highest prevalence occurred in the males between 10-11 years age group.

Prevalence at Comboni and Toklokpo Junior Secondary Schools.

Figure 6 shows the percentage prevalence of urinary schistosomiasis in Comboni and Toklokpo Junior High Schools. For both sexes, Toklokpo Junior High School recorded a higher numerical prevalence of the disease. Males between 10-11 year group of Toklokpo Junior High School recorded the highest prevalence. There was at least one male positive in all the age groups for Toklokpo Junior High School. Though numerically, prevalence rate is higher in Toklokpo than Comboni, the difference is statistically insignificant. See tables below.

Table 2 Prevalence of urinary schistosomiasis by age and sex in Toklokpo J.H.S

	NUMBER EXAMINED			NUMBER WITH OVA			%PREVALENCE		
Age/yr	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**	**Male**	**Female**	**Total**
10-11	1	3	4	1	0	1	100.0	0.0	2.5
12-13	9	14	23	1	1	2	11.0	7.1	8.7
14-15	15	9	24	3	4	7	20.0	44.4	29.2
16-17	4	10	14	2	2	4	50.0	20.0	28.6
18-19	8	2	10	1	0	1	12.5	0	0.0
Total	37	38	75	8	7	15	21.6	18.4	20.7

Table 3 Paired Samples Test

Prevalence Rate	**Mean**	**t**	**Sig**
Pair 1 % male prevalence in Comboni - % female Prevalence in Comboni	0.98	0.24	0.83
Pair 2 % male prevalence at Toklokpo – female Prevalence at Toklokpo	24.40	1.2	0.31
Pair 3 % Total male prevalence in Both Schools – Total female Prevalence in both Schools	12.69	1.18	0.27

Table 4 Statistics

Prevalence rate	**Mean**	**Std. Dev**
% male in Comboni	8.48	0.35
% Female in Comboni7	.50	7.46
% Male in Toklokpo	38.70	37.71
% female in Toklokpo	14.30	18.70
Total % male in two schools	23.59	30.55
Total % female in two schools	10.90	13.90

FIG. A MAP OF SOUTH TONGU DISTRICT SHOWING THE LOCATION OF THE STUDY COMMUNITIES

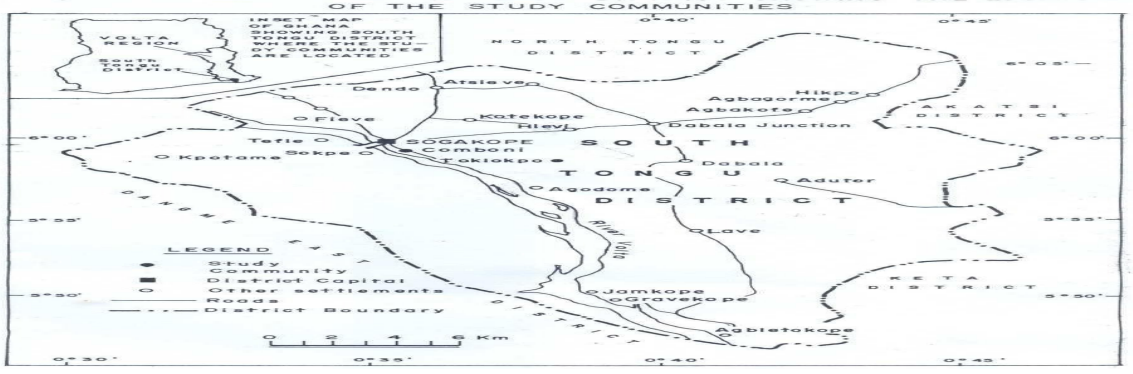
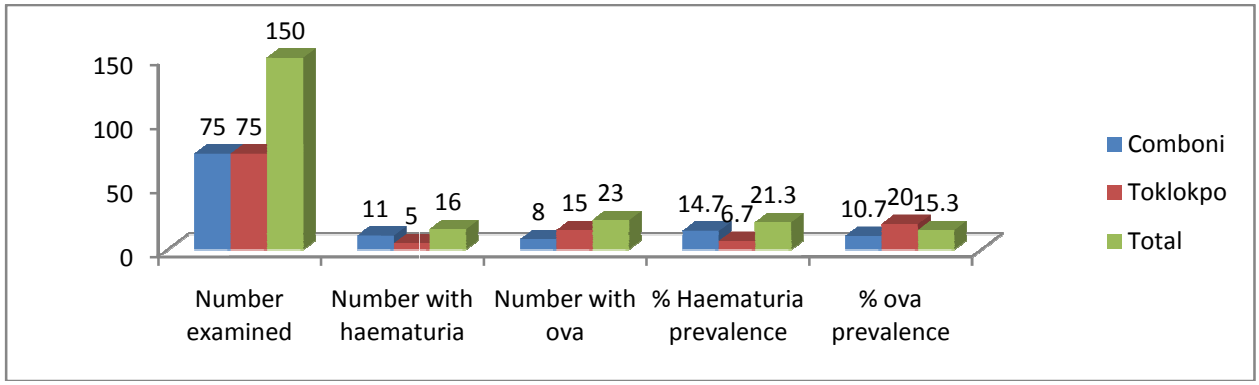


Fig 4 Study Communities



Generally there was a rise in infection in young ones followed by a decrease in older people for both sexes. Infections peaked in individuals aged 14-15 years. Infections found in the 11-15 years age group are similar to the findings of Adeoye (11) that the prevalence of infection reduced to lower levels is in conformity with other reports. In persons older than 19 years, the prevalence of egg counts slowly decline in populations living in endemic areas[2] .These declines in active infection may suggest that individuals have an increasing host immune response or decreasing exposure to contaminated water as they age

Fig. 5 Percentage prevalence of urinary schistosomiasis by age and sex in the Toklokpo and Comboni JHS

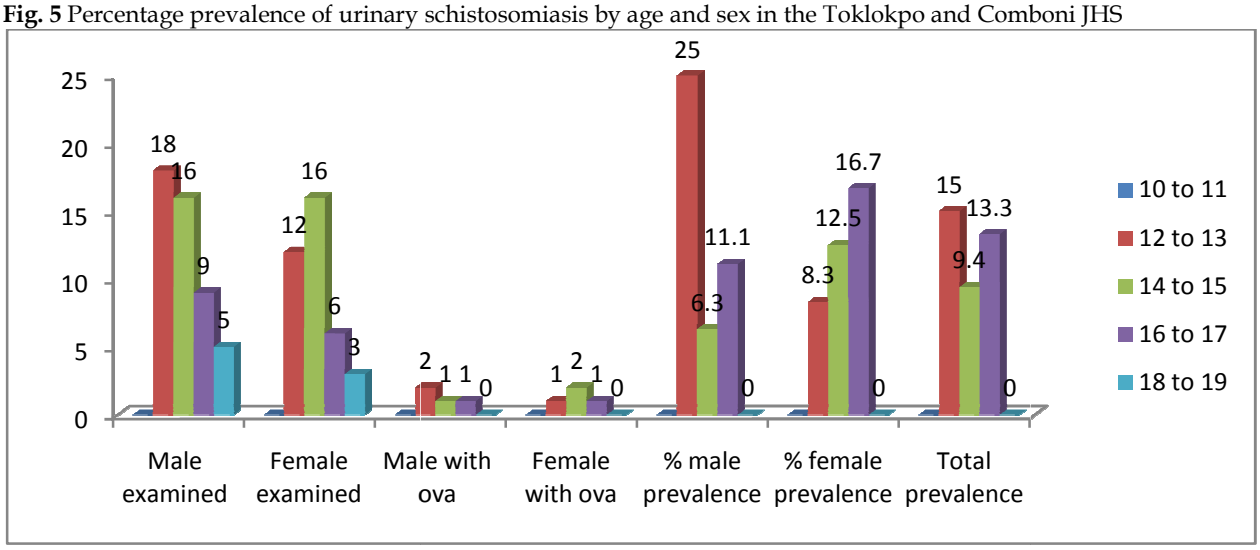


Fig. 5 Percentage prevalence of urinary schistosomiasis by age and sex in the Toklokpo and Comboni JHS

DISCUSSION

The numerical differences in prevalence rate of urinary schistosomiasis between the two schools could be attributed to the frequency of contact with infected water bodies which was higher at Toklokpo JHS than at Comboni JHS, as this is the only means by which the infection could be acquired. Activities of the communities include fishing, swimming, washing and bathing. Majority of these activities took place in the shallower portion of the water bodies usually referred to as the water contact site. As intermediate host snails of schistosomiasis are common at these sites, it is expected that cercarial densities would be higher here. It is also expected that communities that live closer to the water body would have a greater frequency of contact (10).

Numerical differences in prevalence exist between the various age groups in the individual community and the two communities pulled together as shown in Fig 6 but statistically no difference exists between the two communities in terms of means. This could be due to the fact that pupils with ages ranging from, 10-19 years would have different lifestyle relating to swimming, fishing, washing, bathing and fetching

and have different frequencies of contact with the water body.

The numerical prevalence was also higher in males than in females but statistically no difference exists between them. This augments the findings from the lgwun River basin in Bauchi in Anambra State and in the Republic of Mail which indicated that sex was not significant in the distribution of infection (11) Rather the diffrences could be due to variation in behaviour regarding water use and contact. Persons who have greater contact with the breeding foci have higher prevalence of the disease, irrespective of the sex of the individual. In contrast, observations in other parts of Nigeria and Zimbabwe revealed a higher prevalence and intensity of infection in males than females.The difference was attributed to difference in social habits (11). Cardinal among these was the higher tendency among males to swim, play and engage in other activities in the river and other water bodies, besides the domestic activity of washing and collection of water which expose both sexes to infection. He argued that the higher prevalence and intensity rates observed in males in most endemic areas are not due to sex *per se* but to the greater opportunities afforded to males for exposure and that when females assume typical male roles, their risk and prevalence of infection increases. Check Tables 1-4.

