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**PREVALENCE OF HEPATITIS B VIRUS INFECTION AMONG PREGNANT WOMEN IN AN ANTENATAL CLINIC IN PORT HARCOURT, NIGERIA.*****\*Obi, R.K.<sup>1</sup>, Umeh S.C.<sup>1</sup>, Okurede, O.H.<sup>2</sup>, Iroagba I.I.<sup>3</sup>*****<sup>1</sup>Department of Industrial Microbiology, School of Science, Federal University of Technology, Owerri, Imo State,****<sup>2</sup>Dept. of Microbiology, Faculty of Science, Madonna University, Elele, Rivers State, <sup>3</sup>Dept. of Medical Laboratory Sciences, College of Health Sciences, Madonna University Teaching Hospital, Elele, Rivers State, Nigeria.****Abstract**

A total of ten thousand and thirty two (10,032) pregnant women attending ante-natal clinic in Braithwaite Memorial Hospital, Port Harcourt, Nigeria were screened between January 2000 to December 2004 for the possible occurrence of hepatitis B virus using HBV paper strips. The results showed that a total of 290 (2.89%) of the pregnant women tested positive for hepatitis B Virus. The years 2001 and 2002 had the highest prevalence of 61, while 2004 had the least prevalence of 52. No significant difference ( $P<0.5$ ) was however observed in the annual prevalence of the infection among pregnant women in the hospital. Studies of the age distribution of the infection among the studied pregnant women showed that women in the age group of 41-45 had the highest prevalence rate (60%) for the sampled population within that age group, followed by women in the age group of 31-35 with an occurrence rate of 11.04% within that age group. The least rate of occurrence was observed in the age group of 21 – 25 which showed only 1.75%. The prevalence of the deadly hepatitis B virus among pregnant women whose immunity is often compromised by gynaecological and nutritional factors is of grave clinical importance.

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**INTRODUCTION**

Hepatitis is an inflammation of the liver (1). The word is derived from a combination of two Greek words "Hepatos" (Liver) and "itis" (inflammation) (2). The disease can be occasioned by several factors including viral infection. Many viruses cause hepatitis and advances in molecular biology and virology techniques have led to the identification of pathogens responsible for acute and chronic hepatitis (3). To date at least six hepatitis viruses

have been recognized, and these have been named: Hepatitis A, B, C, D, E, and G. Acute hepatitis may also occur as part of the clinical course of a number of viral infections including human cytomegalovirus, Epstein – Barr virus, herpes simplex virus, yellow fever virus and rubella (4).

Hepatitis B is one of the major diseases of mankind and is a serious global public health problem. Of the 2 billion people who have been infected with the

hepatitis B virus (HBV), more than 350 million have chronic (lifelong) infections. These chronically infected persons are at high risk of death from cirrhosis of the liver and liver cancer, diseases that kill about one million persons each year (5). In endemic areas, where carrier rates are >5%, most individuals are infected perinatally, by vertical transmission, or in early childhood (6).

Hepatitis B infection is caused by hepatitis B virus (HBV) which is a circular double stranded DNA virus in the family, *Hepadnaviridae*. The HBV genome has four genes, *pol*, *env*, *precore* and *X*, that encode the DNA polymerase, envelop proteins, precore protein (which is processed to viral capsid) and protein X respectively. The function of protein X is not clear, but it may be involved in the activation of host cell genes (7). The virion also known as a Dane particle has a diameter size of 42 to 47nm, with an electron-dense core of 27 nm. Three well-defined antigens are associated with the virus. These are HbcAg and HbeAg (described as core proteins and contained in the nucleocapsid) and HBs Ag (described as surface coat protein) which is found in the outer envelope of the virus (8).

HBV is a hardy virus that can exist almost on any surface for up to one month. The virus remains infective for days in dry blood and for months when stored in serum at room temperature. (9). Hepatitis B infection is an ancient disease that has been found in all populations though the incidence and risks are high among people living under crowded conditions, drug addicts, the sexually promiscuous and people in certain occupations involving blood or blood products (9).

Hepatitis B is usually transmitted parenterally through transfusions of blood and blood products, sharing of needles and razors, tattooing and acupuncture, renal dialysis, organ donation and sexual intercourse.

Horizontal transmission is possible in children, families, and 'close personal contact'. Vertical transmission occurs perinatally from a carrier mother to her baby through the placenta and during delivery (4; 10).

This study was designed to create awareness as to the prevalence and menace of hepatitis B virus infection and its possible consequences on maternal and infant health in Port Harcourt, Rivers State, Nigeria in order that appropriate preventive steps could be taken by Medicare providers to safeguard pregnant women and their babies from hepatitis B virus infection.

## MATERIALS AND METHODS

### Study Population:

A total of 10,032 pregnant women who presented themselves at Braithwaite Memorial Hospital, Port Harcourt, Nigeria were used for this 5-year study which spanned from 2000 to 2004. The purpose of the study was fully explained to them and their informed consent obtained prior to the study as recommended by the World Health Organisation (11).

### Sampling:

The presence/absence of the virus was determined using hepatitis B virus (HBV) strips according to the method of Levy *et al* (12) based on the principle that hepatitis B antibody present in serum binds to the hepatitis B antigen present in the strip forming an immune complex. The reaction is visualized by the presence of a chromogene impregnated in the strip showing a red coloration which indicates a single red line for a negative reaction and two red lines which indicate a positive reaction (12).

Briefly, a blood sample was collected from each patient with the aid of a syringe and was left to stand before centrifuging at high speed for 15 mins. The

resulting serum was left to equilibrate at room temperature and then the paper strip was dipped into the serum (vertically) for 15 seconds. Each strip was then placed on a flat non-absorbent white tile and the results read after 15 minutes taking appropriate precautionary measures (12).

## RESULTS AND DISCUSSION

Table 1 presents the annual distribution of cases of hepatitis B surface antigen among pregnant women attending Braithwaite Memorial Hospital, Port Harcourt, Nigeria. The results indicate that 2001 and 2002 had the highest occurrences of pregnant women with the HBsAg (61 cases each) while 2004 had the least occurrence of 52 cases. There is therefore no significance difference ( $P < 0.5$ ) in the annual distribution of hepatitis among pregnant women in the hospital. Table 1 also shows the monthly

occurrences of HBsAg. The result shows that the mean occurrence of hepatitis B was highest in January (31 patients) followed by October (30) while the least occurrence was in the month of July (14) followed by May (15). The occurrences of hepatitis B surface antigen among pregnant women attending the hospital was therefore significantly lower ( $P < 0.5$ ) in July and May and significantly higher ( $P < 0.5$ ) within the months of October and January (except for December) suggesting that the disease occurred more during the dry season.

Table 2 shows the age distribution of hepatitis B in pregnant women in Braithwaite Memorial Hospital. The highest prevalence was recorded between the age bracket of 41 - 45 (60%) while the lowest was between the age bracket of 21 - 25 (1.75%).

**Table 1:** Annual distribution of hepatitis B among pregnant women attending Braithwaite Memorial Hospital.

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept.	Oct.	Nov.	Dec.	Total
2000	9	8	4	5	2	6	2	4	6	7	3	4	60
2001	4	6	3	6	2	6	3	5	8	4	8	6	61
2002	9	4	6	5	3	7	5	3	5	7	2	5	61
2003	4	5	2	5	4	7	4	5	3	6	6	5	56
2004	5	3	5	3	4	3	4	5	2	6	8	4	52
<b>TOTAL</b>	<b>31</b>	<b>26</b>	<b>20</b>	<b>24</b>	<b>15</b>	<b>29</b>	<b>18</b>	<b>22</b>	<b>24</b>	<b>30</b>	<b>27</b>	<b>24</b>	<b>290</b>

**Table 2:** Age distribution of Hepatitis B Prevalence in the studied hospital in Port Harcourt, Nigeria.

Age interval	Total Screened	HbsAg Positive	HBsAg negative	% Positive
15- 20	519	30	489	5.78
21 - 25	3762	66	3696	1.75
26 - 30	3820	94	3726	2.46
31 - 35	480	53	427	11.04
36 - 40	1441	39	1402	2.70
41 - 45	10	6	4	60

The fact that there was no significant difference ( $P < 0.5$ ) in the annual distribution of hepatitis B antigen in the hospital within the five years interval indicate that there was no annual variation in the disease prevalence. This would then mean that the incidence of the disease in the hospital may have been there long before our research. Our results are therefore of clinical significance to the hospital because high hygienic conditions will need to be maintained to ensure that some of the positive cases are not from nosocomial infections. This need is further heightened by the fact that the pregnant women will readily transmit the infection to the fetuses and/or the neonates. Hepatitis B virus has a high rate of vertical transmission causing fetal and neonatal hepatitis. Hepatitis A, C and E are rarely transmitted trans-placentally (13).

Also, the prevalence of the infection was higher between the months of October and January. This could be due to increased exposure to the risk factors within this period and also due to the season. Exposure to risk factors as well as season of transmission are known determinants of the possible intensity of infection (4). In the months of May and July the prevalence of the infection was at its lowest ( $P < 0.5$ ). This could also be as a result of reduction in the rate of exposure to the risk factors.

The dangers inherent in the observed cases of hepatitis are legion and call for conscious efforts to address them especially as it has been reported that infection acquired perinatally and in early childhood is usually asymptomatic, becoming chronic in 90% and 30% respectively. But in those people who experience disease, the severity of symptoms and the aftermath of hepatic damage vary widely. Liver damage is usually mild during childhood; severe liver disease, including cirrhosis and hepatocellular carcinoma (HCC) may develop

insidiously for 2-7 years (14). Yet it is known that approximately 90% of infants of HBs Ag seropositive mothers become chronic hepatitis B surface antigen (HbsAg) carriers. When a woman goes into labour there is a massive exchange of blood; the virus can therefore be passed from the mother's blood to the newborn through the umbilical cord. The blood exchange occurs before delivery and so even a caesarean section will not prevent infection (15, 16).

Finally, we recommend that all pregnant women be screened for hepatitis B surface antigen HbsAg during antenatal visits. It is already recommended (17) that all infants be vaccinated against hepatitis B at birth to further reduce any potential risks of infection. It is also important that blood given to pregnant women in need of it be screened before transfusion. It is also important that pregnant women reduce the rate of their patronage of commercial pedicure and manicure outfits since their instruments, shared among their numerous customers, could be a potential abode of hepatitis B virus. The present observations are also of national and international interest since it is assumed (especially in Nigeria) that hepatitis B infection is of little gynaecological importance.

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**EVALUATION OF SIDA ACUTA SUBSPECIE ACUTA LEAF/FLOWER COMBINATION FOR ANTIMICROBIAL ACTIVITY AND PHYTOCHEMICAL CONSTITUENTS****SAGANUWAN, ALHAJI SAGANUWAN & GULUMBE, MOHAMMED LAWAL**

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**ABSTRACT**

*Sida acuta subspecie acuta* leaf/flower combination was evaluated for antimicrobial activity and phytochemical constituents using methanol, hexane, chloroform and aqueous method of extractions. The antibacterial activities were exhibited by the four extracts on *E. coli*, *S. pyogenes*, *P. multocida* and *S. typhimurium* as there was no activity exhibited on *S. tyhi*, *S. pneumoniae* and *K. pneumoniae*. Phytochemical analyses revealed the presence of alkaloid, tannin, flavonoid and saponin whereas steroid and glycoside were absent.

**Key words:** Evaluation, *Sida*, *acuta*, leaf, flower, antimicrobial, phytochemical, constituents.

**INTRODUCTION**

*Sida acuta subspecie acuta* (Horn-beam-leaved sida) is herb of about 0.7m high with numerous erect branches, the leaves are oval, dentate, about 6.5-7.5cm long and 1-2cm broad (1). Inflorescence consists of flowers that are solitary, axillary and sometimes with terminal pauciflorous glomerule, although, fruits are more or less globular and covered on top with golden hairs as inside the fruit is ovoid containing black numerous seeds (1). Pan tropical wild species, grow around roadside, and on waste land medicinally used for malaria, gonorrhoea, abortion, breast cancer (1),

inflammation and poisoning (2). Phytochemically, *Sida acuta subspecie acuta* contains saponin, tannin and prostaglandin (3).

Infectious diseases are the world's leading cause of premature deaths, killing almost 50,000 everyday (4,5). The discovery of effective antimicrobial agent was considered to be one of the greatest contributions to medicine in the 20<sup>th</sup> century (6). The changing pattern of bacterial aetiology of infections and their altered sensitivities to antimicrobial agents employed in their treatment call for intensive regular exploration of indigenous plants. This will help us

identify plants with antimicrobial values that will not only serve as resource for our indigenous pharmaceutical industries but will also serve as an alternative/complementary medicine. Orji and co-workers reported that a particular characteristic of a plant is that, different chemical substances are obtained in members of even the same species in different areas (7).

In view of these, this study was carried out to investigate and evaluate the antimicrobial activities and phytochemical constituents of *Sida acuta subsp. acuta* leaf and flower combination using different methods of extraction.

## MATERIALS AND METHODS

**Plant:** Fresh leaves of *Sida acuta subsp. acuta* were collected from Katcha town in Niger state identified and authenticated in herbarium of Biological Sciences department, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.

**Extraction:** The leaves of *Sida acuta subsp. acuta* were collected fresh, macerated with mortar and pestle then dried in sun for about 45 minutes and grounded using mortar and pestle.

Twenty (20g) of the powdered leaf was weighed into 4 conical flasks and 100mls each of hexane, chloroform, methanol and water added to the flasks. The mixtures were thoroughly shaken and allowed to stand for 24 hours. The mixtures were filtrated separately through whatman No. 1 filter paper into measuring cylinders and concentrated to dryness using water bath and dessicator. The dried residue was stored at 4°C until ready to use.

**Preparation of extract:** Serial concentrations: 70, 80, 90 and 100% of the hexane, methanol, chloroform

and water extracts were prepared and sterilized through 0.45µm membrane filter paper.

**Bacterial isolates:** The bacterial isolates of *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*, *Salmonella typhimurium* and *Pasteurella multocida* were donated by the department of Veterinary Public Health and Animal Production, Usmanu Danfodiyo University Sokoto, Nigeria, but authenticated by chemical and serological tests as described by Cheesebrough (10) although preserved on blood agar slant and stored at 4°C until ready to use.

**Other materials:** include nutrient agar plates (70), isotonic sodium chloride solution, Mueller-Hinton agar, pieces of dried petri dish plates (70), Whatman (No.1) paper, measuring ruler and distilled water.

**In vitro test:** The isolates of *S. pyogenes*, *S. pneumoniae*, *K. pneumoniae*, *E. coli*, *S. typhi*, *S. typhimurium* and *P. multocida* were subcultured overnight at 37°C on nutrient agar plates, 10 plates per microorganism. The suspension of each bacterial were prepared as described by John et al (11) using isotonic sodium chloride solution.