Urinary schistosomiasis has been recognized as an important public health problem in the Sogakope schools (Comboni and Toklokpo) and this call for active intervention. Therefore urgent control measures with emphasis on the regular surveillance and public health interventions, such as access to safe water, improved sanitation, immunizations, education, health communication and access to medical care with appropriate case management should be put in place by Government of Ghana and Donor agencies in order to help control the menace in school children. Village-based health workers should be used in providing free diagnosis and treatment through primary health facilities of the local government. There should be adequate health education for the Sogakope people on the disease, feasible control strategies as well as other preventive measures. This will increase their knowledge on schistosomiasis, consequently modifying their attitudes and behavioural practices related to urinary schistosomiasis transmission and other diseases and also, a mass chemotherapy involving every individual in these two schools as well as the surrounding communities should be carried out. And finally prevalence of intestinal schistosomiasis should also be determined in the two schools to reduce the infection rate in order to produce healthy people in the area for a sound mind is the healthy body.

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REFERENCES

1. World Health Organisation. The control of schistosomiasis; second report of the WHO Expert Committee, Geneva, WHO Technical report, 1998 Vol. 830

2. Hachman, A. Impact of *Schistosoma haematobium* infection and of praziquantel treatment on anemia of primary school children in Bertouna, Cameroon. Journal of Tropical Medicine and Hygiene, 1997; 96(4): 225-325

3. Rollin, S.G. Classification of Schistosomes. NIH publication No 99-4625. Bench Aid for the Diagnosis of Urinary Parasites. World Health Organization. Geneva, 1987.

4. Behrman, J. K. Schistosomiasis. Tropical Diseases paper No. 2 Progress in the prevention and control of schistosomiasis, 2006; 4 (3): 651-655.

5. Ross, A. G. Schistosomiasis Diseases Information. The UNICEF-UNDP-World Bank WHO Special Programme for Research and Training in Tropical Diseases. Geneva, 1992

6. Odai M.A. The effect of the Akosombo dams (at Akosombo Kpong) on the ecology of schistosomiasis, 1998

7. CDC. Some Preliminary observations on the distribution of bilharzias Schistosomiasis in U.S. Peace Corps volunteers-Malawi, 1993. 42: 565-570.

8. Stephenson, P. Relationship of Schistosoma haematobium, hookworm and malaria infections and metrifonate treatment to hemoglobin level in Kenyan school children. American Journal of Tropical Medicine and Hygiene 1986, 34(3): 519-528

9. Dazo, B.C. Two new field techniques for detection and counting of Schistosoma haematobium eggs in urine samples with an evaluation of both methods. Bulletin of the World Health Organization, 1974 P51: 399-408
10. Piekarski, G. In Medical Parasitology Springer-Verlag Berlin Berlin Heidelberg, 1989
11. Muller, R Webbe. In worms and Diseases, A manual of medical Helminthology, World Health Organisation, Genva1975, P52
12. Ferguson, W. Workshop on the role of human/water contact and shistosomiasis transmission and control. WHO Document TDR/SER/HWC/1979.3 World Heath Organisation, 1998.
13.Adeoye, W. The control of schistosomiasis. Methodology and interpretation of parasitological surveillance of urinary schistosomiasis, 1988; 86(4): 532-654.

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TOXOPLASMA ANTIBODIES AMONGST HIV/AIDS PATIENTS ATTENDING THE UNIVERSITY TEACHING HOSPITAL YAOUNDÉ, IN CAMEROON

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ABSTRACT

Toxoplasmosis is caused by an obligatory intracellular protozoon. It causes a wide range of diseases with toxoplasma encephalitis commonly encountered in HIV/AIDS patients. This work was carried out to determine the seroprevalence of toxoplasma antibodies (IgM and IgG) in HIV/AIDS patients attending the Yaoundé University Teaching Hospital (UTH) in Cameroon. Sera were collected from 133 HIV/AIDS patients at the out-patient department and the ELISA technique was employed serologically to determine toxoplasma antibodies. Of the 133 patients 83 (62.4%) were females and 59 (37.6%) were males; ninety three (69.9%) were positive for toxoplasma antibodies. Fourteen (10.8%) of the 93 of seropositive patients presented with both IgG and IgM-antibodies in their sera while fifty six (42.1%) and 8 (6.0%) were only sero-positive for toxoplasma IgG or IgM-antibody respectively

This rate of infection was not dependent on the patient's sex or age (X^2=11.49, P>0.05). The data provides enough evidence to conclude that 64.7% of the positive cases were due to reactivated infection.

Key words: Toxoplasmosis, HIV/AIDS, Pregnancy, Risk factors, Prevalence, Yaounde, Cameroon.

INTRODUCTION

Toxoplasmosis is caused by an obligatory intracellular protozoan; *Toxoplasma gondii* of worldwide distribution (1). It has gradually evolved over the pass decades to be among the most common opportunistic diseases in HIV/AIDS in developing countries (2). The prevalence of sero-positivity for antibodies against *Toxoplasma gondii* has been estimated at 3% in Western Europe and 67% to 90% in tropical countries. Transmission is mainly by ingestion of tissue cysts in raw and undercooked meat, exposure to oocysts in contaminated vegetable or contact with faeces of a felid (2).The development of a cell-mediated immunity after an acute infection results in the control of the disease, but not the eradication of the infection. The issuing of a chronic or latent phase of infection is characterized by the presence of the persistence form (cysts) of the organism in tissues (skeletal muscles, heart and brain). An infected individual who develops a defective cell-mediated immunity is at risk of reactivated infection (1, 2, 3). In Cameroon such data are scarce, and information used is usually from developed countries. This study was therefore carried out to determine the sero-prevalence of *T. gondii* among HIV/AIDS patients to help guide health care providers initiating treatment.

MATERIALS AND METHODS

Study Area

This study was carried out at the University Teaching Hospital in Yaoundé (UTHY), located in the Central Region of Cameroon. Yaoundé is the political capital of Cameroon; with a cosmopolitan population of 3,098,044 (~16%) inhabitants of a total population of 19,406,100 peoples. The town therefore attracts people from all regions of the country. The University Teaching Hospital (UTH) Yaoundé, where the study was conducted is one of the 4 reference hospitals in Yaoundé, which handles the diagnosis, treatment and management of many diseases including toxoplasmosis and HIV/AIDS.

Study Population and Sample Collection

This study was carried out from February 2010 to May, 2010. We used volunteer patients already diagnosed and confirmed to be HIV positive. Written informed consent forms were distributed to the participants one week prior to the beginning of the samples collection. Only HIV positive volunteers who returned their informed consent forms duly signed were recruited irrespective of their gender or clinical state of the disease. All age groups were recruited in the study. We collected at least 2ml of venous blood from each patient. The samples were then centrifuged after the blood had clotted to obtain serum that was collected into serum-tubes and stored in the freezer for at most three days. Collection was done every week day

from 8 am to 1pm and sample processing was done twice a week or once a batch reaches 9 samples

Sero-analysis

The ELISA technique was employed to estimate the levels of toxoplasma IgG and IgM antibodies in patients' sera collected. *Toxoplasma gondii* antibody enzyme ELISA kit is a solid phase enzyme-linked immune-sorbent assay (ELISA). The ELISA method relies basically on the formation of an antigen-antibody complex, on which an enzyme conjugate binds and then reacts with a substrate that is detectable by the ELISA machine. Therefore if a patient has toxoplasma antibodies, they will bind on the antigens coated on the walls of the micro-wells. The intensity of the reaction between a substrate and the conjugate enzyme is reflective of the degree of the formation of the antigen-antibody complex and hence, estimates qualitative and quantitative toxoplasma antibodies in the patient's serum. The procedure was carried out as described by Calderaro et al.(4)

Reporting Results

The ELISA gives results in terms of absorbance (optic density). The values of IgM and IgG are calculated using a calibration curve converted in the standard international units/ml (IU/ml).

For IgG values > 73IU/ml positive
= 60 to 73 IU/ml equivocal
< 60 IU/ml negative

For IgM values > 37 IU/ml positive
= 30-37 IU/ml Equivocal
< 30 IU/ml negative

Statistical Analyses

The Chi-Square test was used to test the association between patient characteristics of the sample population and *Toxoplasma gondii* sero-positivity data. A confidence level of 95% was chosen (P-value = 0.05) and values of chi-square computed using Yates correction.

collected.

RESULTS

A total of 133 immune compromised (HIV/AIDS) patients participated in this sero-survey. Ninety three (69.9%) of the patients were sero-positive for toxoplasma-antibodies and 40 (29.1%) were sero-negative. Fourteen (10.8%) of the 93 (69.9%) of the sero-positive patients presented with both IgG and IgM-antibodies in their sera. Fifty six (42.1%) and 8 (6.0%) were only sero-positive for toxoplasma IgG or IgM-antibodies respectively. Seven (5.2%) were sero-positive for IgG-antibodies and ambiguous in IgM titers, and 2 (1.5%) were only sero-positive for IgM-antibodies and ambiguous for toxoplasma IgG-antibodies. Three (2.3%) were serologically ambiguous for both toxoplasma IgG- and IgM titers. Two (1.5%) were serologically ambiguous for toxoplasma IgG-antibodies and negative for IgM while 1 (0.7%) was ambiguous for IgM and negative for IgG antibodies (Table 1).

From the 93 positive patient samples analyzed, 119 positive titers where obtained. 77 (64.7%) and 7 (5.9%) IgG titers were fully established and ambiguous respectively, and 24 (20.2%) and 11 (9.2%) IgM titers were fully established and ambiguous respectively. The data provided a P-value > 0.5 (table2).

Of the 133 HIV/AIDS patients that participated in the human sero-survey, 83 (62.4%) were females and 50 (37.6%) were males. Fifty nine (71.1%) of the 83 (62.4%) females and 34 (68.0) of the 50 (37.6%) males were sero-positive for Toxoplasma-antibodies (Table 3).

The age of the studied population ranged from 17 to 55 years of age. The data was categorized into 8 ranges with a class width of 4. The modal class was 26 – 30 years of age, with a frequency of 39 (29.3%) and an observed sero-positivity frequency of 32 (24.1%) (P<0.05) (Table 4).

TABLE 1: TOXOPLASMA ANTIBODIES IN HIV/AIDS PATIENTS AT THE UNIVERSITY HOSPITAL YAOUNDÉ

		TOXOPLASMA IgG ANTIBODIES			
		Positive No (%)	**Equivocal No (%)**	**Negative No (%)**	**Total No (%)**
Toxoplasma IgM Antibo dies	**Positive**	14 (10.52)	2 (1.50)	8 (6.01)	**24** (18.04)
	Equivocal	7(5.26)	3 (2.25)	1 (0.75)	**11** (8.27)
	Negative	56 (42.10)	2 (1.50)	40 (30.07)	**98** (73.78)
	Total	**77 (57.89)**	**7(5.26)**	**49 (36.84)**	**133 (100.00)**

TABLE 2: DISCRIMINATIVE DISTRIBUTION OF TOXOPLASMA IGM AND IGG TITERS IN HIV/AIDS PATIENTS

	Established positive No (%)	Equivoque No (%)	Total No (%)
IgG	77 (64.70)	7 (5.88)	**84** (70.58)
IgM	24 (20.16)	11 (9.24)	**35** (29.41)
Total	**101** (84.87)	**18** (15.12)	**119** (100)

Df = 1 P-value = 0.05 $X^2 = 9.81$

TABLE 3: DISTRIBUTION OF OXOPLASMA SERO-POSITIVITY ACCORDING TO SEX

Sex	Positivity No (%)	Negative No (%)	Total No (%)
Male	34 (25.56)	16 (12.03)	50 (37.59)
Female	59 (44.36)	24 (18.04)	83 (62.40)
Total	93 (69.92)	40 (30.07)	133 (100.00)

Df = 1 P-value = 0.05 $X^2 = 0.037$

TABLE 4: DISTRIBUTION OF TOXOPLASMA SERO-POSITIVITY ACCORDING TO VARIOUS AGE RANGES

Class (age)	Positive No (%)	Negative No (%)	Total No (%)
16 – 20	**1 (0.75)**	**4(3.01)**	**5(3.76)**
21 – 25	**23 (17.29)**	**14(10.53)**	**37(27.82)**
26 – 30	**32 (24.06)**	**7(5.26)**	**39(29.32)**
31 – 35	**17 (12.78)**	**6(4.51) ()**	**23(17.29)**
36 – 40	**13(9.77)**	**2(1.50)**	**15(11.28)**
41 – 45	**5 (3.76)**	**3(2.26)**	**8(6.02)**
46 – 50	**1(0.75)**	**4(3.01)**	**5(3.76)**
51 – 55	**1(0.75)**	**0 (0.00)**	**1(0.75)**
Total	**93 (69.92)**	**40(30.08)**	**133 (100.00)**

Df = 7 P-value = 0.05 $X^2 = 11.49$

DISCUSSION

The *T. gondii* seroprevalence estimated for human population varies greatly among different countries, among different geographical areas within the same country, and among different ethnic groups living in the same area (5). In this study the prevalence of *Toxoplasma gondii* antibodies in an HIV-positive population at the UTHC Yaoundé was found to be 69.9%. This figure does not differ much from that obtained in other African countries : 75.4% in Nigeria (6), 60% from AIDS patients in Cote d'Ivoire (7), 58.4% in Tunisia (8), 53.6% in Benin (9), 40.2% in Senegal (10), 34.1% from pregnant women in Sudan (11), and 28.5% in HIV positive women in Benin (5) and confirms the geographical variation. The absence of prevention strategies on serious risks of acquiring primary infection during pregnancy in these countries may account for these important prevalence values.

In Cameroon, a similar study carried in 1992 (12), revealed a toxoplasmosis sero-prevalence of 77.1% in pregnant women. The difference in the sero-prevalence of toxoplasmosis obtained in this study can be associated to the fact that in some HIV/AIDS patients, the failing immune system may not produce detectable amounts of antibodies, hence may account for a low sero-prevalence in the sample. Recently, highly active antiretroviral therapy (HAART) against HIV infection has been advocated for improving the immune status of patients thus reducing the incidence of opportunistic infections (13); The studied population was on antiretroviral treatment regimens thank to the implementation of a national decentralization programme for HIV care in 2006 that led to existing health infrastructures being overwhelmed by a huge demand for treatment (14). This could explain the fact that most patients with antibodies presented with no clinical symptoms of the disease. However non-adherence remains a major concern as the antiretroviral treatment (ART) programs scale up and as more patients are expected to remain on this life-long therapy, this necessitates the need for the development of additional interventions to maintain optimal adherence to ART and consequently to reduce patients' vulnerability to opportunistic infections (15). The particular clonal lineage may be different compared to the rest of the continent and the world. However, studies by Lindström et al. (16) described the genotype distribution in Uganda to be very similar to that in Europe, where type II allele of SAG2-locus was the most common. As compared to other areas (Uganda where the prevalence was 80%), this area presents a better socio-economic condition, rapid urbanization, improved access to potable water and sanitation facilities though constant breakdowns from time to

time, continued awareness around good hygiene, education and rare exposure to stray cats are characteristics of the Yaoundé region. The global variation in prevalence may also be attributed to different cultural habits. The high prevalence has been linked to the practice of Buddhism in Thailand which has significantly led to the increase in amount of stray cats, hence increased possibility of human infection (13). In Mexico and Brazil, cats are fed with raw viscera and leftover, increasing the chance of human infection (17). A 64.7% IgG to 20.2% IgM titer provide enough statistical evidence to conclude that there is a 95% possibility of any random patient of presenting with at least a high IgG titer for toxoplasma antibodies. This can be surely attached to their failing immune system increasing the patient's vulnerability and the fact that IgG-antibodies last longer than IgM-antibodies. It is therefore possible most of the toxoplasmosis cases in HIV/AIDS are due to a reactivated infection (18). A 3/5 ratio of male to females ($p>0.05$) did not provide enough statistic evidence that positivity of toxoplasma antibodies is not sex dependent. Hence, any random gender with equal risk behaviors can be positive or negative. The modal class in this study was within the range of 26 to 30 years of age and could be due to the fact that this age group constitute the most active part of a population and are therefore exposed to many risk factors. However, the statistics did not provide enough evidence to incriminate a particular HIV/AIDS age group to positivity with toxoplasma antibodies.

CONCLUSION

This human sero-survey has shown the presence of toxoplasmosis amongst HIV/AIDS patients at UTHCY, with a prevalence of 69.9%. This infection rate does not depend on the patient's sex or age. The data also provide enough evidence to conclude that 64.7% of the positive cases are due to reactivated infections. Pregnant women and immune-compromised patients should be screened for previous *Toxoplasma gondii* exposure to ensure adequate clinical and individual precautions to prevent an acute or a reactivated infection.

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REFERENCES

1 Tenter A. M., Heckeroth A. R., Weiss L. M. *Toxoplasma gondii*: from animals to humans. Intern. J. Parasitol. 2000; **30**: 1217-1258.

2. Montoya J. G., Rosso F. Diagnosis and management of toxoplasmosis. Clin Perinatol. 2000; **32**: 705-726.

3. Luft B. J., Remington J. S. Toxoplasmic encephalitis in AIDS. Cli Infect Dis. 1992; **15**: 211-222.

4. Calderaro A, Piccolo G, Peruzzi S, Gorrini C, Chezzi C and Dettori G. Evaluation of Toxoplasma gondii Immunoglobulin G (IgG) and IgM Assays Incorporating the New Vidia Analyzer System. Clin. Vacc. Immunol, July 2008, p. 1076-1079, Vol. 15, No. 7

5. Simpore J., Savadogo A., Ilboudo D., Nadambega M. C., Esposito M., Yara J., Pignatelli S., Pietra V., Musumeci S. Toxoplasma gondii, HCV, and HBV seroprevalence and co-infection among HIV-positive and - negative pregnant women in Burkina Faso. J Med Virol. 2006; 78:730–733.

6. Onadeko M. O., Joynson D. H., Payne R. A, Francis J. The prevalence of Toxoplasma antibodies in pregnant Nigerian women and the occurrence of stillbirth and congenital malformation. Afr. J. Med. Sci.1996; 25:331–334.

7. Adou-Bryn K. D, Ouhon J., Nemer J., Yapo C. G., Assoumou A. Serological survey of acquired toxoplasmosis in women of childbearing age in Yopougon (Abidjan, Cote d'Ivoire). Bull. Soc. Pathol. Exot. 2004; 97:345–348.

8. Bouratbine A., Siala E., Chahed M. K., Aoun K., Ben Ismail R. Seroepidemiologic profile of toxoplasmosis in Northern Tunisia. Parasite Immunol. 2001; 8:61–66.

9. Rodier M. H., Berthonneau J., Bourgoin A., Giraudeau G., Agius G., Burucoa C., Kekpazo A., Jacquemin J. L. Sero-prevalences of toxoplasma, malaria, rubella, cytomegalovirus, HIV and treponemal infections among pregnant women in Cotonou. Repub. Benin. Acta Trop. 1995; 59:271-277.

10. Faye O., Leye A., Dieng Y., Richard-Lenoble D., Diallo S. Toxoplasmosis in Dakar. Seroepidemiologic sampling of 353 women of reproductive age. Bull. Soc. Pathol. Exot. 1998; 91:249–250. 11. Elnahas A., Gerais A. S, Elbashir M.I, Eldien E. S., Adam I. Toxoplasmosis in pregnant Sudanese women. Saudi. Med. J. 2003; 24: 868–870.