Dried petidish, 10 per each microorganism of Mueller-Hinton agar were flooded with the appropriate suspensions of the bacterial isolates. Sterile 6mm diameter absorbent filter papers (punched out from No.1 Whatman paper were impregnated with the appropriate concentrations: 700, 800, 900 and 1000mg of the hexane, chloroform, methanol and water extracts and placed on the corresponding inoculated 70 plates ten per microorganism. After the incubation at 37°C for 24 hours, all the plates were observed for zones of growth inhibition and the diameters of these zones measured in millimeter using measuring ruler.

## RESULT

**Table 1: Serial concentrations of the hexane extract and their corresponding zones of inhibition.**

Conc. of hexane extract (mg)	Diametric zone of inhibition in millimeter (mm)						
	S. pneumoniae	P. Multocida	K.pneumoniae	S.pyogenes	S.typhimrium	S.typhi	E. coli
700	0	0	0	0	0	0	0
800	0	0	0	0	0	0	8
900	0	10	0	16	0	0	13
1000	0	14	0	10	0	0	15
Mean: 850	0.0	6.0	0.0	6.5	0.0	0.0	9.0

*E. coli*, *S. pyogenes* and *P. multocida* exhibited zones of inhibition at concentration ranges between 700-1000mg as there was no zone of inhibition shown by other microorganisms at concentration ranges between 700-1000mg. *E. coli*(9.0mm) has the highest mean followed by *S. pyogenes* (6.5mm) and lastly *P. multocida*(6.0mm) see table 1.

**Table 2: Serial concentrations of the chloroform extract and their corresponding diametric zones of inhibition.**

Conc. of chloroform extract (mg)	Diametric zone of inhibition in millimeter (mm)						
	S. pneumoniae	P. Multocida	K.pneumoniae	S.pyogenes	S.typhimrium	S.typhi	E. coli
700	0	0	0	0	0	0	0
800	0	0	0	0	0	0	0
900	0	10	0	8	0	0	0
1000	0	15	0	12	0	0	16
Mean: 850	0.0	6.25	0.0	5.0	0.0	0.0	4.0

*E. coli*, *S. pyogenes* and *P. multocida* showed zones of inhibition at concentrations between 900 and 1000mg with *P. multocida* (6.25mm) showing the highest followed by *S. pyogenes* (5.0mm) and then *E. coli* (4.0mm). But there was no zone of inhibition shown by other microorganisms between concentration ranges 700-1000mg (table 2).

**Table 3: Serial concentrations of the methanol extract and their corresponding zones of inhibition.**

Conc. of methanol extract (mg)	Diametric zone of inhibition in millimeter (mm)						
	S. pneumoniae	P. Multocida	K.pneumoniae	S.pyogenes	S.typhimurium	S.typhi	E. coli
700	0	0	0	0	0	0	0
800	0	0	0	0	0	0	8
900	0	8	0	5	0	0	10
1000	0	13	0	9	0	0	13
Mean; 850	0.0	5.25	0.0	3.25	0.0	0.0	3.0

*P. multocida*, *S. pyogenes* and *E. coli* showed zones of inhibition between 900-1000mg concentration with *P. multocida* (5.25mm) having the highest mean, then *S. pyogenes* (3.25mm) and lastly *E. coli* (3.0mm). Though there was no zone of inhibition exhibited by the rest of microorganisms at any concentration (table 3).

**Table 4: Serial concentrations of the water extract and their corresponding zones of inhibition**

Conc. of water extract (mg)	Diametric zone of inhibition in millimeter (mm)						
	S. pneumoniae	P. Multocida	K.pneumoniae	S.pyogenes	S.typhimurium	S.typhi	E. coli
700	0	0	0	0	0	0	0
800	0	0	0	0	0	0	0
900	0	0	0	0	0	0	0
1000	0	0	0	0	6.5	0	6
Mean; 850	0.0	0.0	0.0	0.0	1.6	0.0	1.5

*S. typhimurium* and *E. coli* exhibited zones of inhibition at 1000mg concentration with *S. typhimurium* (1.6mm) having higher mean and *E. coli* (1.5mm) as there was no zone of inhibition exhibited by the rest of microorganisms at concentration ranges between 700-1000mg (table 4).

**Table 5: Phytochemical analyses of *Sida acuta* subspecies *acuta* methanol, hexane, chloroform and aqueous leaf extracts.**

Extract	Alkaloid	Tannin	Glycoside	Flavonoid	Steroid	Saponin
Methanol	-	+	-	+	-	+
Hexane	+	+	-	+	-	+
Chloroform	+	+	-	+	-	+
Water	-	+	-	+	-	+

Phytochemical analyses revealed the uniform presence of saponin, flavonoid and tannin in all the four extracts. Nonetheless, alkaloid was present in hexane and chloroform extracts as it was absent in methanol and aqueous extracts of *Sida acuta* subspecies *acuta*, but steroid was absent in all the four extracts (table 5).

## DISCUSSION

The antibacterial activity exhibited by hexane, chloroform methanol and aqueous leaf extracts of *Sida acuta* subspecies *acuta* on *E. coli*, *S. pyogenes*, *P. multocida* and *S. typhimurium* at various concentrations agrees with the report of Orji et al (7) that Nigeria has an interestingly rich flora because it has varied climatic conditions ranging from the mangrove swamps and rainforest in the south to the Savanna and torn bush regions in the north. The result confirmed the report of Shahidi Bonjar and Rashidi Farrokhi that natural resources, especially plants and microorganisms are potent candidates for new drugs (12) as Orji et al (7) reported that a particular characteristic of plants is that different chemical substances are obtained in members of even the same species in different areas. *Sida acuta* subspecies *acuta* may be used to avert the emergence of antimicrobial resistance. This has not only resulted in increased morbidity and mortality, but also in higher health care cost (13). Infectious diseases are the world's leading cause of premature deaths killing almost 50, 000 people every day and control of such diseases has posed serious problem especially on the developing nations (14). Differences in the zones of inhibition shown by the

hexane chloroform and aqueous extracts might be due to differences in the methods of extraction. It was reflected in the result of phytochemical analyses whereby all the four extracts uniformly revealed the presence of saponin, flavonoid and tannin as alkaloid was present in hexane and chloroform extracts. Whereas methanol and aqueous extracts did not reveal the presence of alkaloid. This agrees with the report of Gill that *Sida acuta* subspecies *acuta* contains saponin, tannin and prostaglandin as it also confirmed absence of steroid and glycoside in all the four extracts(3).

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**RESISTANCE PATTERNS OF *STREPTOCOCCUS PNEUMONIAE* ISOLATED FROM THE UPPER RESPIRATORY TRACT OF PERSONS ATTENDING VARIOUS CLINICS OF A UNIVERSITY TEACHING HOSPITAL IN LAGOS, NIGERIA –A preliminary study.****<sup>1</sup>Oduyebo, OO., <sup>1</sup>Nwaka, DU., <sup>2</sup>Nwaowolo, C., & <sup>1</sup>Ogunsola, FT.****<sup>1</sup>Department of Medical Microbiology & Parasitology College of Medicine, University of Lagos, Lagos, &****<sup>2</sup>Department of Surgery College of Medicine, University of Lagos, Lagos, Nigeria****Correspondence to: Dr. O. O. Oduyebo E-mail: oyinoduyebo@yahoo.com Tel; 234-8023163319****Abstract**

The upper respiratory carriage rate, serotypes and resistance patterns of *Streptococcus pneumoniae* in persons attending four clinics of the Lagos University Teaching Hospital (LUTH) were determined. Nasal swab specimens were collected from a total of 372 persons, 175 of whom were males and 177 were females. Their ages ranged from 14 weeks to 65 years. The upper respiratory carriage rate found in the total population of both adults and children was 9.9%, but the rate was highest in children less than 5 years (18.6%). Among the 17 isolates that were available for serotyping, there was no significant serotype, though resistant serotypes like 23F, 19F, 6A and 14 were identified. Initial oxacillin screening for penicillin resistance revealed that 12 out of 36 isolates were clearly sensitive, but combined with the result of Etest, penicillin resistance was found to be 6.8%. Susceptibility testing by disc diffusion revealed that 80.5% of isolates were sensitive to ceftriaxone and 94.4% to chloramphenicol. Sixty one percent were sensitive to erythromycin while 94.4% were resistant to co-trimoxazole and 80.5% to tetracycline. Except for amoxicillin and cefotaxime which showed high activity, sensitivity pattern by Etest was found to be similar to that of disc diffusion. The result of this study makes it possible to formulate hypothesis for a larger study. In this study, the carriage rate of *S. pneumoniae* probably ranged from 2.6% to over 18.6% depending on the study population. If the limitations of this study are excluded in a larger study, the rate most likely will be higher. Also, penicillin resistance in carriage strains would be up to 6.8%, probably higher, but may be intermediate, so penicillin could still be useful for treatment of pneumonia and probably otitis media, but not meningitis. There is reason to watch out for increased resistance to penicillin, cephalosporin and erythromycin. Most pneumococcal isolates would likely be resistant to tetracycline and co-trimoxazole



## Introduction

*Streptococcus pneumoniae*, normal flora of the nasopharynx is an important agent of both community and hospital acquired pneumonia. It is also implicated in otitis media, sinusitis and meningitis (1,2). Till 1978, *S. pneumoniae* was generally susceptible to penicillin, but penicillin resistant pneumococci (PRP) and multiply antibiotic resistant strains are increasingly being reported from all over the world.(3,4). PRP are highly resistant to penicillin and as many seven classes of antibiotics like other beta lactams, tetracycline, macrolides, chloramphenicol, rifampicin and co-trimoxazole (3).

This increasing resistance has led to a change of treatment protocols and diagnostic guidelines in the affected countries. Guidelines have been developed by the World Health Organisation for the treatment of PRP infections and to differentiate between highly resistant, moderately resistant strains and strains with low level of resistance. The site of infection is also taken into consideration in the guidelines. Treatment of life threatening infections like meningitis requires the use of extended spectrum cephalosporins like ceftriaxone while for otitis media, amoxicillin clavulanate would be recommended<sup>5</sup>.

Though many serotypes of pneumococci have been associated with antibiotic resistance, some like 23F, 19F, 14 and 6A are more often reported than others. These common serotypes are also associated with invasive disease (6,7). Many of these serotypes are carried by healthy persons who have been shown to be important reservoirs of *S.pneumoniae* and high carriage rates apart from favoring dissemination, also, precede disease in infected individuals (8). In Nigeria PRP has not been documented as a problem and there is therefore no new guideline on the treatment of

pneumococcal infections. This situation is largely due to the fact that very few laboratories have consistently isolated *S.pneumoniae* in the past 10 years mainly because of widespread antibiotic abuse (9). This study was therefore carried out as a preliminary study to determine the carriage rates of serotypes and resistance patterns of *S.pneumoniae* in patients attending selected outpatient clinics in the Lagos University Teaching Hospital.

## Methodology

### Patients and Methods

Between March and December 2004, nasal swabs were collected from all adults and children who attended the following 4 LUTH clinics: Child health and immunization clinic, Ear, nose and throat clinic, Staff clinic and paediatric clinic. The specimen was collected each time patients attended the clinic, regardless of their complaints. The only exclusion criterion was consumption of antibiotics in the previous 2 weeks. Ethical clearance for the study was obtained from the Ethics and Research committee of the Lagos University Teaching Hospital and informed consent was obtained from the participants or their parents.

### Procedure

The nasal specimen was collected with a sterile swab (sterilin). This was immediately inoculated on Columbia agar base (Oxoid) to which 5-7% sheep blood and 5mcg/ml gentamicin had been added. Incubation was in air in 5- 10% CO<sub>2</sub> at 37°C for 24 hrs. Alpha haemolytic gram-positive diplococci were tested for sensitivity to optochin and bile solubility. Optochin sensitive and bile soluble isolates were identified as *Streptococcus pneumoniae* (10).

### **Optochin susceptibility test.**

Optochin disk was applied to a quarter of sheep blood agar plate that has been streaked with a few colonies of alpha haemolytic streptococcus isolated. Culture plates were then incubated at 35°C in 5-10% CO<sub>2</sub>. A zone > 14mm with a 5 ug (6 mm diameter) disk was indicative of inhibition and identified isolates as *S. pneumoniae*. Isolates with smaller zones of inhibition were then subjected to bile solubility test.

### **Bile solubility test**

0.5 ml of 2% sodium deoxycholate was added to 0.5 ml of a (0.5 Mc Farland) saline suspension of the isolate.

#### **Incubation**

was at 35°C for up to 2 hours. A clearing in the presence of deoxycholate indicated a positive bile solubility test, which identified the organism as *S. pneumoniae*.

### **Storage**

Isolates were stored in skim milk tryptone glucose glycerol broth (STGG) at -70°C until antibiotic sensitivity and serotyping were performed.

### **Oxacillin screening**

This was used to identify isolates susceptible to penicillin and select isolates for resistance testing. Imeg oxacillin disk was used.

Isolates were considered sensitive to penicillin if the zone of inhibition was >20mm. For isolates with zones < 20mm, Etest was performed to confirm whether they were actually resistant to penicillin (10).

### **Sensitivity testing**

Sensitivity testing was by Disc diffusion and Etest methods in accordance with the manufacturer's instructions and interpretations of antimicrobial susceptibility results were in accordance with Clinical and Laboratory Standards Institute (formerly NCCLS) (11). Antibiotics included in the Etest were penicillin G, amoxicillin, chloramphenicol, Cefotaxime, Cotrimoxazole, and tetracycline. Apart from oxacillin, antibiotics tested by disc diffusion included cotrimoxazole(25ug), tetracycline, ceftriaxone, erythromycin and chloramphenicol (30ug) (11).

Serotyping of isolates was undertaken at Professor Richard Adegbola's laboratory at the Medical Research Council Laboratories in The Gambia. It was carried with capsular and factor-typing sera (Statens Serum Institute, Copenhagen Denmark) using the Neufeld (Quellung reaction) method (12).

## **RESULTS**

Nasal swab specimens were collected from a total of 372 patients. One hundred and thirty samples came from the Children's health and immunization clinic, 128 from the Ear/nose and throat clinic, 86 from the Staff clinic and 28 from the Pediatric clinic. One hundred and ninety five of the patients were males while 177 were females. Their ages ranged from 14 to weeks to 65 years (Table1)

*Streptococcus pneumoniae* was isolated from 36(9.9%) out of the 372 specimens collected. Carriage rate was highest in children less than 5 years. Rates reduced with age till 25 years as shown in table 1. More females (13.5%) were colonized compared to males (6.1%). This difference was statistically significant at 0.05 level. Most isolates came from the immunization clinic and the least number from the staff clinic.

Oxacillin screening showed that only 12 (33.3%) out of 36 isolates were clearly sensitive to penicillin (table2). E-test performed on 17 of the 24 isolates which had equivocal penicillin sensitivity revealed that two were resistant to penicillin. One had an intermediate resistance with an MIC of 0.125u/ml and the other was fully resistant (table3)

Susceptibility pattern of all 36 isolates as determined by disc diffusion is shown on (table2). Many isolates were sensitive to ceftriaxone (80.5%) and chloramphenicol

(94.4%). Sixty one percent were sensitive to erythromycin while the majority were resistant to cotrimoxazole (94.4%) and tetracycline (80.5%). Except for amoxycillin and cefotaxime, which showed high activity, sensitivity pattern by E-test was found to be similar to that of disc diffusion (tables 2&3).

There was no predominance of any serotype though resistant serotypes like 23F, 19F, 6A and 14 were identified.

**TABLE 1: CARRIAGE RATES OF *S.PNEUMONIAE* IN PATIENTS ATTENDING SOME LUTH CLINICS**

AGE GROUP(YEARS)	NUMBER OF PATIENTS	NUMBER COLONISED	CARRIAGE RATE
0-5	134	25	18.6%
6-10	32	4	12.5%
11-15	26	1	3.8%
16-20	17	1	5.8%
21-25	24	1	4.1%
26-30	27	1	3.7%
>30	112	3	2.6%
TOTAL	372	36	9.9%

**TABLE 2; ANTIMICROBIAL SENSITIVITY PATTERN OF 36 CARRIAGE STRAINS OF *S.PNEUMONIAE* BY DISC DIFFUSION METHOD**

ANTIMICROBIAL AGENT	NO.(%) SENSITIVE	INTERMEDIATE SENSITIVITY (%)	NO. (%) RESISTANT
Oxacillin	12(33.3)	-	24(66)
Chloramphenicol	34(94.4)	1(2.7)	1(2.7)
Erythromycin	22(61.1)	11(30.8)	3(8.3)
Tetracycline	4(11.1)	3(8.3)	29(80.5)
Ceftriaxone	29(80.5)	7(19.4)	-
Cotrimoxazole	1(2.7)	1(2.7)	34(94.4)

**Table 3: Sensitivity of *S. pneumoniae* by E-test**

Antibiotic	No.(%) tested	No. (%) sensitive	Intermediate resistance (%)	No.(%) resistant
Penicillin G	17	15 (88.2)	1 (5.88)	1 (5.88)
Amoxycillin	17	17 (100)	-	-
Chloramphenicol	17	17 (100)	-	-
Cefotaxime	16	15 (93.73)	-	1 (6.25)
Co-trimoxazole	17	4 (23.52)	2 (11.64)	11 (64.7)
Tetracycline	17	5 (29.41)	-	12 (70.58)

## Discussion

The carriage rate of 9% found in this study is quite low, probably because it includes rates for both children and adults. Highest rates are usually found in children while very low rates are found in adults (13,14). As expected, the rate in this study was highest in children less than 5 years. However this rate of 18.6 % is still low when compared with a similar study carried out in Ghana in 2002, in which, a much higher rate of 51% was reported<sup>15</sup>. This finding is not surprising because the population studied in Ghana was 6 months to 2 years, the age group that has been found to have the highest carriage rate all over the world (13,14,16,17). In our study, the

lower carriage rate found in children in the immunization clinic is bound to be an under-estimate and may be attributed to the fact that nasal rather than nasopharyngeal samples were collected. Unfortunately, the mini wire loop required for collecting material from the posterior pharynx, recommended for carriage studies by the WHO was not available for our study hence the use of nasal swab (18). Nasal swabs even though not ideal have been found in some studies to be more efficient than oropharyngeal swabs for isolation of *S. pneumoniae* in the upper respiratory tract (19).

Initial oxacillin screening for penicillin resistance revealed that 12 out of the 36 isolates obtained were clearly sensitive to penicillin while 24 had equivocal penicillin sensitivity which had to be confirmed by quantitative methods. Due to storage difficulty E-test was performed on only 17 of these and only two out of the 17 were eventually found to be resistant to penicillin. If the results of Oxacillin screening and E-test were combined then only two out of 29 isolates were resistant to penicillin making the resistance rate 6.8%. Anecdotal reports suggest that high rates of penicillin resistant pneumococci exist in Nigeria and a limited survey carried out in 1978 put the prevalence at 20% (20). Out of the two penicillin resistant strains, one had a high level resistance, while the other had intermediate resistance. Infection with highly resistant isolates has to be treated with cephalosporins. In case of intermediate resistant isolates, serious potentially life threatening infections like meningitis or septicaemia must be treated with cephalosporins. However pneumonia and otitis media can be treated with high dose penicillin (5,21,22). All the penicillin resistant isolates were sensitive to amoxicillin. It is recommended that amoxicillin sensitivity of oxacillin resistant isolates be routinely confirmed by E test as oxacillin resistance is said to be generalizable for all beta lactams. Fortunately studies around the world, have shown that most oxacillin resistant isolates remained sensitive to amoxicillin (23,24).

Of concern in this study is the reduced sensitivity to erythromycin (22 out of 36 sensitive), which has been a valuable drug for pneumococcal infections especially as an alternative for persons allergic to penicillin. This finding has to be confirmed and monitored because of increasing global emergence and spread of Macrolide resistance<sup>25</sup>.

There is still a high level of sensitivity to chloramphenicol, although it does not provide adequate therapy for penicillin resistant pneumococcal meningitis because despite in-vitro susceptibility chloramphenicol has been associated with significant therapeutic failures in meningitis caused by penicillin – resistant pneumococci. This is thought to be due to loss of autolysins in penicillin – resistant strains resulting in chloramphenicol being bacteriostatic rather than bactericidal against such strains (26,27). So far, most isolates are still sensitive to the third generation antibiotics which is recommended for the therapeutic management of meningitis caused by penicillin resistant Pneumococci, but it is obvious that resistance is developing with >6% of isolates showing high level resistance. This trend needs to be monitored as resistance to cephalosporins is expected to rise with a rise in penicillin resistance since they share a similar mechanism of resistance (28).

The high level of tetracycline and co-trimoxazole resistance is consistent with the high level of penicillin resistant pneumococci and this situation has been in reported other parts of the world. In such settings a widespread and indiscriminate use of antibiotics, which is also found in Nigeria, is associated with high carriage rates and high level of antibiotic resistance. Rationale use of antibiotics when introduced in such settings led to reduction in antibiotic resistance and carriage rates (29).

Only 17 isolates were available for complete sensitivity testing. Unfortunately 19 were lost because of storage problem. Ideally *S. pneumoniae* should be stored at –70°C. This study is ongoing and -70°C freezer has since been purchased. Another major limitation of the study was the small sample size, a result of the exclusion criteria of

antibiotic use in the previous 2 weeks. This however should be seen as reflecting the reality of unregulated antibiotic use in Lagos. Nevertheless, in Nigeria where there are very few data, the results of this study enable one to formulate hypothesis for further studies.

In this study, the carriage rate of *S. pneumoniae* probably ranged from 2.6% to over 18.6% depending on the study population. If the limitations of this study are excluded in a larger study, the rate most likely will be higher. Also, penicillin resistance in carriage strains would be up to 6.8%, probably higher, but may be intermediate, so penicillin could still be useful for treatment of pneumonia and probably otitis media, but not meningitis. There is reason to watch out not only for increased penicillin resistance but also reduced cephalosporin and erythromycin sensitivity. Furthermore, most pneumococcal isolates would likely be resistant to tetracycline and co-trimoxazole

#### Acknowledgement

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**IMMUNOPROTECTIVITY OF ATTENUATED TURKEY POXVIRUS IN TURKEY POULTS AND BROILER CHICKS.**ODOYA, E.M.<sup>1</sup>; ABEGUNDE, A.<sup>2</sup>; AGYOGBO, B.G.<sup>3</sup>; OMOTAINSE, S.O.<sup>1\*</sup>; OGO, N.I.<sup>2</sup>; OGO, M.F.<sup>2</sup> AND GIDA, P.N<sup>1</sup>Nigerian Institute for Trypanosomiasis Research, Vom Plateau State Nigeria, <sup>2</sup>National Veterinary Research Institute, Vom, Plateau State Nigeria, <sup>3</sup>Department of Veterinary Pathology University of Agriculture Abeokuta, Nigeria, <sup>4</sup>Ministry of Health, Jos. Plateau State Nigeria.

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**ABSTRACT**

In Nigeria, fowl pox vaccine is used in all categories of poultry. However there has been reports of outbreak of turkey pox virus in poults previously vaccinated with fowl pox vaccine.

Pox Lesion from poults was excised for isolation of virus and viral propagation in chorioallantoic membrane. Turkey pox virus were isolated from the infected turkeys and confirmed by infecting susceptible turkeys with the isolate to reproduce the disease. Persistence of both turkey poults and broiler chickens to challenge after vaccination with attenuated turkey poxvirus was confirmed.

**INTRODUCTION**

Avian Pox is prevalent worldwide and birds of all ages sexes and breeds are susceptible to the virus (4). Avian pox is caused by four strains of poxvirus. Turkey poxvirus, Fowl poxvirus, Pigeon poxvirus and Canary poxvirus. Report shows that there is antigenic relationship between the poxviruses of the different avian species and it has been shown experimentally that the virus of one type of avian pox can give rise to disease in another and that infection in one may stimulate protection against another. (2)

However, turkey poxvirus is immunogenically distinct from fowl poxvirus (1). This is collaborated by

report in Vom, Nigeria that turkey poults suffered outbreak of pox after previous vaccination with fowl pox vaccine (4). The disease still remains a common complaint and constraint to growth of turkey industry in Nigeria.

**This work was carried out.**

- i. To isolate turkey pox virus
- ii. To attenuate the isolated virus
- iii. To ascertain the effectiveness of the attenuated turkey poxvirus in the control and prevention of turkey pox disease in turkeys.

## **MATERIALS AND METHODS**

Nodular lesion of turkey pox was excised from five mixed-bred turkey poults of eight weeks old. Excised lesions were weighed and ground with aid of sterile sand. A heat 20% w/v suspension was prepared with P.B.S., centrifuged at 2,500 r.p.m. for 30 minutes. The supernatant was decanted and stored in the deep freezer at  $-20^{\circ}\text{C}$ .

### **REPRODUCING THE DISEASE IN SUSCEPTIBLE TURKEYS**

3.0ml concentrated turkey poxvirus suspension containing  $10^3$  EID<sub>50</sub> was inoculated subcutaneously at multiple points on the head region of 5 (4 months old) Local bred susceptible turkeys.

$1.0\text{ml} \times 10^3$  EID<sub>50</sub> was inoculated subcutaneously on both wing web of 5 (5 weeks old) Local bred turkey poults.

### **PREPARATION OF MASTER SEED**

The master seed was prepared as a 50% solution with stabilizer. (Equal volume of infected membrane with equal volume of stabilizer). This was then dispensed into vaccine vials in 2mls aliquots and stored at  $-20^{\circ}\text{C}$  in the deep freezer.

### **PROTECTION OF BROILER CHICKEN AND TURKEY POULTS WITH TURKEY POX ATTENUATED VIRUS**

7 Broiler Chicks at 6 weeks old and 7 (8 weeks old turkey poults were immunized with 20<sup>th</sup> passage chorioallantoic membrane turkey pox virus. 2 poults and 2 broiler chicks were left unimmunized for control. The method was by subcutaneous inoculation of 0.5ml attenuated suspension containing  $10^{5.6}$  EID<sub>50</sub>. Birds were left for 3 weeks for observation and were later challenged with virulent fowl poxvirus.

## **TEST FOR PROTECTION**

Fowl pox challenge virus was used and the protected broiler chicken above were challenged together with the controls. The combs were scarified. Challenge fowl pox virus was dissolve in 2mls P.B.S. and was applied uniformly on the scarified comb protected turkeys and the control above were challenged by infecting subcutaneously at multiple points with 2mls P.B.S. dissolved fowl pox challenge virus.

## **RESULTS**

### **Reproducing the disease on susceptible turkey**

5 days post infection, multiple nodular lesion were observed on the head and upper neck region of the 4 month old susceptible turkeys. In the five weeks old poults, lesion appeared 4 days post infection.

### **Protection of Broiler chicken and turkey poults with turkey pox attenuated virus**

'Vaccine take' was observed at different points in all birds 3 days post inoculation.

### **Test for protection**

3 weeks after challenge the protected 7 broiler chicken and 7 turkeys showed no reaction to challenge while the control came down with the disease.

## **DISCUSSION**

It is the author's experience in previous fieldwork that fowl pox vaccine could not confer lasting immunity in turkeys but rather may be protective if used in chicken (4).

Cutaneous infection alone ordinarily cause low or moderate mortality and the affected birds generally return to normal upon recovery (1) Nodules though

progressively increase in size with scab formation but terminate with desquamation of the degenerated epithelium.

The result most significantly indicates that attenuated turkey poxvirus could protect strongly both chicken and turkeys from pox infection. Resistance of turkey poults to challenge after vaccination with attenuated turkey poxvirus confirms the immunogenicity of turkey pox antigen in turkeys.

It is recommended that turkey pox vaccine be developed specifically from turkey pox virus in the control and prevention of turkey pox disease. This will boost the growing turkey industry in Nigeria.

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**HIV SEROPREVALENCE RATES AMONG PROSPECTIVE SERVICE PERSONNEL IN A NIGERIAN SECURITY FACILITY****\*NWOKEDI, E. E., \*\*\* TAURA, AMINU A. \*\*DUTSE, A. I. \* BUKAR, A.****DEPARTMENTS OF MEDICAL MICROBIOLOGY / PARASITOLOGY, INTERNAL MEDICINE AND  
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**BACKGROUND:** There is no doubt that the greatest health problem threatening the human race in these times is the HIV/AIDS pandemic. The greatest burden of this scourge is in sub-Saharan Africa. According to the joint United Nations Committee on HIV/AIDS (UNAIDS), over 40 million people have been infected with the disease as of the end of 2001 and of which over 28 million are in sub-Saharan Africa. These are the group presenting themselves for paramilitary recruitment in Nigeria.

**OBJECTIVES:** The aim of this retrospective study is firstly to analyse the results of the HIV antibodies screening and confirmatory tests in order to determine the seroprevalence rate of HIV infection among this prospective service personnel. Secondly, to compare the prevalence rates between the males and females. Finally, to compare the seroprevalence rate in 2003 with that of 2002 among similar group.

**METHODOLOGY:** A total of 900 consecutive prospective recruits were screened for HIV antibodies using double technique. Confirmatory tests were then performed on positive sera using Immunoconfirmatory kits. Chi square was used to analyse the results.

**Of the 900 tested, 817 are males while 83 are females**

**RESULTS:** The prevalence is 1.4%. Overall in 2002, out of 431 officers screened in both groups, 8 (1.86%) were positive for HIV antibodies comprising 5 men (1.16%) and 3 women (0.69%). In 2003, Out of a total of 900 recruits tested for HIV seropositivity, 13 (1.4%) were found to be seropositive with a 95% confidence interval of 1.0% to 1.8%.

**DISCUSSION AND CONCLUSION:** Our results show a seroprevalence of 1.44% among members of this security outfit and it is quite low compared to the national average of 5%. Our findings are low compared to seroprevalence rates among ANC clients, Tb patients, STD clients and blood donors<sup>1</sup>. However, it is comparable to 1.7% among another group of paramilitary in 2002, 1.4% among people with leprosy and 1.8% in Jigawa State sentinel survey. Health education is advised.