12. Ndumbe P. M., Andela A., Nkemnkeng-Asong J., Watonsi E., Nyambi P. Prevalence of infections affecting the child among pregnant women in Yaoundé, Cameroon. Med. Microbiol. Immunol. 1992; **181**:127-130.
13. Sukthana Y., Chintana T., Supathanapong W., Siripanth C., Lekkla A., Chiabchalard R. Prevalence of toxoplasmosis in Selected Populations in Thailand. J Trop Med Parasitol 2000;23:53-58
14. MOH (Ministry of Public Health). National strategy plan to fight against HIV/AIDS. National Committee to FightAIDS, Cameroon. 2006-2010,Yaounde, Cameroon. 2009
15. Kamga H. L. F., Assob J. C. N., Njunda A. L, Nsagha D. S., Nde Fon P., Atanga M. B. S., Weledji P., Achidi E. A. Puinta D. P. The kidney function trends in HIV/AIDS patients. Lambert Academic Publishing, Germany. 2011
16. Lindström I., Kaddu-Mulindwa D., Kironde F., Lindh J. Prevalence of latent and reactivated Toxoplasma gondii parasites in HIV-patients from Uganda. Acta Trop. 2006; 100: 218-222.
17. Sukthana, Y. Toxoplasmosis: beyond animals to humans. Trends Parasitol., 2006; 22:137-142
18. Rougemont M., Stoll B. E, Elia N., and Ngang P. Antiretroviral treatment and its determinants in sub-Saharan Africa: a prospective study at the Yaounde Central Hospital, Cameroon. AIDS Res. Ther. 2009; 6:21.

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IN-VITRO EFFICACY OF ANTIMICROBIAL AGENTS USED IN THE TREATMENT OF BACTERIAL EYE INFECTIONS IN IBADAN, NIGERIA

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Running Title: *In-vitro* efficacy of antimicrobial agents in Ibadan, Nigeria

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Abstract
Failure to cure eye infections, and reduced potency in topical antimicrobials had been observed in South Western Nigeria, this study sought to evaluate *in vitro*, the efficacy of antimicrobial agents in the treatment of ocular infections. A total of 46 ocular bacterial isolates were recovered from the diagnostic laboratory of the University College Hospital, Ibadan, from conjunctival swabs of patients having underlying eye diseases (Cataracts, glaucoma and esotrapia), and from patients presenting with other symptoms of eye infections. The pathogens incriminated were *Staphylococcus aureus* (73.5%), Coagulase negative *Staphylococci* (13.3%), *Klebsiella* species (10.3%), and *Pseudomonas aeruginosa* (2.0%). Disc diffusion tests (Bauer-Kirby method) were carried out using ciprofloxacin, gentamicin, chloramphenicol, erythromycin, augmentin, cefuroxime and levofloxacin. Broth dilution techniques were thereafter performed using gentamicin, chloramphenicol and ciprofloxacin. The microlide- erythromycin was 63.0% efficacious, augmentin and cefuroxime showed 71.1% and 76% efficacy. Minimum inhibitory concentrations (MIC) of commonly used topical antibiotics however showed different levels of resistance. Resistance to the aminoglycosides was marked, yielding 53.4% with MIC50= 8, MIC90 > 256, Resistance to chloramphenicol was even more marked 69.6% with MIC50= 16, MIC90= 64, the fluoroquinolones showed high efficacy- levofloxacin and ciprofloxacin showed 93.4% and 82.6% susceptibility respectively with MIC50 < 0.5, though slightly demonstrable resistance was observed (MIC90= 8). The study thus recommends the discontinuation of empirical therapy by physicians in order to stem the tide of resistance; it justifies the inclusion of the fluoroquinolones in susceptibility testing of ocular bacterial isolates, and its first line of choice if cure is warranted.

Introduction

Ocular bacterial flora includes *Corynebacterium xeroisis, Moraxella catarrhalis,* and *Staphylococcus epidermidis* (1). The coagulase negative *staphylococci,* a subject of debate in the 1980s, regarding its role in pathogenicity, are remarkable for its opportunism. It has thus been incriminated in chronic blepharitis (2), corneal ulcers, and endophthalmitis after traumatic eye surgery (3). Globally, *S. aureus* is the leading cause of conjunctivitis (3). The incidence of methicillin resistant *Staphylococcus aureus* (MRSA) in ocular infections is on the rising side (4). In Onitsha, Nigeria, *S. aureus* is the leading cause of conjunctivitis and keratitis (5). *Pseudomonas aeruginosa* is also leading causes of corneal ulcers especially among contact lens wearers (6). *Neisseria gonorrheae* and *Chlamydia trachomatis* cause severe conjunctivitis in the newborn - ophthalmia neonatorum (7). *Haemophilus species, Streptococcus pneumoniae* (8) and non fermenting *coliforms* have been implicated in ocular infections (6).

The aminoglycosides gentamicin and tobramycin are well established as first-line therapy for external ocular infections, and possess a broad spectrum of activity against Gram positive and Gram negative organisms (9,10). However, resistance to these antibiotics has been reported. For example, resistance to topical aminoglycoside therapy may be encountered in as many as 8% to 10% of ulcerative keratitis cases caused by *Pseudomonas aeruginosa* (11). Resistance appears to be even greater in ocular infections caused by Gram positive organisms (12). The *in-vitro* studies of antibacterial susceptibility tests by various authors have shown increasing resistance of commonly used antibacterials; gentamicin (21%) (4,13), chloramphenicol, though potent against MRSA strains, has just been demonstrated to show significant reduction in its bacteriostatic action in Europe (14.1%), (14). However, ciprofloxacin, which is still comparatively the most efficacious, has also shown reduced potency; resistance at 35% was shown in Pittsburg, USA (15), ciprofloxacin has also shown reduced potency against ocular MRSA isolates in the United States (16).

These differing levels of resistance impel an evaluation of these drugs in Nigeria in order to ascertain their efficacy and have a documented level of susceptibility to these agents. The study was therefore aimed at evaluating *in-vitro* susceptibility patterns of ocular clinical isolates to commonly used antibiotics, with emphasis on gentamicin, chloramphenicol and ciprofloxacin due to the availability of their topical applications (eye drops).

Materials and Methods

Bacterial strains

A total of 46 bacterial isolates were isolated by standard procedures (17) from 136 eye swabs and scrapings sent to the diagnostic laboratory of Medical Microbiology and Parasitology department, University College Hospital, Ibadan from January to October 2009.

Disc susceptibility testing

Varying concentrations of antibiotics discs; gentamicin (10 µg), ciprofloxacin (10 µg), methicillin (5 µg), chloramphenicol (10 µg), erythromycin (5 µg), ampicillin (10 µg), cloxacillin (5µg), cefuroxime (30 µg), augmentin (10 µg), and levofloxacin (10 µg) were used. Inhibition zone diameters around the discs were measured to the nearest millimetre using a calibrated transparent ruler. The susceptible inhibition zone diameter break point used throughout the study for each antibiotic to the various organisms was based on CLSI recommendation (18). The diameters of the zone of inhibition were recorded. Growth within the zone of inhibition was recorded as resistant (18). Sensitivity patterns for *Staphylococcus*, *Pseudomonas* and *Klebsiella* were compared with the standard *S. aureus* ATCC 29213; *P. aeruginosa* NCTC 10662 and *E. coli* NCTC 10418, respectively.

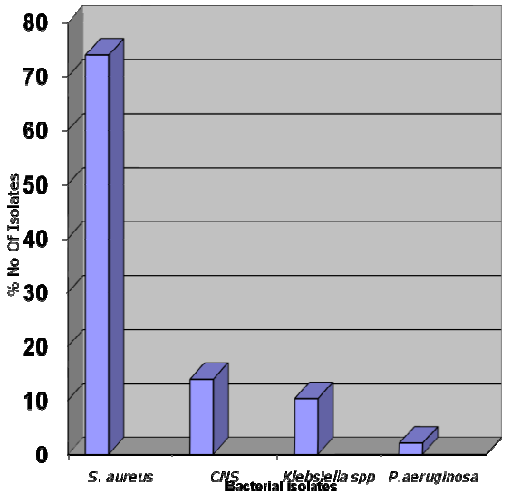
Minimum inhibitory concentration

The minimum inhibitory concentration (MIC) of 3 antibiotics; gentamicin, chloramphenicol and ciprofloxacin for all the bacterial strains was determined as described by Goldstein and Acar (19). The antibiotics were supplied in powdery formulations, namely; gentamicin, chloramphenicol and ciprofloxacin by SIGMA-ALDRICH, U.K. Serial doubling dilutions of these antibiotics were made, ranging from 0.0625 to 512 µg/ml. A drop (0.02 ml) of standard inoculum (0.5 Macfarland) of organisms was introduced and these were then incubated at 37˚C for 18 hours. MIC was interpreted as the least concentration or highest dilution with no observable turbidity. Controls were set up namely; sterility control: Mueller Hinton broth only, viability control: Mueller Hinton broth and test organism, positive control: Mueller Hinton broth with antibiotics and the control organisms. They were incubated at 37ºC overnight.

Result

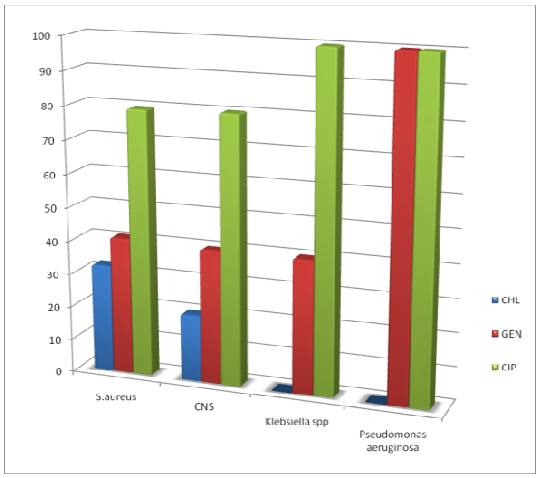
Bacteria isolates recovered were *Staphylococcus aureus* 34 (73.9%), coagulase negative *staphylococci* (CNS) 6 (13%), *Klebsiella* species 5 (10.8%) and *Pseudomonas aeruginosa* 1 (2.2%). The distribution of the various isolates with underlying eye conditions, and ocular infections are shown in Figure 1.

Figure 1: Percentage (%) distribution of conjunctival bacterial isolates.



All the strains examined showed resistance to one or more of the eight antibiotics used for this study. The results depicted a high level resistance. The fluoroquinolones showed slightly lower level of resistance than the rest of the antibiotics including the third generation cephalosporins. More isolates were sensitive to levofloxacin (93.4%) and ciprofloxacin (76%) than all the antibiotics tested (Table 1). Similarly, susceptibility of the strains to methicillin was 10.4%; 30.4% for chloramphenicol while gentamicin had 41.3% (Figure 2). It is noteworthy that chloramphenicol had no activity against *Klebsiella* species and *Pseudomonas aeruginosa* in this study.

Figure 2: Percentage susceptibility to chloramphenicol, gentamicin and ciprofloxacin (disk diffusion test).



The MIC results also showed that the level of resistance to many antibiotics was high. MIC50 and MIC90 of ciprofloxacin were lower than the rest of the antibiotics. MIC_{90} of gentamicin to the strains was very high with value >256 µg/ml (Table 2).

Discussion

The study showed *S. aureus* as the most frequently incriminated conjunctival pathogen, this is consistent with previous studies in Nigeria (20), and outside the country (21). Methicillin-resistant *S. aureus* (MRSA) and Methicillin resistant coagulase negative *staphylococci* (MRCNS) are intraocular pathogens. They all however have a common source; the anterior nares (22), which via the nasolacrimal duct, may reach the conjunctiva or infect deeper ocular structures; endophthalmitis may ensue (23). The isolation of MRSA from patients having cataracts and glaucoma (92.3%) is therefore alarming. The definitions of these conditions do not presuppose a microbial cause. Their incrimination may thus imply the likelihood of subsequent infections that further destroy ocular tissues, and increased susceptibility of these patients to ocular infections. Ultimately, if it be ascertained that these pathogens are simply microflora, it should be alarming from a general epidemiological perspective of resistance, as well as the serious dangers posed these patients if a surgery would be carried out to correct these conditions.

Table 1: Susceptibility patterns of bacterial pathogens using disc diffusion

Organism	MET			CHL			GEN			CIP			ERY			AUG			LEV			CXM		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
S. aureus (34)	3	0	31	11	0	23	14	2	18	26	2	6	24	0	10	27	0	7	31	0	3	30	0	4
CNS (5)	2	0	3	1	0	4	2	0	3	4	0	1	3	0	2	3	0	2	5	0	0	3	0	2
Klebsiella spp (5)	0	0	5	0	0	5	2	0	3	4	0	1	2	0	3	2	0	3	5	0	0	2	0	3
P. aeruginosa (1)	0	0	1	0	0	1	1	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0	0	1

MET- methicillin, CHL- chloramphenicol, GEN- gentamicin, CIP- ciprofloxacin, ERY-erythromycin, AUG- augmentin, LEV- levofloxacin, CXM- cefuroxime, S- susceptible, I- intermediate, R- resistant.

Table 2: Minimum inhibitory concentrations of common antimicrobials to the pathogens

Organism	Antimicrobial Agent	MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	Range (µg/ml)
MRSA (30)	Chloramphenicol	24	64	0.25 – 256
	Gentamicin	8	> 256	0.25 – 256
	Ciprofloxacin	0.25	8	0.25 – 256
MSSA (3)	Chloramphenicol	16	32	0.25 – 256
	Gentamicin	4	> 256	0.25 – 256
	Ciprofloxacin	0.25	8	0.25 – 256
Klebsiella spp (5)	Chloramphenicol	8	16	0.25 – 256
	Gentamicin	64	> 256	0.25 – 256
	Ciprofloxacin	2	8	0.25 – 256
P. aeruginosa (1)	Chloramphenicol	-	64	0.25 – 256
	Gentamicin	-	64	0.25 – 256
	Ciprofloxacin	-	4	0.25 – 256

The coagulase negative staphylococci generate fewer controversies on its pathogenicity these days. They have been incriminated in chronic blepharits (2), Keratitis (24), and endophthalmitis (22). In Nigeria, the pathogenicity of the CNS has been established and is incriminated in various disease conditions; Ogbolu *et al.* thus advocate processing when isolated from repeated cultures (25). Their incrimination and inclusion in this study is thus not surprising, and their resistance patterns justify the discourse, which further show that resistance mechanisms are equally been evoked by these organisms, thus increasing their endemic status and enhancing their pathogenicity, especially when they reach deeper structures.

Most ocular isolates showed resistance using disc diffusion techniques to gentamicin (54.6%) but more marked levels of resistance was demonstrated (MIC_{50} = 32, MIC_{90} >256 µg/ml) by broth dilution technique. Resistance to Gram negative rods and *Staphylococcus aureus* including MRSA strains was demonstrated. Third generation fluoroquinolone - levofloxacin was most efficacious using the disc diffusion test technique (93.4%). The efficacy of this drug buttresses the reliability of the fluoroquinolones against conjunctival bacterial pathogens, especially the MRSA. Ciprofloxacin also had demonstrable clinical efficacy using both techniques. Susceptibility of MRSA strains was 83% using disc diffusion test, confirmed by broth dilution techniques (MIC_{50} < 0.5). Resistance was however also demonstrated, with MIC_{90} = 8.0 µg/ml. These results are in sharp variance with Kotlus *et al*'s observation in the United States, where 94% resistance to ciprofloxacin was observed, with MIC_{50} = 8.0 µg/ml, in the study, gentamicin was most efficacious (16). The efficacy of ciprofloxacin has also been demonstrated in Nigeria, this conforms to the study of Idu *et al.* where it was found to be most potent among other topical fluoroquinolones (20).

Abuse of fluoroquinolones in the U.S may have led to the potent drug, whereas it's comparatively new status in Nigeria still ensures its potency.

On definitive prophylaxis pre-ocular surgery, this study may be quick to recommend the use of ciprofloxacin in topical formulations in Nigeria. However, discouragement of empirical therapy for the treatment of ocular infections is essential considering the increasing levels of resistance to commonly used antibiotics as has been shown in this study. The study affirms the high levels of the superbug MRSA among these species in eye infections in Nigeria, having varying resistance patterns. It also quantitatively validates reports of significantly reduced potency to commonly used topical antimicrobials - gentamicin and chloramphenicol. It high rate of resistance to this 1 however shows the fluoroquinolones as unparalleled in the treatment of ocular infections. We therefore advocate for discontinuation of empirical therapy by physicians, and advise isolation of the causative bacterial agents, and subsequent susceptibility testing which should include the fluoroquinolones. The inability to isolate typically fastidious ocular pathogens like *Streptococcus pneumoniae, Haemophilus influenzae,* and *Haemophilus aegypticus,* should impel a clamour for the use of transport swabs in routine diagnosis of eye infections. Periodic re-evaluation of antimicrobial agents is essential in order to guide therapy, as well as to track and monitor resistance by organisms in this sensitive organ of vision.

REFERENCES

1. Hentges DJ (1993): The anaerobic microflora of t he human body, *Clinical Infectious Diseases*. 16(Suppl4): S175.
2. McGulley JP, Dougharty JM, Deneam D (1982): Classification of chronic blepharitis. *Ophthalmology* **89:**1173-1175.
3. Mozayemi RM, Lam S (1996): Phyctenular keraconjunctivitis and marginal *Staphylococcal* Keratitis. *Indian Journal of Ophthalmology*.1392-1395.
4. Fukuda M, Ohashi H, Matsumoto C, Mishima S, Shimomura Y (2002): Methicillin-resistant *Staphylococcus aureus* and methicillin resistant coagulase-negative Staphylococcus ocular surface infection efficacy of chloramphenicol eye drops. *Cornea*. 21 (Suppl): S86–S89.
5. Asonye CC, Ezelum C (2003): Bacterial aetiology of eye infections in Onitsha, Anambra, Nigeria. *National Journal of Science.* **(2):** 42-50
6. Houang E (2001): Microbial Keratitis in Hong Kong: Relationship to climate, environment and contact lens disinfection. *Transactions of the Royal Society of Tropical Medicine and Hygiene*. 95: 361-367.
7. Nagayama A, Nakao T, Taen H. In vitro activities of ofloxacin and four other new quinoline-carboxylic acids against Chlamydia trachomatis. *Antimicrob Agents Chemother* 1988; 32: 1735-7.
8. Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. In-vitro efficacy of antibacterials against bacterial isolates from corneal ulcers. *Indian J Ophthalmol*. 50: 109-14
9. Jarudi N, Golden B, Hoyme J, Tyson MD, Harter JG. Comparison of antibiotic therapy in presumptive bacterial conjunctivitis. *Am J Ophthalmol* 1975; 79: 790-4.
10. Cagle GD, Abshire RL. Quantitative ocular bacteriology: a method for the enumeration and identification of bacteria from the skin-lash margin and conjunctiva. *Invest Ophthalmol Vis Sci* 1981; 20: 751-7.
11. Gelender H, Rettich C. Gentamicin-resistant *Pseudomonas aeruginosa* corneal ulcers. *Cornea* 1984; 3: 21-6.
12. Mehta NJ, Webb RM, Krohel GB, Smith RS. Clinical inefficacy of tobramycin and gentamicin sulfate in the treatment of ocular infections. *Cornea* 1984; 3: 228.
13. Arlene G (1992): Topical ofloxacin compared with gentamicin in the treatment of external ocular infections. *British Journal of Ophthalmology*. **76(12):** 714-718.
14. Morrissey I, Burnett R, Viljoen L, Robbins M (2002): Surveillance of the susceptibility of ocular bacterial pathogens to the fluoroquinolones, vancomycin and gatifloxacin in Europe during 2001-2002. *Journal of Infections* **49:** 109-114.
15. Goldstein MH, Kowalski RP, Gordon YJ (1999): Emerging fluoroquinolone resistance in bacterial keratitis. *Ophthalmology*. **206:** 1313–1318.
16. Kotlus B, Wymbs R, Vellozi E, Udell I (2003*): In vitro* activity of Fluoroquinolones, Vancomycin, and Gentamicin against Methicillin Resistant *Staphylococcus aureus* Ocular isolates. *American Journal of Ophthalmology*. 95(**4):** 361-367E.
17. Barrow GI, Feltham RKA. Characters of Gram-negative bacteria. In: Cowan and Steel Manual for Identification of Medical Bacteria. 3rd edition, Cambridge University Press, 1993; 94-149.
18. Clinical and Laboratory Standard Institute, CLSI (2007): Performance standards for antimicrobial susceptibility testing; seventh informational supplement M100-S17 Vol. 27 No. 1.
19. Goldstein FW, Acar JF (1995): Epidemiology of quinolones resistance: Europe and North and South America. *Drugs* (suppl 2): 36-42.
20. Idu FK, Odjimogho SE (2003): Susceptibility of conjunctival bacterial pathogens to fluoroquinolones: A comparative study of ciprofloxacin, norfloxacin and ofloxacin. *Online Journal of Health Allied Sciences*; 2: 1
21. Baum JL (1997): Bacterial conjunctivitis: Diagnosis and Treatment. *APUA Newsletter.* **15(4):** 1, 4-5, 8.
22. Tsuyoshi K, Seiji H (1998): Methicillin Resistant *Staphylococcus aureus* and Methicillin Resistant