## INTRODUCTION

There is no doubt that the greatest health problem threatening the human race in these times is the HIV/AIDS pandemic. The greatest burden of this scourge is in sub-Saharan Africa. According to the joint United Nations Committee on HIV/AIDS (UNAIDS), over 40 million people have been infected with the disease as of the end of 2001 and of which over 28 million are in sub-Saharan Africa (1,2,3). Although the first case of AIDS in Nigeria was in 1986, the epidemic has now reached an alarming proportion. The national median prevalence of HIV infection in the general population in Nigeria from the sentinel surveys was found to be 5% in 2003 up from 1.8% in 1991. HIV infection has spread slowly in Nigeria than in many other African countries. For example, in several South African and East African countries, HIV prevalence among 15 to 49 years old is now estimated at 15-30%. No one is quite sure why the epidemic has spread more slowly in Nigeria and in some other West African countries. However, Nigeria's HIV prevalence rate is one of the highest in West Africa, second only to Cote d'Ivoire (9.7%) (1). Due to its large population size, Nigeria now has the fourth highest number of HIV infected people in the world, behind South Africa, India and Ethiopia (2,3). Evidence from sentinel surveillance suggests that HIV prevalence is still on the rise in Nigeria. Analysis based on the ANC HIV seroprevalence surveys show that the prevalence in the 15-49 year age group in Nigeria is still rising, going from 1.8% in 1991 to 3.8% in 1994 to 5.8% in 2001. Experience in other parts of Africa suggests that HIV prevalence in Nigeria could rise higher than at present (1). The impact of this on the nation will be immense viz increase in new AIDS cases yearly, AIDS deaths among 15-49 years of age, cumulative AIDS deaths, AIDS orphans, number of persons with

tuberculosis etc and decrease in the work force, life expectancy and of general population (1).

The largest numbers of HIV infected individuals are heterosexual young men and women living in sub-Saharan Africa including Burkina Faso, Cote d'Ivoire, Ghana and Nigeria. This group accounts for about 60% of the world total HIV infected persons and almost 90% of the current 28 million HIV infections in adults and adolescents in Africa (2). WHO (4) reported that the rates of newly acquired HIV infections are highest in the 15-40 year old groups among both females and males in most sub-Saharan Africa. These are the productive age groups that are presenting themselves for recruitment into the various security agencies in Nigeria. Within the military, police, and other uniformed services, which occupy critical positions within Nigerian society, AIDS control units exist but these are under funded and not as effective as they could be. The police are actually a larger uniformed service than the military and share many of the same risk factors. However, the police response to the epidemic needs bolstering (1). As with most employers of labour in Nigeria, a medical certificate of fitness is now required from a government hospital. This paramilitary outfit is no exception. During the 2002/2003 recruitment exercises, those intending candidates were counselled and offered voluntary testing for HIV among other laboratory tests. The aim of this retrospective study is firstly to analyse the results of the HIV antibodies screening and confirmatory tests in order to determine the seroprevalence rate of HIV infection among this prospective service personnel. Secondly, to compare the prevalence rates between the males and females. Finally, to compare the seroprevalence rate in 2003 with that of 2002 among similar group.

## **MEN, MATERIALS AND METHODS**

A total of 900 consecutive prospective recruits were screened for HIV infection at Kano Teaching Hospital between December 2002 and January 2003. Of these officers who are apparently healthy, eight hundred and seventeen (817) were males and eighty three (83) were females. Following counselling, standard aseptic procedures were used in sample collection and processing. Only 4-5ml of venous blood was obtained. Using Capillus, a rapid agglutination test and method, Biotech Ireland (5), all serum samples were screened for HIV antibodies. Every positive serum sample with the first kit was subjected to Immunocomb HIV 1 and 2 Bispot. Another different method and kit (Immuno-comb orgenics Israel) (6). Immuno confirmatory kit (HIV 1 & 2 Immunoconfirm II) (7) was then employed for greater assurance. The result was subjected to statistical analysis and was later released afterwards with strict confidentiality and necessary counselling.

## **RESULTS**

The ages of the recruits ranged from 21 to 39 years with a mean age of  $28 \pm 2.3$  years. Out of a total of 900 recruits tested for HIV seropositivity, 13 (1.4%) were found to be seropositive with a 95% confidence interval of 1.0% to 1.8%. When considered separately of the 817 male recruits, 7(0.9%) were HIV seropositive compared to 6(5.6%) of the 83 females. This difference was

statistically significant ( $X^2=14.8$  at  $P<0.001$ ) as shown in table I.

Similarly when divided into age groups, there was an increasing trend in seropositivity among all recruits from 1.1% in the youngest age group (20-24 years), through 1.6% in (25-29) years age group, to 1.9% among those within the 30-34 years age bracket. However, none of the older recruits (35-39 years) was seropositive, this increasing trend was not statistically significant ( $X^2$  trend = 0.08 at 3df and  $P=0.77$ ). Table II.

Overall in 2002, out of 431 officers screened in both groups, 8 (1.86%) were positive for HIV antibodies comprising 5 men (1.16%) and 3 women (0.69%). In 2003, Out of a total of 900 recruits tested for HIV seropositivity, 13 (1.4%) were found to be seropositive with a 95% confidence interval of 1.0% to 1.8%. When considered separately of the 817 male recruits, 7(0.9%) were HIV seropositive compared to 6(5.6%) of the 83 females.

## **DISCUSSION**

Our results show a seroprevalence of 1.44% among members of this security outfit and it is quite low compared to the national average of 5%. Our findings are low compared to seroprevalence rates among ANC

**TABLE I DISTRIBUTION OF HIV SEROPOSITIVITY BY GENDER IN PARAMILITARY RECRUITS**

Gender	No. positive (%)	No. negative (%)	Total
Male	7 (0.9)	810 (99.1)	817 (100.0)
Female	6 (7.2)	77 (92.8)	83 (100.0)
<b>Total</b>	<b>13 (1.4)</b>	<b>887 (98.6)</b>	<b>900 (100.0)</b>

$\chi^2 = 14.8$  at 2dF and  $P < 0.001$

**TABLE II DISTRIBUTION OF SEROPOSITIVITY BY AGE AMONG PARAMILITARY RECRUITS**

Age Group	Seropositive	Seronegative	Total
	No. (%)	No. (%)	
20 – 24	3 (1.1)	279 (98.9)	282
25 – 29	6 (1.6)	362 (98.4)	368
30 – 34	4 (1.9)	206 (98.1)	210
35 – 39	-	40 (100.0)	40
<b>Total</b>	<b>13 (1.4)</b>	<b>887 (98.6)</b>	<b>900 (100.0)</b>

$\chi^2$  trend = 0.08 at 3dF and  $P = 0.77$ . Not significant.

However, it is comparable to rates among other recruits in 2002 (1.7%) (4) and leprosy patients (9). Moreover considering that about half of those positive (5.6%) are women, there is need to be concerned. This difference between females and males is statistically significant ( $X^2=14.8$ ). Projecting this into the future with the HIV infection long incubation period (5-15years) and adventurism among this sexually active group, the thirteen recruits today may translate into tens of Nigerians that may live with HIV/AIDS. There is therefore the need to educate the members of all paramilitary groups on the ABC's of STD control and behavioural change in order to avert any disease spread in the population. There is need to incorporate health education among this group since there is no cure for now and the treatment is life-long, expensive and out of reach of most Nigerians. In our preliminary report last year, the prevalence rate was 1.7% among 400 paramilitary group while it is 1.4% a year later among 900 members. The difference is not statistically significant ( $X^2$  at Pvalue 0.05 and IdF = 0.36). Based on our findings above, we are tempted to conclude that the prevalence of HIV infection among some security groups in Nigeria is quite low 1.56%. The national average is 5.8%. HIV infection rate among female members is generally higher than their male counter part.

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**"PREVALENCE OF INTESTINAL PARASITES AND ITS ASSOCIATION WITH SOCIODEMOGRAPHIC, ENVIRONMENTAL AND BEHAVIORAL FACTORS IN CHILDREN IN POKHARA VALLEY, NEPAL"**

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**ABSTRACT:**

Intestinal parasitosis in children is the commonest infection across the globe. Epidemiological research carried out in different countries has shown that prevailing socioeconomic condition is the single most powerful determinant of prevalence of parasitic infection in a society. The objectives of this study were to assess the prevalence of intestinal parasitosis in different pediatric age group (1-14 year) children, to identify the causative socio-demographic determinants and also to assess the controlling factors. 5236 stool samples of children of both sexes, aged 1-14 years were processed at Manipal Teaching Hospital, Pokhara, Nepal. Out of 5236 stool samples, male were 3004 (57.4%) and females were 2232 (42.6%). *Giardia lamblia* showed highest prevalence both in male and female children 21.50% and 20.25% respectively. Other parasites in male children were *Entamoeba histolytica* (8.05%), *Ascaris lumbricoides* (2.86%), *Enterobius vermicularis* (1.66%) and *Ancylostoma duodenale* (0.73%). The same in female children were as follows: *Ascaris lumbricoides* (1.34%), *Ancylostoma duodenale* (0.94%) and *Hymenolepis nana* (1.03%). Highest prevalence of dual pathogenic protozoan seen in <5 years of age in male was 41.8% whereas in older males >10 years was 35.9%. Older girls of 10-14 years had 22.3% *Entamoeba histolytica* and *Giardia lamblia* parasitosis as opposed to the same age group boys who had 20.84% dual protozoal infestation. Mostly single pathogenic species was detected from all the samples except 10 patients, who had double parasites e.g., *Giardia lamblia* with *Entamoeba histolytica*, *Hymenolepis nana*, *Entamoeba coli* or *Ascaris lumbricoides*. A significantly low ( $p < 0.05$ ) prevalence rate of intestinal parasitosis in children using toilet (15.74%) was noted as compared to those who were not using toilets (69.40%). Similarly, significant ( $p < 0.05$ ) was the lower prevalence rate of intestinal parasitic positivity in urban children (26.33%) as compared to that of those who were residing in rural areas (54.19%). Children from upper caste belonging to higher socio-economic group had significantly low ( $p < 0.05$ ) intestinal parasitosis (20.27%) as opposed to lower caste children belonging to lower socio-economic group (49.16%). According to the results, low level of education and consequently poor socio-economic and hygienic condition of families appear to be powerful determinants of intestinal geoparasitosis.

**KEY WORDS:** Intestinal parasites, children, Pokhara valley, Nepal.

## INTRODUCTION:

Intestinal parasitic infection is one of the commonest and major causes of public health and in turn socio-economic problems in the world especially in developing nations like India, Nigeria, Bangladesh and other countries (1,2,3). People from poor sanitation, improper hygienic practices and lower socio-economic groups are reported to have a very high burden of intestinal parasitic infestation with prevalence rate as high as 40–59% (4, 5). Nearly 200 million people are infected with *Giardia lamblia* while *Entamoeba histolytica* infects about 10% of the world population (5). In 1997, it is estimated that amoebiasis, 45 million people carried *Entamoeba histolytica* in their intestine and 1/10<sup>th</sup> of them suffer from invasive form of amoebiasis which accounts for 70,000 deaths per year (4). Intestinal parasites reportedly affect both on nutritional and immune status of individuals. The reported prevalence of intestinal parasitosis in Nepal vary considerably from one study to another with over 90% prevalence in some areas<sup>6</sup>. Manipal(6). Teaching Hospital, Pokhara valley, Nepal serves as a referral center for pediatric and young adolescent population in and around Western region of Nepal. Most of the patients belong to low socio-economic strata having inadequate safe water supply and lack of proper environmental sanitary facilities. This two-year study reports a prospective analysis of serious enteric parasites and associated epidemiological factors. Similar study in school children has also been done in Bangladesh and Malaysia with comparable results (3, 9).

## MATERIALS & METHOD:

**Study area:** This was a cross sectional prospective study done in Manipal Teaching Hospital from Jan, 2000 to Dec, 2002. Children attending to out-patient as well as in-patient pediatrics department of Manipal Teaching Hospital

which caters a specialized tertiary patient care services to around 4.5 lakhs of population in and around Pokhara valley, situated in the Western region of Nepal. Patients of both rural and urban population from neighboring 40 VDCs (village development committee) in Pokhara and Lekhnath municipality of Kaski district under Gandaki zone were seeking medical services from this hospital. Children belonging to upper caste Brahmin-Chhetri were mainly coming from urban area and lower caste Gurung, Thapa, Tamang, Magar, Limbu, Sarki and Sunar children were mainly from villages under those above mentioned districts. In those villages, there is no direct road links. Water supply from natural sources of dhara (flowing water), dugwell, stream, brooklets and community piped water supply from hill-top fountains. Excreta disposal in villages are primitive type e.g., open field defecation, self-made pit-latrines or rarely seen sanitary latrines, made by non governmental organizations. **Study subjects and sample collection:** A total of 5236 stool samples over 2 year period were examined. Stool samples obtained from children of both sexes aged 1–14 years were found to contain one or other type of intestinal parasites. In this study, children were coming from the above mentioned neighbouring urban and rural areas. The samples were collected from individual patients who were attending the out-patient or in patient department of hospital with complaints of anaemia, failure to thrive, nausea, vomiting, loose motion and abdominal pain. The samples were sent to the laboratory for immediate processing without any delay. A proforma was made regarding age, sex, presenting complaints, duration of illness, number of siblings, dietary habits, housing, types of latrine used, personal hygiene and any medication used prior to presentation. A written informed consent was taken from each patient.

Parasitic examination: 4 - 5 grams of stool samples were collected in a Universal plastic container [10 ml capacity] without any preservative and necessary precaution was taken to avoid contamination. For suspected pinworm cases, Scotch tape similar to NIH (National Institute of Health) swabs were improvised as well as perianal examination was done. All samples were examined on the same day of collection. The samples were then observed making a saline and an iodine preparation by formal-ether concentration method (7). The method of estimation of parasitic burden in the intestine was adopted as mentioned by Cheesebrough (8). Nearly 9-10 stool samples were examined per clinic day in addition to routine urine, full blood count including absolute eosinophil count and serum electrolytes were done according to requirement of individual patient.

Drug Distribution: All stool positive children were given anti-parasitic medications according to the specific types

of parasite detected in each case namely albendazole, mebendazole, metronidazole, praziquantel and piperazine citrate.

Statistical analysis: The results obtained were analyzed with Epi-info version 6 programmes of WHO/CDC. Chi-square test and simple distribution of the variables were used to quantify the "p" value of the relationship of the variables.

## RESULTS:

During the 2-year period, 5236 stool specimens were examined at Manipal Teaching Hospital Pathology department. Out of 5236 samples examined, 3004 (57.4 %) were from male and 2232 (42.6%) were from female children. Out of 5236 stool specimens, 2036 (38.8%) stool specimens were positive for various types of intestinal parasites.