Coagulase Negative *Staphylococcus* from conjunctiva from pre-operative patients. *Japan Journal of Ophthalmology*. **42:** 461-465.

23. Scott IU, Flynn HW Jr, Feuer W (1995): Endophthalmitis after secondary intraocular lens implantation. A case-control study. *Ophthalmology* . **102:**1925–31.

24. Khosavri AD, Mehdinejad M, Heidari M (2007): Bacteriologic findings in patients with ocular infections and antibiotic susceptibility pattern of isolated pathogens. *Singapore Medical Journal* **48(8):** 741-745.

25. Ogbolu DO, Daini OA, Alli OAT, Adesina OA, Odekanmi AA, Okanlawon BM, Olusoga-Ogbolu FF, Oni AA (2009): Coagulase Negative *Staphylococci* distribution in clinical samples in a tertiary hospital in Ibadan, Nigeria. *Nigerian Journal on Health and Biomedical Sciences*. 8(1): 36-40

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ORAL *CANDIDIASIS* AMONGST CANCER PATIENTS AT QODS HOSPITALS IN SANANDAJ

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ABSTRACT

Background: Within the past two decades, *Candida* species have emerged as major human pathogens and are currently the fourth most common cause of nosocomial infection. Propose of this study was to determine the occurrence of oral Candidiasis among cancer patients at Qods hospitals in Sanandaj.

Materials and Methods: Sixty cancer patients were examined for oral candidiasis. For all patients, the clinical diagnosis had to be confirmed microbiologically by the presence of yeasts and / or hyphae or pseudohyphae on potassium hydroxide–treated smears of oral swabs. Oral samples were obtained and cultured on Sabouraud's dextrose agar and CHROMagar.

Results: 25 out of the 60 patients (41.7%) were males and 35 (58.3%) were females ranging in age from 15 to 79 years. Gastro-intestinal cancer and Breast cancer were the most frequent cancer in the studied group, accounting for 65 % and 18.4 % respectively. The mean weight of the patients was 52.67 Kg (range, 38– 80 Kg). Similarly, the mean of hospital stay was 3.58 days (range; 1-9 days).

From these patients, 19 Candida spp were isolated; *C. albicans* alone outnumbered other species and accounted for 73.68% episodes of trash.

For *C. albicans* isolates, the MIC values ranges from 1 to 9 µ g / ml µg / ml for polyenes and from 0.03 to 16 µ g / ml for the azole antifungals. All the *Candida albicans* had closely related MFCs values.

Conclusion: In conclusions, the finding of our study strongly suggest that oral candidiasis is a frequent complication among cancer patients, being *C. albicans* the main etiological agent.

Keywords: Cancer, Oral candidiasis, *Candida albicans*, Antifungal agents

INTRODUCTION

The ubiquitous *candida spp* are important cause of oropharyngeal candidiasis and nosocomial infections including life threatening infections among cancer patients. Indeed oropharyngeal candidiasis is a common infection in cancer patients and ranks as the most common fungal diseases [1]. Among *candida spp*, the most frequently encountered clinical problem is caused by *C. albicans* [2]. Actually many people are colonized by *Candida spp* as a commensal organism. For this reason, cancer patients must be strictly monitored for the clinical presence of yeast.

The use of broad – spectrum antibiotics, steroids, or other immunosuppressive agents, diabetes mellitus, cancer patients and organ transplantation can increase the risk for candidal infections [3]. The prevalence of oral candidiasis in various countries varies among studies according to location, age of the patients, and the site sample, and has been reported to range from 20- 75% [4]. The incidence of Candida spp isolated from the oral cavity has been reported to be 45 % - 65% in children, 50-65% in people with removable dentures, 65-88% in those residing in acute and long term care facilities, 90% in patients with acute leukemia, 95% with HIV [4-6]. Among cancer patients, infection can spread through the bloodstream, leading to sever infection with significant morbidity and mortality [7]. A routine oral examination of cancer patients has revealed a greater incidence of Candida infections than that in most types of patients. Almost all surveys on fungal infections in cancer patients come from USA, Europe, and other developed countries, and little is known about this problem in developing countries particularly Iran. For the first time we report on occurrence rate of oral *Candidiasis* among cancer patients at Qods hospitals in Sanandaj and in-vitro susceptibility to antifungal agents were also determined.

MATERIALS AND METHODS

This study was conducted over a period of 16 months at Qods hospital of the Kurdistan University of Medical Sciences. Patients who had developed oral thrush and were treated at the department of medical oncology were eligible for the study.

All the patients had different types of cancers and after taking the sample, had received different types of chemotherapy / radiotherapy prescribed by attending physicians. Oral candidiasis was clinically diagnosed by investigator. The clinical diagnosis was based on lesions clinically recognized as creamy, whitish, curd-like plaques or pseudomembranes involving the oropharyngeal mucosa and the tongue. For all patients, the clinical diagnosis had to be confirmed microbiologically by the presence of yeasts and / or hyphae or pseudohyphae on potassium hydroxide–treated smears of oral swabs. Swab was also used for yeast cultures on plates with Sabouraud dextrose agar. Cultures were considered positive if ≥10 CFU appeared on the plate. Candida spp were identified by classical methods [8]. The differential medium Chromagar Candida was used to confirm the results by colony morphology and pigmentation according to the manufacture's instructions.

Minimum Inhibition Concentration (MIC) was determined by serial broth dilution method [9]. Briefly, a serial dilution was made from the stock solution of the antifungal agents to have the final concentration ranges from 0,03 to 16 µg / ml for amphotericin B, Ketoconazole and miconazole; 0.125 to 64 µg / ml for fluconazole, and 0.7 to 18.5 µg / ml for nystatin.

The prepared inocula of *Candida spp* were incubated with different antifungal concentration at 30^0C.

Aliquot from each isolate showing inhibition was inoculated on the surface of SDA plate and incubated at 30^0C for 24 – 48 hours to determine the MFC of the respective antifungal agent.

RESULTS

During a 16 months period (March 2009 to September 2010), 60 patients from Qods hospital in Sanandaj were analyzed for oral *Candidiasis* among cancer patients. Twenty five out of the 60 patients (41.7%) were males and 35 (58.3%) were females ranging in age from 15 years to 79 years.

Gastro-intestinal cancer and Breast cancer were the most frequent cancer in the studied group, accounting for 65 % and 18.4 % respectively. The mean weight of the patients was 52.67 Kg (range, 38– 80 Kg). Similarly, the mean of hospital stay was 3.58 days (range; 1-9 days).

From these patients, 19 Candida spp were isolated; *C. albicans* alone outnumbered other species and accounted for 73.68% episodes of trash.

For *C. albicans* isolates, the MIC values ranges from 9 to 18 µ g / ml for polyenes and from 16 to 64 µ g / ml for the azole antifungals. All the *Candida albicans* had closely related MFCs values.

TABLE 1: DEMOGRAPHIC CHARACTERISTICS OF CANCER PATIENTS WITH CANDIDIASIS

Patient characteristic	
Sex	No. (%)
Male	25 (41.7)
Female	35 (58.3)
Age (years)	
Range	15 – 79
Mean	49.88
Weight (Kg)	
Range	38-80
Mean	52.67
Days admitted in hospital (days)	
Range	1-9
Mean	3.58
Cancer type	
GI	39 (65)
Lung	6 (10)
Breast	11 (18.4)
Head and Neck	2 (3.3)
Others	2 (3.3)
Total	60

TABLE 2: FREQUENCY OF ISOLATION OF *CANDIDA SPECIES* FROM 60 CANCER PATIENTS WITH ORAL CANDIDIASIS

Candida spp	**Number (%)**
Candida albicans	14 (73.68)
Candida krusi	05 (26.32)
Total	19 (100)

DISCUSSION

Candidal infections are a major problem in the world, especially among the cancer patients [10-11]. The epidemiology of *C. albicans* and other yeasts from the oral cavity of patients with cancer is quite varied.

Our patient population consisted of 60 individuals with seven different types of cancers. Gastro-

intestinal cancer and Breast cancer were the most frequent cancer in the studied group, accounting for 65 % and 18.4 % respectively which is in accordance to other investigation [12].