**Table 1. Prevalence of *G. lamblia* and *E. histolytica* by age-sex distribution**

	Age (in years)	Total Tested	<i>G.lamblia</i> (%)	<i>E.histolytica</i> (%)	Both parasites(%)
MALE	Less than 5	1272	330(25.95)	118(9.2)	448(41.8)
	5 to9	1070	210(18.7)	92(8.85)	302(41.1)
	10 to 14	662	106(16.01)	32(4.83)	138(20.84)
	Total	3004	616(21.50)	242(8.05)	888(29.56)
FEMALE	Less than 5	892	174(19.50)	46(5.15)	220(24.66)
	5 to9	854	194(22.7)	52(6.07)	246(28.7)
	10 to 14	484	84(17.3)	24(4.9)	108(22.3)
	Total	2232	452(20.25)	122(5.46)	574(25.71)

**Table 2. Prevalence of individual parasite among male and female children**

	MALE		FEMALE		TOTAL
	(3004)		(2232)		(5236)
Parasites	Positive	Percentage	Positive	Percentage	
G. lamblia	646	21.5	452	20.25	1098 (20.97)
E. histolytica	242	8.05	122	5.46	364 (6.95)
E. coli	136	4.52	119	5.33	255 (4.87)
A. lumbricoides	86	2.86	30	1.34	116 (2.2)
Taenia spp.	20	0.66	18	0.80	38 (0.72)
H. nana	28	0.93	26	1.18	54 (1.03)
A. duodenale	50	1.66	18	0.80	68 (1.29)
E. vermicularis	22	0.73	21	0.94	43 (0.82)
Total	1230	40.94	806	36.11	2036 (38.88)

**Table 3. Prevalence of intestinal parasitosis among children having toilets and no toilets at their houses.**

Toilet	Total no	Positive no	percentage
Yes	2945	446	15.14
No	2291	1590	69.40
Total	5236	2036	38.88

**Table 4. Prevalence of intestinal parasitosis among children of rural and urban dwellings**

Dwellings	Total no	Positive no	Percentage
Urban	2878	758	26.33
Rural	2358	1278	54.19
Total	5236	2036	38.88

**Table 5. Prevalence of intestinal parasitosis among children of higher and lower social status.**

Social status	Total no	Positive no	Percentage
Higher	1864	378	20.27
Lower	3372	1658	49.16
Total	5236	2036	38.88

intestinal parasites is shown in Table 1. The distribution was not uniform for all ages. The prevalence rate of common intestinal protozoa *E. histolytica* and *G. lamblia* in 10-14 year old male children (20.84%) was lower as compared to female children (22.3%) of same age, which was statistically significant ( $p < 0.05$ ). In male children, prevalence rate of *Ascaris lumbricoides* (2.86%), *Ancylostoma duodenale* (1.66%) and *Enterobius vermicularis* (0.73%) were noted. A prevalence of *E. histolytica* (8.05%) and non-pathogenic *E. coli* (4.52%) were recorded in male children. The least common intestinal parasite *Enterobius vermicularis* was found in 0.73% in male and 0.94 % in female children, conspicuous by their relatively low occurrence. *H. nana* and *Taenia* spp. were detected in 1.03% and 0.72 % respectively. We could not differentiate the various species of Taeniasis. *Ancylostoma duodenale* was detected in male and female children 1.66% and 0.80% respectively. Mostly single pathogenic species was detected from all the samples except 10 patients, who had double parasites e.g., *Giardia lamblia* with *E. histolytica*, *Hymenolepis nana*, *E. coli* or *Ascaris lumbricoides*. A significantly low ( $p < 0.05$ ) prevalence rate of intestinal parasitosis in children using toilet (15.14%) was noted as compared to those who were not using toilets (69.40%). The urban dwellers were having 26.33% as opposed to rural dwellers of 54.19% of intestinal parasitosis. In Table 5, it is clearly shown ( $p < 0.05$ ) that those belonging to higher socio-economic group of children, residing in urban settlement, were having intestinal parasitosis (20.27%) as opposed to those who belonged to lower socio-economic status. According to the results, low level of education and consequently poor socio-economic and hygienic condition of families appear to be powerful determinants of intestinal geoparasitosis.

## DISCUSSION

In Manipal Teaching Hospital, Pokhara valley, Nepal from Jan 2000 - Dec 2002, 5236 stool samples were processed from children who presented with abdominal pain, diarrhea, vomiting, anemia and failure to thrive. Out

of the total 5236 stool specimens, 2036(38.88%) samples were positive for all parasites. Of the 5236 samples examined, 3004(57.37%) were from male and 2232(42.62%) were from female children. Present study reveals that (1230/3004; 40.9%) of boys and (806/2232; 36.11%) of girls of Pokhara valley suffered from one or other type of intestinal parasitosis. This study also showed that the parasitic prevalence was uniformly distributed in both sex groups which are in conformity with other similar studies done else where in Nepal, India, Bhutan, Thailand and other South Asian countries (6,9a,9b,9c).

The caste system and class distinction of Nepali society has made great impact on the health and education in Nepal. Children from upper caste belonging to higher socio-economic group had significantly low ( $p < 0.05$ ) intestinal parasitosis (20.27%) as opposed to lower caste children belonging to lower socio-economic group (49.16%). According to the results of this study, low level of education and consequently poor socio-economic and hygienic conditions of families appear to be powerful determinants of intestinal geoparasitosis. Significantly higher prevalence rate ( $p < 0.05$ ) among the lower socio-economic group (Sunar, Sarki, Nepali, Magar, Gurung, Thapa, Pun, Tamang and other backward classes) appeared to be associated with their relatively low literacy rate, unhygienic habits and low socio-economic status compared with upper caste Nepalese (Brahmin and Chettri). The prevalence of intestinal parasitosis found to be associated with socio-economic status, dwelling condition, family size, sanitary disposal and toilet use, type of water supply for cooking and drinking and practice of personal hygiene and habits. A significantly lower prevalence rate ( $p < 0.05$ ) in upper caste Nepalese

was associated with their relatively higher literacy rate and health awareness as compared with the lower caste Nepalese as was evident from our study results. A significantly high prevalence among young children (aged 10 years) appeared to be associated with their unhygienic habit and age. A significantly low ( $p < 0.05$ ) prevalence rate of intestinal parasitosis in children using toilet (15.74%) was noted as compared to those who were not using toilets (69.40%). There was a significant difference ( $p < 0.05$ ) in parasite prevalence among children having toilets (pit latrines) and without toilets indicated that the parasitic burden was directly related to excreta disposal in the environment. This is in conformity with similar type of study done in Cuba (10) and Iran (11). This was attributed mainly due to the open air defecating habits of lower caste children. Though not investigated but the significantly higher prevalence of parasitosis in children drinking piped water could be due to the fecal contamination of drinking water supply system as has been seen else in other parts of the country. A very high percentage of fecal contamination of drinking water has been reported even in the capital Kathmandu (12). All stool positive children were treated with anthelmintic and anti-protozoal medications. They had no facility of adequate safe water supply and sanitary latrines. Other factors responsible were lacwere inadequate environmental sanitation, poor personal hygiene, low literacy and hilly inaccessible part on the mountain top where health care facility i facilities were unreachable. Poor living condition with lack of portable water, and last but not the least low socio-economic status of status of this underprivileged group of people.

In all the district areas, there was both inbuilt sewage system as well as open drains in many places even

in the heart of the city. Similarly, significant ( $p < 0.05$ ) was the lower prevalence rate of intestinal parasitic positivity in urban children (26.33%) as compared to that of those who were residing in rural areas (54.19%). In the rural areas, people living under no brick-built dwellings where sanitary latrine system was almost non-existent. The inhabitants used open fields for defecation and urination, which was more a habit than a real necessity. The children playing in the rural areas in the dirt and soil, children used to get the ova stick to their nails. *Ascaris lumbricoides*, *Giardia intestinalis*, *E. histolytica*, *Hymenolepis nana* and *E. coli* are common intestinal parasites constituting a public health problem in the communities of poor hygienic standard, lack of sanitation and low socioeconomic status.

Older female children of 10-14 years were found to harbor intestinal protozoa more (108/484; 22.3%) than the same age group male children (138/662; 20.84%) and overall the older children above 10 years were harboring less number of parasites than the younger group of children as evident from Table 1. The higher rate of prevalence of parasites (22.3%) in 10 to 14 years age group female children may be due to their close physical proximity to their sick siblings in the family as a helping hand to the mother in accordance with the local tradition and custom. Mahendra Raj S et al in 1997 reported similar findings from Malaysian peninsula (13).

Male children have higher education facility as compared to their peer female children in the Nepali society. *G. lamblia* was found to be the most common protozoa harboured (646/3004; 21.50%) in male and (452/2232; 20.25%) in female children. In this study no AIDS, cystic fibrosis or hypogammaglobulinemic cases documented which were closely associated with *G. lamblia*

infestation (14). A study done by Zikan <sup>14</sup> in Saudi Arabia had also shown the inexplicably high prevalence of *G. lamblia* infestation among the school going children in Saudi Arabia (15). The next common parasite was *I. histolytica* (242/3004; 8.05%) in male and (122/2232; 5.46%) in female children whilst the third most common parasite (a non-pathogenic one) was *Entamoeba coli* (255/5236; 4.87%) was documented in our study. *Ancylostoma* spp. ova were infrequent though the bare foot walking and drinking contaminated water were common habits among the rural children. Similar findings have been reported in the work of Steiner et al in 199<sup>16</sup>. Our results showed that < 5 years age group male have the highest (448/1272; 41.8%) rate of infestation. There seems to have an influence of age on the prevalence of infestation of parasites ( $p < 0.05$ ). Other investigators have also mentioned the age related variation of prevalence of parasites<sup>17</sup>. The reduction of parasitic infestation with increasing age may be explained by the fact that older children have more awareness of personal hygiene than the younger ones.

Hookworm infestation is seen mainly in moist and warm climate in tropic and subtropics between 45°N and 30°S of the equator (e.g., Asia, Africa, Central and South America). The paucity of hookworm, *Ancylostoma duodenale* (68/5236; 1.29%) in this study was interesting. Probably environmental factors did not play a significant role in its transmission<sup>18</sup> or were detrimental for the survival of the hookworm ova and larvae in the hilly regions of Nepal. The Scotch tape and perianal examination when required revealed the overall prevalence of *Enterobius vermicularis* was only (43/5236; 0.82%) which is in conformity with other reports from

other endemic zones as studied by Sung, J. F. et al, in Taiwanese school children<sup>19</sup>.

The low prevalence of *A. lumbricoides* (116/5236; 2.2%) was remarkable. Lack of surface water of plane land and presence of relatively less humid dry weather of hilly regions of Nepal might be detrimental for the survival of these parasites.

Children presented with vague abdominal, anaemia, failure to thrive and diarrhoea were always suggested to undergo stool examination. The higher or lower percentage of stool testing did not coincide with the higher or lower percentage of stool positivity. Therefore to find out all agents associated with intestinal symptoms further investigations in the form of routine stool microscopy as well as stool concentration methods were performed. During this study 16 samples, which were negative by direct microscopy, were found to be positive by Formalin-ether concentration technique. This indicates that concentration method should be compulsory for the microscopy of the negative stool specimen which might give a higher yield of positivity. *Giardia lamblia* was more often found in faecally contaminated water. In Pokhara valley, both rural and urban people used natural sources e.g., dhara (flowing water), dug well, streams, brooklets and community-made piped water or DWSC (Drinking Water Supply Corporation; non governmental organization funded) water for drinking or cooking purposes although transmission through dug well water or ova and cyst contaminated unwashed fruits or vegetables could not be ruled out. Similarly, person-to-person contact among family members, especially among siblings, may also be another factor for transmission of *G. lamblia*. The importance of animal reservoirs like beavers, cats, dogs and other mammals as a source of

human infection was unclear because the parasites in the intestine of man and other mammals are indistinguishable. Zoonotic transmission has also been suggested for *Giardia lamblia* (20) but its possibility in Pokhara region was very remote because of the absence of beavers and other reservoir rodents in this region. The isolation rate of *G lamblia* was a little higher as compared to other study done by Ram R et al in Indian born Nepali children in Darjeeling district (21). It may be inferred that *G lamblia* is primarily associated with abdominal symptoms among these children although there may be inapparent infection with other intestinal pathogens as well. Based on the two techniques used, the results from the Scotch tape provided a higher sensitivity for the detection of *Taenia* spp. and *Enterobius vermicularis* eggs as had also been done in Saudi Arabia (22). Intestinal helminthic polyparasitism was seen only in 10 cases. A programme to fight against geohelminths in school children should be initiated as a public health priority. Albendazole, among other antiprotozoal and anthelmintic medications was the drug of choice. Frequency of drug distribution should be based on the prevalence of geohelminths in specific endemic areas. Prevalence and intensity of infection was low probably due to periodic antihelminthic treatment (funded by non governmental organizations) offered by the local health authorities. Improvement in mother's level of education brought about decrease in prevalence of intestinal helminthiasis among the children belonging to upper caste society residing in the urban settlement. Similar observation had also been noted in Haitian children in Latin America (23). Therefore, improvement in female education in lower caste Nepalese residing in the rural

areas should be encouraged to reduce the incidence of communicable disease in the family.

#### CONCLUSION:

The study has shown that protozoan cysts have been the main cause of gastrointestinal manifestations among children in Pokhara valley, Nepal. This study must create awareness among the local community and health authority, which should actuate them to take necessary steps to minimize the transmission of parasite in children. This study also represents not only intestinal parasitosis in children of Pokhara valley, Nepal but also depicts real picture of parasitic infestation in other 3<sup>rd</sup> world countries of Asia, Africa and Latin America. Therefore, suitable prophylactic measures, early detection and anti-protozoal and anthelmintic treatment will go a long way in containment and lessen the burden of parasitic infestations among children in this part of the globe as well as socio-economically deprived section of people in developing nations of the world. School-based health education should be implemented in order to prevent and control intestinal parasitosis.

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## RECENT TRENDS IN MANAGEMENT OF MALARIA IN PREGNANCY

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### ABSTRACT

Malaria remains a significant causal factor in both maternal and fetal morbidity and mortality in this environment though it is essentially preventable. There are increasing incidence rates worldwide, including those areas of the world where, hitherto, malaria infection was rare.

More women than before now present with clinical malaria in pregnancy with both obstetric and non-obstetric complications, including severe anaemia, IUGR, miscarriage etc. Recent rapid diagnostic tests are available, more sensitive and specific than microscopy but their applications are limited in scope.

Antimalarial Combination Therapy (ACT), Intermittent Preventive Treatment (IPT), Insecticide Treated Nets (ITNs), good and adequate antenatal (ANC), intrapartum and postpartum care will ensure optimal health and reduction in the incidence rate of malaria infection in pregnancy.

**KEYWORDS:** RECENT TRENDS, MALARIA IN PREGNANCY, ROLL BACK MALARIA, ANTI-MALARIA COMBINATION THERAPY (ACT), INSECTICIDE TREATED NETS (ITNs).

### INTRODUCTION

Malaria continues to be a scourge in tropical and subtropical regions of the world (1,2). Currently, it is endemic in about 100 countries, affecting 40% of world's population. The worldwide prevalence of the disease is estimated to be between 300 – 500 million clinical cases annually. Annual mortality due to malaria is estimated to be between 0.5 – 2.5 million people. More than 90% of world's malaria occurs in Sub-Saharan Africa. Malaria has been eliminated or effectively suppressed in several parts of the world

in past decades but is now undergoing resurgence (3). It is returning to areas from which it had been eradicated as well as spreading into new areas such as central Asia and Eastern Europe. Despite global economic development, people are dying from malaria now than 30 yrs ago.