TABLE 3: MIC AND MFC PROFILE OF *CANDIDA SPP.* ISOLATED FROM CANCER PATIENTS WITH ORAL CANDIDIASIS

Antifungal agents	**MIC** **MFC**	***C. albicans***
Amphotericin B	**MIC** **MFC**	**16 µ g / ml** **≤ 16 µ g / ml**
Nystatin	**MIC** **MFC**	**≥9 -18 µ g / ml** **≤ 18 µ g / ml**
Fluconazole	**MIC** **MFC**	**≥ 32 -64 µ g / ml** **≤ 64 µ g / ml**
Ketokonazole	**MIC** **MFC**	**16 µ g / ml** **≤ 16 µ g / ml**

Results obtained in this study established several points pertinent to the prevalence of oral candidiasis consistent with published data [13-14]. Nineteen Candida spp were isolated from the oral cavity of 60 cancer patients. As shown in Table 2, *C. albicans* alone outnumbered other species and accounted for 73.68% episodes of oral candidiasis. Our findings were consistent with that noted by other investigators [15-16].

Bagg et al. [17] showed that patients with advanced cancer have demonstrated a high incidence (51%) of oral colonization with non-*C. albicans* yeasts [18].

Antifungal drug resistance of Candida spp continues to increase in response to the widespread of application of antifungal agents in treatment of cancer patients. MIC and MFC data for the two polyenes (amphotericinn B and nystatin) and for the two azoles (flucobazole, and Ketoconazole) are in general agreement with others studies conducted in close geographical regions [19-20].

In conclusions, the finding of our study strongly suggest that oral candidiasis is a frequent complication among cancer patients, being *C. albicans* the main etiological agent; Most isolates of *Candida spp.* tested were very resistant to Polene as well as azole groups. The frequent occurrence of *Candida albicans* in oral cavity of cancer patients indicates a need for effective management of the infection prior to any anticancer treatment, as severe complications can otherwise result.

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References

1. ***Reichart P A****, Samaranayake L P, Philipsen H P. Pathology and clinical correlates in oral candidiasis and its variants: a review. Oral Dis2000;**6**:85–91.*
2. ***Nguyen M H****, Peacock J EJr, Morris A J, et al. The changing face of candidemia: emergence of non-Candida albicans species and antifungal resistance. Am J Med 1996;**100**:617–23.*
3. Daniluk T, Tokajuk G, Stokowska W, Fiedoruk K, Ściepuk M, Zaremba ML, Ro□kiewicz D, Cylwik-Rokicka D, Kędra BA, Anielska I, Górska M, Kędra B. Occurrence rate of oral *Candida albicans* in denture wearer patients. · Advances in Medical Sciences · Vol. 51O c · c2u0rr0e6n c ·e Srautpep ol.f 1o r ·al. 77-80.
4. Hana M, Khaled H, Ali Z, and Mawieh H. Isolation and characterization of Candida spp. In Jordanian Patients: Prevalence, Pathogenic Determinants, and antifungal sensitivity. Jpn. J. Infect. Dis. 57, 279-284. 2004.
5. Abu_Eltenn K, and abu lateen R. 1998. The prevalence of Candida albicans populations in the mouths of complete denture wearers. New Microbiol; 21: 41-48.
6. Oppenheim B. 1998. The cahngeing pattern of infection in neutropenic patients. J. Antimicrob. Chemother. 41: 7-11.
7. Akpan a and Morgan R. 2002. Oral Candidiasis: a review. Postgrd. Med. J. 78: 455-459.
8. Forbes Betty A, Danine F Sahm and Alice S Weissfeld. Bailey & Scott's diagnostic Microbiology. 12th edition. Elsevier Health Sciences. 2002.
9. National Committee for Clinical Laboratory Standards (1997). Refence method for broth dilution antifungal susceptibility testing of yeats. M27 – A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. Safdar A, Armstrong D. Infectious morbidity in critically ill patients with cancer. *Crit Care Clin* 2001; **17:** 531-570.
11. Uzun O, Ascioglu S, Anaissie EJ, Rex JH. Risk factors and predictors of outcome in patients with cancer and breakthrough candidemia. *Clin Infect Dis* 2001; **32:** 1713-1717.

12. M. DiNubile, D.Hille, C.Sable, N.Kartsonis. Invasive candidiasis in cancer patients: observations from a randomized clinical trial. *Journal of Infection*. 2008; 50(5):443-449.

13. Farzad Katiraee, Ali Reza Khosravi, Mahboubeh Hajiabdolbaghi, Ali Asghar Khaksar, Mehrnaz Rasoulinejad, and Mir Saeed Yekani nejad. Oral candidiasis in Human Immunodeficiency Virus (HIV) infected individuals in Iran. Tehran University Medical Journal; Vol. 68, No. 1, Apr 2010: 37-44.

14. **Spencer W. Redding, Richard C. Zellars, William R. Kirkpatrick, Robert K. McAtee, Marta A. Caceres, Annette W. Fothergill, Jose L. Lopez-Ribot, Cliff W. Bailey, Michael G. Rinaldi, and Thomas F. Patterson.** Epidemiology of Oropharyngeal *Candida* Colonization and Infection in Patients Receiving Radiation for Head and Neck Cancer. Journal of Clinical Microbiology, December 1999, p. 3896-3900, Vol. 37, No. 12.

15. Mirhendi H, Adin H, Shidfar M, Kordbacheh P, Hashemi S, Moazeni M, Hosseinpur L, Rezaie M. Identification of Pathogenic Candida Species: PCR-Fragment Size Polymorphism (PCR-FSP) Method. Tehran University Medical Journal; Vol. 66, No. 9, Dec 2008: 639-645.

16. Mirhendi H, Makimura K, Khoramizadeh M, Yamaguchi H. A oneenzyme PCR-RFLP assay for identification of six medically important Candida species. *Nippon Ishinkin Gakkai Zasshi* 2006; 47: 225-9.

17. Bagg J , Sweeney M, Lewis M, Jackson M, Coleman D, Al Mosaid A, Baxter W, McEndrick S, McHugh S. High prevalence of non-albicans yeasts and detection of anti-fungal resistance in the oral flora of patients with advanced cancer. Palliative Medicine, Vol. 17, No. 6, 477-481 (2003).

18. Davies A, Brailsford S, Broadley K , Beighton D. 2002. Oral yeast carriage in patients with advanced cancer. Oral Microbiology and Immunology. **Volume 17 Issue 2, Pages 79 – 84.**

19. Hanan M, Khaled H, Ali Z, and Maweih A. Isolation and characterization of Candida spp in Jordanian canbcer patients: Prevalnce, pathogenic determinats and antifungal sensitivity. Jpn.J. infect. Dis. 57; 279-284: 2004.

20. Pfaler M, Jones R, Doern G, Sader h, Messer S, Houston A, Coffman s, Hollis R and the SENTRY Participant Group. 2000. Bloodstream infections due to Candida spp: SENTRY antimicrobial surveillance program in North America and Latin America. 1997-1998. Antimicrob. Agents Chemother. . 44: 747-751.

CASE REPORT
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DEMODECOSIS IN A DOG

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ABSTRACT
Dogs are the most common pet animals worldwide. They sometimes harbour a wide range of parasitic diseases with zoonotic potentials, predisposing humans to health risk. Demodecosis is a parasitic disease that is often seen in young dogs of less than a year, immunodeficient adults and old dogs. Generalized demodecosis when it occurs in a dog may take months and years to treat. Infected animal has persistent puritis due to intense itching from *Demodex canis*. Such animal often inflicts wounds on the body due to persistent scratching and therefore should not under go aesthetic surgery until demodecosis is completely treated. The constant scratching of the body by the dog could traumatize the surgical site perphaps leading to evisceration and death of the animal if not promptly handled.
Keywords: Dog, evisceration, generalized demodecosis, aesthetic surgery.

INTRODUCTION

Amongst all the species of animals, dogs are the closest to man because of their sophisticated social behaviors (1). The different breeds serve various purposes to man ranging from draught dogs for work, guard dogs for protection, assistant dogs to aid the blind and physically challenged, detective dogs to aid in criminal investigations and most importantly pet dogs for mans' companion (2). Most pet owners have a close relationship with their dogs and often spend their leisure time cuddling them.

These pets are sometimes faced with various forms of skin diseases such as "mange" infection which often isolate them from man. Mange otherwise known as ascariasis is a persistent contagious skin disease of animals and man caused by parasitic mites (3). The disease is common among canine species and manifests in three different forms (4, 5, 6). Cheyletiella mange is the least pathogenic. It manifest as dandruff and slight itching (5). Sarcoptic mange also known as canine scabies is more serious, highly contagious and zoonotic (6). Canine scabies can infect all species of animals including man (3). Clinical signs manifests in form of intense itch, injury to the skin, weeping skin lesions, crusts and scab formations often seen at the elbow and ear region (5, 7).