### EPIDERMIOLOGY AND CLINICAL FEATURES

Malaria in pregnancy remain a notable cause of maternal and perinatal morbidity and mortality, often associated with maternal illness, maternal anaemia, low

birthweight, preterm delivery and perinatal loss especially in the primigravidae (1-4). In semi-immuned pregnant women, malaria infection may be asymptomatic, pregnant women are at risk of clinical disease compared to non-pregnant women at all levels of endemicity (5). There is also destruction of both parasitized and unparasitized blood cells leading to a greater level of anaemia than can be explained on the basis of the RBCS parasitization alone (2,4).

It has been suggested that HIV positive pregnant women have malaria like non-immunes (5) and as such holoendemic regions like Africa, face the risk of the deleterious effects of malaria in previously semi-immuned pregnant women.

Primigravidity is a known risk factor in malaria in pregnancy. It not only becomes more prevalent in primigravidae but also more intense (4-7). The use of antimalarials in HIV/AIDS patients is thought to alter the glutathione levels and may exacerbate the oxidation-reduction imbalance attendant on HIV infection.<sup>6</sup> There is increasing incidence in traveller's malaria noted in USA, Eastern Europe etc - this may be more related to the effect of globalisation, spread of chloroquine resistant strains of plasmodium species. Also the transmission rate and the degree of severity is worse in *P. falciparum* malaria than others hence worse severity is noted in African pregnant women.

The peak prevalence of parasitaemia will be altered by prior anti-malarial ingestion. In a study from Madan, Papua New Guinea, the peak prevalence in primigravidae studied reached 55% to compared to 86% in another study from Kenya (4,8,9). Studies have also suggested that the highest prevalence of infection occurs in the 2<sup>nd</sup> trimester with infection rates at delivery and in the postnatal period

approximating to levels in non-pregnant women possibly due to immunity boosting during the course of pregnancy (7).

Severe forms of the disease as reported by several authors (10-12) with hypersplenism (TSS) was prevalent then but it appears the prevalence of such severe form of malaria is rarer today especially in those semi-immuned patients who are routinely on malaria prophylaxis throughout the pregnancy (5,12,13) and linear growths have been reported in such primigravidae. Malaria is associated with miscarriages, preterm labour, Intrauterine growth retardation(IUGR) and even Intrauterine fetal death(IUFD). These effects are produced by maternal and fetal hyperpyrexia, severe maternal and fetal anaemia as well as placental parasitization. It was previously thought that malaria parasites do not cross the placenta into the fetal circulation. However recent studies have confirmed that congenital malaria is common in areas of high endemicity and is related to the immunological status of the mother (1-4,15,16).

#### **The role of Chondrotic sulphate A (CSA).**

Women living in endemic areas who were resistant to malaria between their pregnancy tended to lose this protection when they became pregnant.

This observation has often been interpreted as a consequence of the immunosuppression that is necessary to protect the fetus from being rejected by the mother's immune system. However, in malaria - endemic areas this increased susceptibility to malaria is disproportionately higher in primigravidae, a fact that is not easily explained by the fetus - related immunosuppression explanation.

The parasitized RBCs found in the placenta of primigravidae show a remarkable preference to binding to Chondroitin sulphate A (CSA), which is a ligand that is present on the placental syncytiotrophoblast but is not

readily accessible on cells elsewhere in the body. The high frequency of parasite found in the placenta that bind CSA can explain the susceptibility of primigravidae to clinical malaria and points to the importance to protection of immunization that is specific for parasite variants (14,15).

According to this hypothesis, any parasites that have adhesion specificity for CSA are eliminated from a non-pregnant individual, owing to a lack of a suitable adhesion receptor on the host cells, and presumably before they have induced appreciable levels to the CSA – specific plasmodium falciparum gene variants. In contrast, because CSA becomes available in the developing placenta of primigravidae, parasites that are able to bind CSA and are present in the blood can suddenly multiply unhindered. With successive pregnancies, it can be assured that the levels of antibodies that are directed against the gene variant molecules that can bind CSA increase, and are therefore able to limit the multiplications of CSA binding parasites; protection against pregnancy-associated malaria can be gradually acquired in this way (15).

The concept of Roll Back Malaria (RBM) Initiative is the only global initiative for the control of malaria. RBM is a global partnership to fight malaria, relying on national programmes on malaria control, international agencies/organisations, private sector participants and NGOs coming together to control or eradicate malaria. It is to further strengthen the existing strategies and interventions in order to maximize the impact of contribution from major stakeholders. This RBM initiative is to span a 10 year period (2001-2010) and has the following important elements:

- Bringing reliable, sustainable malaria prevention and early treatment to affected population.
- Investing in research and development of effective and affordable tools.

- Evaluating achievements against clearly defined goals; and.

- Building human and institutional resources (17).

The six critical elements of RBM which work together to help break the cycle of malaria transmission, cure patients and support developments are as follows:

- Evidence-based decisions using surveillance, appropriate response and building community awareness.
- Rapid diagnosis and treatment supporting home care, direct access to effective medications and wide availability of health services.
- Multiple prevention using insecticide-treated, net, environmental management to control mosquitoes and making pregnancy safer.
- Focused research to develop new medicines, vaccines and safe insecticides.
- Well coordinated action for strengthening existing health services, policies and providing technical support and
- Harmonized action to build a dynamic global movement.

## DIAGNOSIS OF MALARIA

Prompt and accurate diagnosis is the key to effective disease management, one of the main interventions of the global malaria control strategy (19,20). Recent efforts have yielded more tools to diagnose reliably and accurately malaria other than the old reliable though cumbersome microscopic examination of blood.

Microscopy can still be considered as the 'gold standard' so long as highly qualified professionals are involved, quality of equipments and starting reagents are maintained and the type of blood smears (thick and thin films) are considered amongst others (19).

Rapid tests offer a complete package with all accessories needed, and their performance should be reliable, easy and safe to use even for junior staff and in

rural settings where microscopic examination of blood and all that it entails is cumbersome.

Rapid Diagnostic Tests (RDTs) are used to detect serum antigens of the plasmodia species using fixed antibodies onto strips of paper. The 2 main types are:

-Those that detect parasite enzyme lactose dehydrogenase (PLDH) from all four plasmodium species that affect humans. These can distinguish between *P. falciparum* from other species, but cannot distinguish between *P. vivax*, *P. ovale* and *P. malariae*.

-Those detecting histidine rich protein II (HRPII). This antigen is only produced by *P. falciparum*.

There are a number of disadvantages to the currently available RDTs. HRPII tests detecting only *P. falciparum* infection are not suitable for use in areas where other species are prevalent since none of the current RDTs can differentiate between *P. vivax*, *P. ovale* and *P. malariae* and none are quantitative which decrease their effectiveness for evaluating prognosis or the efficiency of antimalaria drugs.

HRPII may persist for up to two weeks after successful treatments, so tests based on this antigen cannot be used to measure response to therapy (18). The choice of diagnostic test depends on the level of malaria endemicity, the prevalence of drug resistance and on the availability of appropriately trained staff, equipment and financial resources.

All existing RDTs are more expensive than microscopy therefore the widespread use of RDTs in Africa is not justified at present. However, the rapid spread of drug resistance and the anticipated widespread introduction of malaria control measures may mean that in the future, this cost benefit analyses will favour the increasing use of RDTs in Africa (18). Importantly, pregnancy does not appear to interfere with the sensitivity or specificity pattern of the RDTs. Also, the histological and or cytological

examination of placental tissue, polymerase chain reaction (PCR), tests as well as DNA-in-situ hybridization tests on plasmodium antigens are also useful but mainly of research interests.

## **TREATMENT OF MALARIA IN PREGNANCY- RECENT TRENDS**

Treatment of malaria in pregnancy differs from the treatment of the same woman outside pregnancy in several respects:-

-Frequency and severity of infections are more in pregnancy particularly among primigravidae.

-Anaemia is commoner in pregnancy associated with malaria infection as the 'physiological anaemia' of pregnancy is worsened by the destruction of red blood cells (RBCs) occasioned by parasitaemia.

-Placental parasitization limits and reduces nutrients to the fetus, amongst others, thereby predisposing to IUGR or fetal death (IUD).

-Severe malaria in pregnancy increases the risk of abortions, preterm delivery etc.

-Neonatal morbidity and mortality are increased in congenital malaria.

-Proper and judicious antimalarial use both for cure and prophylaxis in tandem with haematinics have been shown to be beneficial to both mother and fetus and can improve growth in teenage mothers as well increase fetal birthweight and outcome.

-Antimalarials useful outside pregnancy may become toxic to the fetus in pregnancy e.g. Halfan, Metakelfin etc.

The pregnant women with malaria infection must be treated both medically and obstetrically, taking care of both maternal and fetal interests.

**The principles of management of malaria in pregnancy will include:**

- Rapid and efficient diagnosis of malaria in pregnancy**
- Adequate treatment of acute episodes of malaria in pregnancy.
- Prevention of complications such as severe uncomplicated malaria, cerebral malaria, anaemia, prematurity, congenital malaria as well as other maternal and fetal complications.
- Proper and continuous evaluation of the fetus during pregnancy
- Mandatory haematinics throughout pregnancy.
- Preventive measures in vector control including Insecticide-Treated-Nets(ITN). Insecticide -Treated Materials (ITM) etc.

It is reasonable to expect confirmation of malaria infection in pregnancy, but majority of cases will present in rural areas (where microscopic diagnosis of malaria infection is limited) and also because they often present late and acutely ill, presumptive treatment with adequate doses is recommended between the result of the tests (2-7).

The choice of a suitable drug is predicated upon many factors, including gestational age of fetus, severity of the disease (whether complicated or not), the resistance of the infecting malaria parasites to the antimalaria drugs, cost of medications and the safety profile of the drug in both mother and the fetus. Ideally, effective care should clear both peripheral and placental parasites (5,6,18,21).

Intermittent Preventive Treatment (IPT) involves providing all pregnant women with at least two preventive doses of an effective antimalarial drug during routine antenatal clinic visits. This approach has been shown to be safe, inexpensive and effective (12-15). A study in Malawi, evaluating IPT showed a decline in placental infection (32% to 23%) and in the number of low birth weight babies(23% to 10%). It also found that 75% of all

pregnant women took advantage of IPT when offered (21).

Commonly used drugs for the treatment of uncomplicated malaria in pregnancy is Chloroquine (CQ) in areas where the parasites are still sensitive, for example, most of West Africa, and Sulphadoxine- pyrimethamine (S-P) in areas of chloroquine resistance but where parasites still retain sensitivity to S-P. Other drugs used, though not commonly, in pregnancy include Amodiaquine Mefloquine, Quinine, Artemeter etc.

Antimalarial combination therapy (ACT) is now recommended by the WHO to treat or prevent drug resistance. Combination therapy enhances the activity and effectiveness of the drugs in synergism; also limits drug resistance. Usually both drugs have independent modes of actions. Commonly used ACTs include CQ/SP and Amodiaquine(AQ)- SP, but less common ACTs include AL-SP(Mefloquine-SP combination), AQ-AS, Proguanil-Dapsone(PG-DP)(LAPDAP) etc. There is increasing worry about the toxic effects of some of these drugs during pregnancy. For example, whereas CQ and Amodiaquine are very safe during all trimesters of pregnancy, SP, Mefloquine, Artemisin / Artemeter / Artesunate, either alone or in combination should be avoided in the first trimester of pregnancy. Lumefantrine and LAPDAP are still considered unsafe in pregnancy and more research efforts are currently directed towards its possible use in pregnant women (5).

In treating complicated or severe malaria in pregnancy, parenteral quinine or artemisin are the commonly used agents. Intravenous quinine is usually of as a loading dose of 20mg/ kg body weight over 4 – 6 hrs and followed 4–6 hrs later by 10mg/kg body weight; this is usually given until oral medication can be tolerated. The full treatment covers 5 – 7 days. When used as part of the ACT, e.g with

SP or artesunate, this treatment can be shortened to 3 days (5).

In complicated malaria in pregnancy, anemia and preterm contractions are common complications. Anaemia can be treated with oral double dose haematinics (ferrous-folic acid combination) or blood transfusion of packed cells. In severe anaemia (Hb <4gm%a) or anaemic heart failure, exchange blood transfusion under diuretic cover may prove more beneficial than administration of straight whole blood transfusion alone. Injectable or oral forms of salbutamol will reduce the uterine contractions; this should be continued for a few more days after the feverish episodes have subsided.

#### **OBSTETRIC MANAGEMENT OF PREGNANT WOMEN WITH MALARIA INFECTION.**

Obstetric management is an integral part of the complete management of the malarious pregnant woman. It consists of antepartum, intrapartum and postpartum care.

#### **ANTEPARTUM CARE OF PREGNANT WOMEN WITH MALARIA.**

Essentially, during the antepartum or prenatal period, the antenatal clinic forms the template upon which the institutional treatment is based. As part of the routine antenatal care, history of malaria (in terms of frequency, severity and treatment history) is obtained; blood tests including full blood count and malaria parasites are routinely requested for. Harrison et al in their study from Zaria, Nigeria (12-14) had advocated for routine administration of oral Chloroquine tablets in curative doses at the booking clinic as well as prophylactic (IPT) doses during the subsequent visits. Other authors (2,5,23) agreed that S-P combination can be an effective substitute in areas where Chloroquine resistance is widespread. There is

usually a concomitant use of haematinics throughout pregnancy.

Insecticide treated Nets (ITNs) are valuable tools in controlling the malaria infection; this has proved very useful in Africa and elsewhere (23). ITNs decrease both the number of malaria cases and malaria death rates in pregnant women and their children. A study in an area of high malaria transmission in Kenya has shown that women protected by ITNs every night during their first four pregnancies produce 28% fewer underweight or premature babies. In addition, ITN use benefits the infant who sleeps under the net with the mother by decreasing the exposure to malaria infection (24). ITNs should be provided to pregnant women as early in pregnancy as possible, and their use should be encouraged for women throughout pregnancy and during the postpartum period. Health education programmes, social marketing and lobbying to reduce the prices of ITNs and re-treatments are helping to encourage the use of ITNs by pregnant women. There are prospects for long-lasting treated nets which are wash-resistant and based on the most recent technological development in the field of bio-active fibres and fabrics. These nets release insecticide over time and maintain their activity for at least 4years (21).

Regular antenatal attendance is of great importance as a single missed monthly clinic visit can result in a two-fold increase in malaria incidence (15). Increased awareness on the effects of malaria and anaemia on pregnancy during the booking period has been known to improve antenatal clinic attendance and compliance to treatment. The Roll back Malaria (RBM) Initiative and Safe Motherhood Programme place the antenatal treatment and control of malaria in pregnant women as a high priority in achieving improved maternal and fetal morbidity and mortality.