Demodectic mange, also known as demodecosis or Red mange, is the third form and the most serious type caused by demodex canis (4, 8, 9). Demodecosis is non contagious and can only be seen in immunodeficient animals, old dogs and young puppies of about 3months to a year infected in utero (4, 7, 10-13). *Demodex canis* burrow deep into the skin feeds and secrete substance that reduces the innate resistance of infected dogs (vii). The disease is thought to be hereditary often seen in oily skinned, short haired, pubescent dogs and rare in puppies raised by hand and young adults (10, 11). Both sexes have the same ability to transmit genetic predisposition to demodecosis (9). However, (8) reported a higher prevalence of disease in long haired dogs. Dogs with localized form of demodecosis develop alopecic areas around the eyelids, lips, mouth and front limb giving the animal a characteristic moth-eaten appearance (7). Infected puppies recover by their fourteenth month as their immunity develops (15). Otherwise, the disease becomes chronic and generalized showing large alopecic areas on various parts of the body which coalesce into large bald areas (6). The hair follicles maybe plugged with debris and *demodex canis* mites with draining sinus tracts giving the animal a strong foal odour (4, 15, 16). At this stage the animals may be at risk of secondary bacterial infection and complications of pododemodecosis affecting the paws (7, 8).
Diagnosis is by deep skin scrapping into KOH or H_2O_2 to digest the debris and release the mites (4). However, most times it's difficult to identify mites under the microscope and treatment is often commenced based on the obvious clinical signs (3). A common simple way of diagnosis is by "Pedal-pinna reflex" technique where by the dog moves its hindlimb in scratching motion as the examiner gently manipulate and scratches the ear (3). This technique is effective for over 95% of most mange cases because these mites proliferate around the ear region at some point in time. Treatment is of a great controversy amongst Vets. Some recommends small daily shots of ivermectin along with medicated bath for 3-4 weeks (5). Others prefer weekly injection of ivermectin shots or orally (3, 7). However, some breeds of dogs especially the herding dogs such as Collies, Shetland sheep dog and old English sheep dogs are sensitive to ivermectin. Such dogs should be

treated with medicated sampoo containing benzylperoxide as the active ingredient. The hair should be first clipped off for ease of penetration into the skin. They may also be treated with multiple insecticide dips containing lyme sulfur, paramite such as dermisil which have been approved by FDA or mitaban bath. Mitaban dip should be avoided in young puppies because of its sedative effect (4, 5, 7). The use of hydrocodone in reliving the itching is also a bone of contention amongst vets. (15) disapproves its use perhaps because of its immunosuppressive effect which will have a negative effect in the recovery of the infected animal. (7) approves it use probably to relief the intense itching and scratching of the body. Newer methods of treatment include the use of interceptors, used in the treatment of heartworm. This method is rather expensive and tidous (7). FDA has recently approved the use of promeris a topical flea and tick medication for treatment of demodecosis (6). Some anecdotal reports claims the use of a mixture of two liters of seawater (salt water) in a half cut calabash and three fresh limes coated all over the affected areas and allowed for the day before rinsing cure mange. The procedure is to be repeated two to three times after which the animal recovers.

CASE REPORT

A six months old local breed of dog, weighing 8kg was presented on 12 th September 2010 for castration at the college of veterinary medicine Michael okpara university of university umudike. Physically examination of the dog shows a case of generalized mange. The lesions were seen on the forelimb, under the neck, the dorsum, ventral abdomen, head and the lateral aspect of the shoulder just below the neck region. The lesions are somewhat circumscribed and spreading with reddish center and necrotic scabs at the periphery. The lesions under the neck region became clearer when the head was raised up. The temperature was 38.7oc, heart rate 120/min, pulse rate 110/ min. Skin scrapings were collected into a container with KOH. The sample was analyzed and demodex canis confirmed at the department of veterinary parasitology through method described by (xvii). After clinical investigations were completed, the animal was castrated and sutured with unabsorbable catguz wire. The patient was given procaine penicillin injection at the dose of 10,000/iu and was discharged. The treatment for demodecosis commenced two days later. The animal was placed on weekly shots of ivermectin. A week later, the client brought the dog for treatment and it was discovered that the dog has loosen its stitches and there was accumulation of blood inside the wound. The wound was re sutured and treated along with ivermectin shot. Two weeks later, the client reported that he found the dog dead with some of its viscera outside.

DISCUSSION/ CONCLUSION/ RECOMMENDATION

According to the case report, the demodecosis was observed in a 6 months old local breed of dog. This agrees with (4, 6, 10-13) who said that demodecosis is mainly seen in dogs of about 3 months to one year of age. This could be as a result of the fact that dogs within the age bracket still have juvenile immune system which is unable to produce specific and sufficient antibodies to clear the demodex canis mites (6, 14, and 18). The generalized demodecosis is in line with the findings of (6) that demodecosis could be localized or generalized as the case may be. The spreading and dissemination of the disease only confirms the inability of the immune system to checkmates and clear the infection (6). The described lesions on the body as circumscribed spreading with reddish center and necrotic periphery agrees with (4). He described the lesions as reddish hence the name red mange. The reddish lesions are thought to be due to intense itching from the mites and constant scratching of the affected areas by the dog and continuously inflicting fresh wounds on the areas. The temperature of 38.9oc, Heart rate of 120/ min and pulse rate of 110/min were all normal for the dog. Other workers on mange recorded similar observations. This could mean that mange is simply a skin disease with no systemic effect except perhaps in secondary bacteria infection (4).

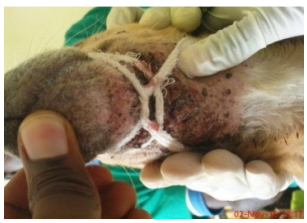

Fig 1: Red mange lesions under the neck region of a dog.

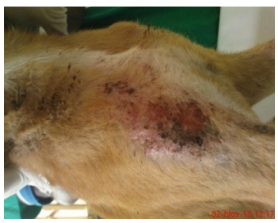

Fig 2: Red mange lesion on the upper fore limb of the dog.

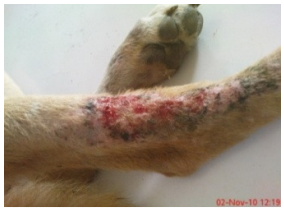

Fig3: Red mange on the right hindlimb of the dog with draining sinus tracts giving the animal a strong foal odour.

The skin scrapping used in the diagnosis of the case conforms to (3, 17) stating that skin scrapings are made into container of 10% KOH. The 10% KOH is for the digestion of the scabs and necrotic tissues,

releasing the mites. The centrifuged medium at 3000rev for 10mins was decanted and the supernatant viewed under the microscope for the presence of *demodex canis* mite (19). However, identification of mites through this method is a little difficult (3, 6). The pedal reflex technique has about 95% accuracy easy to carry out but will not tell the particular specie involved.

Most importantly, the dog after being confirmed to have generalized demodecosis shouldn't have been castrated. This is because demodex canis burrow deep into the skin causing intense itching to the dog (5, 6). The infected dog will constantly be scratching the body inflicting wounds on the body which are liable to bacterial infection (4). Also, while scratching the dog will constantly loosen the stitches and may cause trauma to tissues and if not promptly attended to may lead to evisceration and death of the dog. Secondly, in a normal dog that was castrated; the stitches should be removed by the 7th day post- castration because the wound will be fairly healed by then. Failure of the wound to heal by the 2nd week could mean a possible immunosuppressive effect of the mite on the host. This agrees with (6,7) that demodex canis secretes a substance that reduces the innate immunity of infected animal.

Conclusion/ Recommendation: Dogs with generalized demodecosis should not undergo aesthetic surgeries such as castration, tail docking and ear cropping until the animal recovers. Dog owner should present their pet for routine check up and prophylactic shots of ivermectin at monthly intervals. Treatment of demodecosis should commence immediately on diagnosis and should not stop until two or three negative skin scrapping results are made. Infected animals should be given immune boosters such as vitamin B complex for quick recovery and administration of omega-3 fatty acid supplement for skin restoration.

REFERENCES

1. Ugbomoiko, U. S.; Ariza,.L. and Heukelbach, J. Parasites of importance for human health in Nigerian dogs: high prevalence and limited knowledge of pet owners.Vet Res. 2008; 4: 49.
2. Association of veterinary medical student (AVMS) 2004. Dogs As Man Companion. Brigestome 2004; 1-5.
3. Wikipedia. Mange. Wikimedia Foundation Inc. 2010.
4. Kaminsky, A. What is the Best Treatment for Mange? wiseGEEK.com. 2010.
5. *Mange In Dogs. A Complete Guide To Dog Mange. Mange-In-Dogs.Com. 2010.*
6. Ardeth .B. Treatment of Mange, An explanation of the various types of mange in dogs and cats--all caused by mites--their degree of seriousness, treatment, and prevention. Demandmedia Network. 2002.
7. Nayak, D. C; Tripathy, S. B; Dey, P. C; Ray, D. N; Mohanty, G. S; Parida, S; Biswal and Das, M. Prevalence of canine demodicosis in Orissa (India). J. Vet. Parasitol. 1997; 73: 347-352.
8. Marsalla R. Demodicosis (Red Mange) in Dogs .Petplace.com. 2010.
9. Morris, M. L. Demodex folliculorum canis. Its diagnosis and treatment. J. Am.Vet. Med. Assoc. 1936; 88: 460-467.
10. Koutz, F.R. Demodex folliculorum (iii) Asurvey of clinical cases iin dogs. J. Am. Vet. Med. Assoc. 1954; 123: 131-133.
11. Gaafar, S. M; Smalley, H. E; Turk, R.D. The incidence of demodex species on skin of apparently normal dogs. J.Am. Vet. Med. Asssoc. 1958; 133: 122-123.
12. Mishran, S.C; Sahoo, B; Patel, S. N. Studies on parasitic problems of puppies V. winter populations of helminths and arthropods on puppies at Bhubaneswar. Indian J. Anim. Res. 1974; 8: 89-91.
13. Dog health guide. dog health guide.com. 2010.
14. Smith, H.J. Bovine demodicosis. I incidence in Ontario Cnadan J. comp. Med. 1961; 25; 165-169.
15. Gortel, K. Update on canine demodecosis. Vet. Clin. Small Anim. Pract. 2006; 36: 229-241.
16. Nutting, W. B., Desch, C.E. Demodex canis. Redescription and revaluation. Cornell Vet. 1978; 68: 139-149.
17. Sarkar, P; Mkherjee, J; Ghosh, A; BhattSmith, H. J. Bovine demodecosis. I. Incidence in Ontario Canadian. J. Comp. Med. 1961; 25:165-169.
18. Baker, K.P. Observations on demodectic mange in dogs. J. Small Anim. Pract. 1968; 9: 621-625.