## **INTRAPARTUM MANAGEMENT OF MALARIA IN PREGNANCY**

Active management of labour ensures constant monitoring of the parturient woman. In the event of intrapartum malaria or prelabour malaria, the elevated body temperature and associated dehydration can cause adverse conditions such as maternal and fetal tachycardia which may contribute to fetal morbidity and possible mortality.

Intrapartum management of parturient malarious women should include treatment of the acute malaria with suitable agents (according to the local sensitivity pattern) and avoiding the S-P combination because of its increased tendency to cause neonatal jaundice. In severe cases, Quinine and the Artemisin derivatives are suitable agents. Concomittant rehydration, analgesia and antipyretics are mandatory. Input and output chart intrapartum will closely monitor the renal effect in such women in labour. Presence of proteinuria or haemoglobinuria should make the exclusion of eclampsia or the blackwater fever complicating severe malaria imperative. However, these two can co-exist in an haemoglobin SS disease patient with often severe fatal outcome. Pseudo-toxaemia in pregnancy of such patients needs to be differentiated from the more pathologic severe pre-eclampsia or eclampsia.

Routine intrapartum haemoglobin check is mandatory as ongoing haemolysis or pre-existing maternal anemia can substantially affect maternal and fetal outcome (18). Blood transfusion may be necessary to mitigate against such undesirable outcomes.

## **POSTPARTUM MANAGEMENT OF MALARIA IN PREGNANCY.**

Following delivery, the mother should complete her antimalarials and supportive treatment. The fetus should be properly examined and congenital malaria excluded.

The newborn's peripheral venous blood should be done to check for malaria parasites. Cord blood and placental smears should be taken for analysis. Experience by many authors (4-6) have shown that heavy maternal parasitaemia significantly increases placental parasitization and fetal parasitaemia with consequent fetal anaemia and possible fetal demise.

It is noteworthy that studies have also reported positive neonatal parasitaemia up to the first week in an otherwise negative parasitaemic neonatal at birth; this can be explained by the passage of the parasitized cells out from the hepatic stage usually after three days. It is therefore advocated that in suspicious babies, peripheral blood collection can be done up to the end of the first week since any parasitaemia after the first week may be due to a newly acquired postnatal infection (2,4). Treat the neonatal for malaria infection with suitable agents, which may include Chloroquine, Quinine, Artemisin etc. Avoid the use of S-P, LAPDAP in the neonatal period. Correction of neonatal anaemia and monitoring of the neonatal jaundice level may necessitate exchange blood transfusion (EBT) and physiotherapy.

The use of malaria vaccine in both the mother and the fetus postpartum is still inconclusive and controversial and many trials are underway to ascertain their safety profile and effectiveness.

## **CONCLUSION**

Malaria in pregnancy remains a significant contributor to maternal and fetal morbidity and mortality. Adequate and effective diagnosis as well as judicious antimalarial treatment and supportive

services will help reduce the magnitude of maternal and fetal loss.

The concept of Roll Back Malaria incorporating the use of ITNs, intermittent use of antimalarials antenatally, adequate treatment of malarious attacks etc as well as adequate and effective antenatal, intrapartum and postpartum care of the mother and child should effectively reduce the scourge of the consequences of malaria in pregnancy.

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## VIRUSES AND CANCER – AN OVERVIEW

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### ABSTRACT

Viruses were initially seen as unusual agents that caused cancer in animals but were of no relevance to humans. They are now accepted as bonafide aetiological factors of human cancers. Carcinogenesis is a multistep process and in virally associated human cancers, the viruses appear to be necessary but are not sufficient for tumour development. Viruses possess genes with potential to modulate host responses and through this means, they evade detection and recognition by the immune system. The mechanism of transformation of a normal cell into a neoplastic cell can either be direct or indirect. Better understanding of the role of viruses in human cancer will have therapeutic implication as control can be instituted.

### INTRODUCTION

Viruses are now accepted as bonafide aetiological factors of human cancer.(1) Cancer is seen as accidental side effect of viral replication strategies as the tumour viruses establish long term persistent infections in humans.(1)

The viruses were initially seen as unusual agents that caused cancer in animals but were of no particular relevance to humans.(1) They have however revealed the functional foundations of the genetic basis of cancer and have provided a conceptual framework applicable not only to cancer induced viruses.(1) It is estimated that 15% of all human tumours worldwide are caused by viruses.(2) The percentage of virus-

related cancers is approximately 3 fold higher in developing countries than in developed countries.(1)

Some viruses are associated with a single tumour type e.g. hepatitis B virus (HBV) while others e.g. Epstein Barr virus (EBV), are associated with multiple tumour types.(1)

A tumour-causing virus may produce non-neoplastic disease in some hosts.(3) For example, EBV causes infectious mononucleosis in some young adults undergoing primary infection, while human papillomaviruses (HPV) cause a variety of benign hyperplasia and both HBV and hepatitis C virus (HCV) cause hepatitis. This article seeks to review the molecular relationship between viruses and human cancers

## **CARCINOGENESIS AS A MULTISTEP PROCESS**

Carcinogenesis occurs in a stepwise fashion and a series of discrete complementary events must occur to convert a normal cell to a cancer cell.(4,5)

In those cancers with viral aetiology, the virus appears to be necessary but not sufficient for tumour development.(1) Additional changes must accumulate to complement those mediated by viral functions, in order to disable the multiple regulatory pathways and checkpoints in normal cells and to allow a cell to be completely transformed.(1)

### **MODULATION OF HOST RESPONSES**

Viruses may contain genes that have the potential to modulate host responses.(6) Different viral strategies exist for evasion of detection and recognition by the immune system.(1) These include (i) restricted expression of viral genes and proteins that makes the infected nearly invisible to the host e.g. EBV in B-cells (ii) infection of sites that are relatively inaccessible to immune responses e.g. JC virus and herpes simplex virus in the central nervous system; HPV in the epidermis (iii) variation in viral antigens that allows escape from antibody and T cell recognition e.g. human immunodeficiency virus (HIV) and influenza virus (iv) downregulation of expression of host MHC class I molecules in infected cells (v) inhibition of antigen processing and MHC class I restricted presentation (vi) infection of essential immune cells .(7-9) Despite these elaborate viral evasion mechanisms, the immune system usually prevails e.g. prevalence of HPV may be as high as 50% among young women, but declines with age.(10,11)

Genetic alterations in p53 gene are now recognized as the most common mutations in human cancers, occurring in over 50% of all tumours.(12,13) Cellular endonucleases induced as part of the apoptotic response to damage inflicted by viral infections could degrade replicating viral DNA and block virus replication.(14) Therefore, some viruses are known to encode proteins which suppress or delay apoptosis long

enough to allow for production of progeny virus.(14) For example, adenovirus E1B-19K protein, which is functionally similar to the Bcl-2 family of cellular proteins, blocks p53-dependent apoptosis.(15)

## **CANCERS ASSOCIATED WITH VIRUSES**

Viruses are associated with a variety of types of human malignancies. HBV and HCV cause hepatocellular carcinoma.(16,17) EBV is linked to Burkitt lymphoma, nasopharyngeal carcinoma, post-transplant lymphoma and Hodgkin disease.(18-20) HPV causes cervical cancer, skin cancers in patients with epidermodysplasia verruciformis and possibly head and neck cancers and other anogenital cancers.(10,11,21,22) Human T-lymphocyte virus-1(HTLV-1) induces adult T-cell leukaemia.(3,23) Human herpesvirus-8 (HHV-8), otherwise known as Kaposi sarcoma herpesvirus (KSHV) is related to Kaposi sarcoma and primary effusion lymphoma.(24,25) Simian virus 40 (SV 40) is associated with brain tumours, osteosarcomas and mesotheliomas.(26,27)

## **MECHANISMS OF TRANSFORMATION**

Human tumours display different mechanisms of cell transformation and fall into both direct- and indirect-acting categories.(1) The direct-acting viruses carry 1 or more viral oncogenes, whereas the indirect acting agents do not possess an oncogene.(1)

## **HUMAN PAPILLOMAVIRUS**

DNA sequence of HPV 16 and 18 and less commonly HPV 31, 33, 35 and 51 are found in approximately 85% of invasive squamous cell cancers and their precursors.(28) The HPV viral DNA is usually integrated in to the host genome in cancer, suggesting that integration of viral DNA is important in malignant transformation.(28) The papillomaviruses encode E6 protein, E7 protein and an early protein designated E5.(1) In HPV-induced tumours, p53 mutations are extremely

uncommon, presumably because loss of p53 function is accomplished by binding to the E6 oncoprotein.(28) The E6 oncoprotein also mediates the degradation of BAX, a proapoptotic member of the BCL-2 and it inactivates telomerase.(28) The E7 protein binds to the retinoblastoma (Rb) protein and displaces the E2F transcription factors that are normally sequestered by Rb protein.(28) E7 oncoprotein also inactivates the Cyclin Dependent Kinases /inhibitors (CDKIs) CDKN1 A/p2 and p27. The E5 protein complexes with Platelet Derived Growth Factor (PDGF)  $\beta$ -receptor and activates it in a ligand-independent fashion to mediate a sustained mitogenic signal.(28)

### EPSTEIN BARR VIRUS

EBV is another direct-acting tumour virus that encode a viral oncoprotein LMP-1 that resembles a cell surface receptor.(1) LMP-1 mimics an activated growth factor receptor and mediates its proliferative signals.(29) It binds to and activate a signalling molecule that is normally activated by the CD 40 receptor in B cell, which is the key recipient of helper T-cell signals.(30) LMP-1 activates NFkB and JAK/STAT signalling pathways and promotes B-cell survival and proliferation, thus efficiently co-opting a normal B-cell activation in order to increase the number of cells the virus can infect and inhabit.(31) Several of the EBV-encoded Nuclear Antigens (EBNAs) are necessary for immortalization of B-cells.(32) EBNA-1 expressed consistently in Burkitt lymphoma has been shown to be oncogenic in transgenic mice.(32) EBNA-2 gene transactivates several host genes, including cyclin D and members of the src family, thereby promoting the transition of resting B-cells from G<sub>0</sub> to G<sub>1</sub>.(33) EBNA-2 also activates the transcription of LMP-1 and is a key regulator of viral gene expression.(3)

(1)

2/3

### HEPATITIS B VIRUS

Despite compelling epidemiologic and experimental evidence, the precise role of HBV in the causation of human liver cancer is not clear.(3) It is likely that the effect of HBV is indirect and possibly multifactorial.(34) Chronic liver injury secondary to persistent viral infections leads to necrosis, inflammation and liver regeneration which over time results in cirrhosis, with hepatocellular carcinoma arising out of this background.(35) HBV transactivator protein, the x-protein (HBx) contributes indirectly to liver carcinogenesis by activating the Ras-Raf-miogen-activated protein kinase signalling cascade.(36) HBx can also bind to p53 and it appears to interfere with its growth-suppressing activities.(37-40)

### HEPATITIS C VIRUS

HCV does not carry a classical oncogene but it has been reported that viral non-structural protein NS3 can transform NIH 3T3 and can bind p53.(41-42)

### HUMAN T-LYMPHOCYTE VIRUS-1

HTLV-1 is the only retrovirus accepted as having an aetiologic role in a specific human cancer and it appears to act indirectly in the development of acute T-cell leukaemia.(1) Similar to the AIDS virus, HTLV-1 has tropism for CD 4+ T-cells and hence this subset of T-cells is the major target for neoplastic transformation.(28) It seems the secrets of the transforming activity of the HTLV-1 are found in the TAX gene.(43) The product of the TAX gene can activate the transcription of several host cell genes involved in proliferation and differentiation of T-cells, including c-fos, genes encoding interleukin-2 (IL-2) and its receptor and GM-CSF.(43) TAX protein also dysregulate the cell cycle by inactivating the cell cycle inhibitor p16INK4a and enhancing cyclin D activation.(43) TAX also contributes to malignant

transformation by interfering with DNA repair functions and inhibiting ATM-mediated cell cycle checkpoints activated by DNA damage.(44)

## HUMAN IMMUNODEFICIENCY VIRUS

The role of HIV in carcinogenesis is probably even more indirect.(45) Immunosuppression to HIV infection predisposes those individuals to certain cancers, especially EBV-positive lymphomas, HHV-8 (KSHV)-positive Kaposi sarcoma and HPV-positive tumours.(1) A more direct role of HIV in the genesis of Kaposi sarcoma has been proposed, involving a cellular growth-promoting effect by the HIV tat protein.(46)

## HUMAN HERPESVIRUS TYPE 8

The HHV-8 (KSHV) genome possesses a number of cellular regulatory gene homologues, including genes related to chemokines, cellular proliferation factors, intercellular signalling components and inhibitors of apoptosis.(24, 25, 47)

## CONCLUSION

The role of viruses in the spontaneous and experimental induction of cancer is well established. The study of the role of these viruses has historically provided an appropriate background for understanding the role of oncogenes in carcinogenesis. The number of human cancers associated with viral infections is limited. Better understanding of the role of these viruses in the causation human cancer will have therapeutic implication as control can be instituted.

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## OVERVIEW AND EXPERIENCE WITH THE USE OF FLUOROQUINOLONE IN CHILDREN IN THE TROPICS

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### ABSTRACT

The use of fluoroquinolone is contraindicated in children because of the potential complication of arthropathy. In spite of this, the role of ciprofloxacin is becoming increasingly significant. We report two cases in which organisms that did not respond to the use of some other potent antibiotics clearly responded to the use of ciprofloxacin. A general overview of the fluoroquinolones is also highlighted. It is concluded that ciprofloxacin is a very useful agent in the management of serious infections in children and available data clearly supports its use where the efficacy outweighs any considerable risk. Fluoroquinolone is therefore recommended in children, where it offers a clear therapeutic advantage over other classes of antibiotics but not for routine empirical use.

### Introduction

The demographic and subsequent economic pressures in developing nations have contributed to the increasing levels of antibiotic resistance among both commensals and pathogenic bacteria. This has made empirical options available to diminish by the day. In spite of this, the role of ciprofloxacin in paediatric infection is becoming

increasingly significant (1). Anecdotal observations have shown that certain patients who will otherwise not respond to conventional antibiotics show rapid response with ciprofloxacin. In-vitro laboratory analysis has also consistently shown responsiveness of these organisms to ciprofloxacin. In a study of UTI in hospitalized patients, a lot of the organisms were found to be susceptible to

ciprofloxacin.(2). Yet it is largely contraindicated in children because of rare but well documented cases of joint damage (3-5). This article therefore aims to highlight two remarkable cases in practice where certain antibiotics failed but ciprofloxacin worked satisfactorily with no residual joint damage seen clinically both at discharge and follow-up.

## Case reports

### Case 1

A 9-year-old boy presented with history of progressive post-trauma swelling of the left hip and knee joint and fever of 3 days duration. There was inability to use those joints. Physical examination revealed obvious tender and warm swelling of the left hip and knee joints with decrease range of movement across them. The child was anaemic with PCV of 16%. Needle aspiration of the left hip swelling yielded pus. An assessment of septic arthritis was made and sultamicillin and gentamicin were commenced. He was also transfused raising the PCV to 23%. Fever and pain did not subside with this antibiotics rather abscesses further developed in the anterior chestwall, left forearm and right leg. Meanwhile aspirate from left knee and hip yielded profuse growth of staphylococcus aureus sensitive to pefloxacin, erythromycin, gentamicin, ofloxacin, cefuroxime and ciprofloxacin. Ciprofloxacin was commenced after the child had completed 7 days of sultamicillin and gentamicin with no clinical improvement. Incision and drainage of the abscesses were carried out at the time ciprofloxacin was commenced. Fever subsided fully by 6<sup>th</sup>

day of ciprofloxacin therapy, there was no further spread of abscess and remarkable reduction of pain was noticed by the third day of therapy. He completed 3 weeks of ciprofloxacin and was discharged home. No residual joint damage occurred clinically as full functions returned to both the knee and hip joints.

### Case 2

A 10 months old female child who presented with history of fever and multiple abscesses on the left gluteal region and lower part of the right thigh. There was limitation of movement at the knee joint raising the suspicion of a septic arthritis. However radiograph study of the right knee revealed widening of the joint spaces with no bony involvement. Furthermore, at surgery, pyomyositis of the muscle of the right lower thigh region rather than septic arthritis was found. Incision and drainage of the gluteal abscess was also carried out. Meanwhile she had been commenced on sultamicillin and gentamicin since admission. The culture and sensitivity result of the pus obtained from the pyomyositis and the left gluteal region yielded profuse growth of coliform sensitive to gentamicin, ofloxacin, cefuroxime, ceftriaxone and ceftazidime. Despite the result, both antibiotics were continued since it was sensitive to gentamicin. Both of them had to be discontinued after 10 days when it was noticed that the fever did not completely subside and the right lower thigh continued to be tender. Ciprofloxacin was introduced and fever subsided after 48 hours. He received it for 11 days and became clinically stable. At follow-up the improvement was sustained

## Discussion

In the two children highlighted, there was satisfactory clinical response to ciprofloxacin where sulfamethoxazole and gentamicin failed. The organisms were sensitive to the third generation cephalosporin but the patients could not afford it, hence the consideration of a much cheaper alternative, which informed the trial of ciprofloxacin. Even though we were aware it was contraindicated in children, we were also aware that other workers have successfully tried it in children (6) it therefore turned out that a less often used drug in children became useful. Ciprofloxacin is likely to have worked in those patients because of limited use of ciprofloxacin in the paediatric age group. It is still not recommended as a first line antibiotic, but it should be employed especially in gram-negative septicaemia when all the regular antibiotics have failed. In case 1, the fever clearance time following the use of ciprofloxacin was 6 days while in case 2 it was about 48 hours. It is worth noting that the first patient with septic arthritis developed it prior to commencement of ciprofloxacin. None of them developed arthropathy after the use of ciprofloxacin. No treatment or associated event was recorded in any of the patient both during admission or at follow up. This is consistent with findings of other workers who used bigger sample size (6). However our own sample revealed no single case of treatment associated event compared to 10.9% of children receiving oral ciprofloxacin/18.9% among IV recipients in a group of 1795 children who received treatment courses of IV or oral ciprofloxacin. Overall arthralgia occurred in 31 ciprofloxacin treatment courses (1.5%) and majority of events were of mild to moderate severity and resolved without intervention (6). More than 60% of the arthralgia was in children with cystic fibrosis. We cannot as of yet assemble such a large cohort of patients on either ciprofloxacin or ciprofloxacin

since there is still a high skepticism on its use in children in our environment. We dared to use it in our children for lack of a plausible alternative. A review of the journals indicates its use in children and the rate of adverse event pattern was similar to that observed in adults.

The recommendation that fluoroquinolone should not be used in children resulted from findings in juvenile animal of cartilage damage after administration of high doses. Histopathological examination of the joint surface of affected animals revealed the loss of cartilaginous matrix and chondrocytes and cavitation within the intermediate zone of cartilage accompanied by cartilage fibrillation or chondrocyte clustering or loss of the surface layer which covers the cavitations (loss of outer wall of the cavity (3). The possibility of mutagenesis and joint damage restricted the use of fluoroquinolones in children to serious life saving indication only. The joint damage usually resolves gradually after drug withdrawal and is more frequent with pefloxacin. In practice quinolone is an option for children only when the expected benefit outweighs the risk of joint damage. In the rare cases in which fluoroquinolone is justified, ciprofloxacin is the drug of first choice. Pefloxacin should be avoided.

Fluoroquinolones are generally very safe antibiotics, which do not cause severe or life threatening adverse reactions. The most frequent side effects are gastrointestinal reactions (nausea, dyspepsia and vomiting) and CNS reaction such as dizziness, insomnia and headache. Many of the more severe CNS reactions seem to be due to metabolic interaction with theophylline especially when enoxacin is used. Of the potential serious side effects, photosensitivity has been reported in varying frequencies with the different fluoroquinolones. Caution is necessary when this group of drug especially pefloxacin is prescribed

to patients who will have intense exposure to ultra-violet light during treatment (4).

The quinolones are active against both gram positive and gram-negative bacteria. It is particularly active against gram-negative bacteria including salmonella, shigella, campylobacter, neisseria, bacillus anthracis and pseudomonas. It has only moderate activity against gram-positive bacteria such as streptococcus pneumonia and enterococcus faecalis. It is also active against chlamydia and some mycobacterium.

It should be used with caution in patients with a history of epilepsy or condition that predispose to seizures, in G6PD deficiency, myasthenia gravis (because of risk of exacerbation) in pregnancy, during breastfeeding. It may induce convulsion in patients with or without history of convulsion. Taking NSAIDs at the same time may also induce them. Tendon damage including rupture has been reported rarely in patients receiving quinolones. Tendon rupture may occur within 48 hours of starting treatment; hence its use is contraindicated in patients with history of tendon disorders related to quinolone use. The risk of tendon rupture is increased by the concomitant use of steroids. If tendonitis is suspected, the quinolone should be discontinued immediately. It is given by mouth 10-30 mg/kg in two divided doses or by IV infusion 8-16mg/kg daily in 2 divided doses.

In conclusion, ciprofloxacin is a very useful agent in the management of serious infection in children. Data clearly support its use where the efficacy outweighs any considerable risk. It should also be chosen for indications in which they offer a clear therapeutic advantage over other classes of antibiotics rather than as agent whose broad spectrum prompts routine empirical use.

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## OUTBREAK OF TURKEY POX DISEASE IN FOWL POX VACCINATED POULTS IN VOM PLATEAU STATE OF NIGERIA

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### ABSTRACT

An outbreak of poxvirus infecting 45 turkeys of 8 weeks of age is reported. Poults were previously vaccinated against pox using fowl pox vaccine. The outbreak persisted for 5 weeks with 100% morbidity but no mortality. The cutaneous form only existed. Turkey pox virus was isolated by propagation in chorioallantoic membrane and confirmed by reproducing the disease in susceptible turkeys.

### INTRODUCTION

Turkey pox is a world wide, slow spreading viral infection characterized by proliferative lesion in the skin (cutaneous form) that progresses to thick scabs and by lesion in the upper gastrointestinal and respiratory tract (Diphtheritic form). Transmission is commonly by contact to pen-mate through abrasion of the skin. Mosquitoes, poultry ticks & lice and other biting insects may serve as mechanical vector (3)

Prevention in Nigeria is by vaccination with fowl pox vaccine (cell cultured propagated or chorioallantoic propagated live viral vaccine from

Poults were previously vaccinated at 6<sup>th</sup> week of age against pox with fowl pox vaccines (manufactured

in NVRI Vom, Nigeria). The origin of seed for the production of fowl pox vaccine is chicken. Post

chicken). In this report, despite the initial vaccination with fowlpox vaccine, there was an outbreak of Turkey Pox disease among a flock of turkeys.

### THE OBJECTIVES OF THIS WORK.

- i. To isolate the virus
- ii. To confirm the virus by reproducing the disease in susceptible turkeys.

### CASE HISTORY

An outbreak was reported in the month of June of pox virus infecting 45 turkey (mix bred) poults of 8 weeks of age.

vaccination 'take' was observed 4-5 days P.I. Morbidity rate was 100% over a period of 3 weeks. No death was

recorded. The infection was characterized by formation of progressively increasing nodular lesions in parts of the un-feathered skin, head, upper neck and eyelids.

## MATERIALS AND METHODS

Nodular lesion of turkey pox was excised from five mixed bred turkey poults. Excised lesions were weighed and ground with aid of sterile sand. A neat 20% w/v suspension was prepared with P.B.S. centrifuged at 2,500 r.p.m. for 30 minutes. The supernatant was decanted and stored in the deep freezer at  $-20^{\circ}\text{C}$ .

## REPRODUCING THE DISEASE IN SUSCEPTIBLE TURKEYS

3.0ml concentrated turkey pox virus suspension containing  $10^{-3}$  EID<sub>50</sub> was inoculated subcutaneously at multiple points on the head region of 5 (4 months old) Local bred susceptible turkeys.

1.0ml  $10^{-3}$  EID<sub>50</sub> was inoculated subcutaneously on both wing web of 5 (5 weeks old) Local bred turkey poults.

## RESULTS AND DISCUSSION

### Reproducing the Disease In Susceptible Turkeys

5 days post infection, multiple nodular lesions were observed on the head and upper neck region of the 4 month old susceptible turkeys. In the five weeks old poults, lesion appeared 4 days post infection.

The cutaneous form only with 100% morbidity but no mortality existed in the outbreak. These confirm previous observation that cutaneous infection ordinarily causes low or moderate mortality and the affected birds generally returned to normal upon recovery(1).

The source of infection may be traced to mosquitoes or other biting arthropods because poults were

raised under semi-intensive care in the rainy season, which agrees with the findings that when mosquitoes are plentiful, transmission within a flock may be rapid (2).

The disease may be exacerbated by other pathogens such as viruses of NCD, Infectious bronchitis and fowl pox, and also *Haemophilus paragallinarum* and *Mycoplasma gallisepticum*. Deficiency of vitamin A and excess ammonia in the atmosphere may also predispose to severe disease (4). Interestingly however, is the fact that after observation of post vaccination 'take' poults still came down on the disease. There have been reports on atypical and variant fowl pox virus strains based on the appearance of fowl pox lesions in previously vaccinated chickens. Upon further investigations these viruses have not proven to be variant (4).

Studies have shown that there is antigenic relationship among the pox viruses of the avian species and that the virus of one type of avian pox can give rise to disease in another species as well as may stimulate protection against another (3). Nevertheless, it is equally proven that turkey pox virus is immunogenically distinct from fowl pox virus. Consequently, turkey pox vaccine was developed from turkey pox virus and is now widely used in advanced countries (2). The homologous vaccine has proven to be superior in its immunogenicity in turkey compared to fowl pox vaccine. Yet in Nigeria, live fowl pox vaccine is being used in all categories of poultry.

Our recommendation is that attempt should be made to develop turkey pox vaccine from turkey pox virus for the growing turkey industry in Nigeria.

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## FLORID ANOGENITAL CONDYLOMA ACUMINATA IN A MALE AFRICAN: A CASE REPORT

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### ABSTRACT

Condyloma acuminata are commonly transmitted through sexual intercourse among those in the age bracket of 15 to 49 years. Florid lesions occur in those with immunosuppression, debilitating illness or pregnancy. Clinical diagnosis is often used in Nigeria. Their prevalence among HIV Seropositive patients is unknown. Lesions are often treated with 25% Podophyllin hydrochloride solution or ointments in other combinations. Occasionally surgery is used in giant and obstructive types. This is a case of 34 year old male homosexual that is HIV positive presenting with a Florid Anogenital Condyloma Acuminata. He was undergoing treatment with *HAART* (Nevirapine, Stavudine and Lamivudine). He did well clinically before coming down with this condition. He also responded well to podophyllin treatment without surgery but was lost to follow-up. Clinicians should watch out for similar presentations.

KEY WORDS: Condyloma Acuminata, Warts, Human Papilloma Virus, anogenital.

### INTRODUCTION:

Genital warts or Condyloma acuminata are found in approximately 7 to 20% of the general population, with the highest frequency in the early teenage years. Autoinoculation of virus to contiguous or distant sites is frequent (1). Condyloma acuminata are one of the most

common sexually transmitted infections. They are hyperplastic, sessile or pedunculated neoformations, red or pink, sometimes forming soft exuberant masses, strangulated at their base. The human papilloma virus (HPV) (1,2,3,4) causes them. In men, they occur most commonly in the preputial area, on the coronal sulcus and

the urethral meatus, rarely on the scrotum and anus. In women, they predominate on the posterior vestibule and the vulva, the vagina, the urethra, the perineum and the uterine cervix. Certain clinical conditions such as pregnancy, diabetes and immunodeficiency states accelerate their growth. Warts recur frequently and are quite contagious. Systematic histologic examination of excised pieces is necessary to confirm their sero-types (subtypes). We hereby present a peculiar case of florid anogenital warts in a young male adult homosexual of African descent living with HIV/AIDS.

## CASE REPORT

A 34-year-old male presented to our clinic with a history of weight loss diarrhoea and fever of more than one-month duration. He had visited many herbalists, chemist shops and private hospitals without significant improvement in his clinical status. However, when the symptoms worsened and associated with weakness and tiredness, he was referred to STD/RVD clinic for HIV antibody testing and further management.

History revealed a high risk activity in his sexual orientation (homosexuality) which is uncommon in the general population. His educational, social and occupational history was not significant. Medical history was also not significant until these presenting complaints of the last few months that brought him to our clinic. No previous history of admissions, surgery or significant illness.

Physical examination revealed a young, male, African patient of 1.75metre in height, weighing 50kg

while height, pale++, ill looking, weak, emaciated, febrile to touch and generalized lymphadenopathy. He was well oriented in time, place and person. His chest was clinically clear. Cardiovascular system, abdomen, musculoskeletal system and urogenitals were intact.

HIV screening by double ELISA as well as confirmatory tests by Westernblot were reactive. The follow up tests revealed the following: HbsAg was non-reactive, PCV 26%, WBC of 3,200, blood film was normocytic and normochronic, urea and electrolytes, Liver function test, lipid profile and Serum amylase were all within normal range with only slight derangement. His CD<sub>4</sub> cell count was however low at 290 cells/L. He was then placed on **HAART** comprising Nevirapine, Stavudine and Lamivudine apart from cotrimoxazole prophylaxis, analgesic and antidiarrhoea. Reviews were carried out at one month, three months and six months respectively. At one month, he was slightly better than before following control of fever, diarrhea and tiredness. His weight had increased to 52kg. At three months, his confidence had been regained, his weight appreciated to 56kg while his CD<sub>4</sub> count had risen to 320 cells/L. No more fever, tiredness, weakness and loss of appetite were reported. At six months, he now came down with florid anogenital warts with some difficulty in moving bowels. There was no fever, pain, itching or other complaints. He was actually looking much better and stronger. He had visited several surgeons in private hospitals where he was charged exorbitantly. Since he could not afford it, he decided to consult us at the Sexual Transmitted Disease/Retroviral Disease (STD/RVD) clinic.

We then examined him, evaluating him for optimal treatment and concluded that he could benefit from podophyllin application following a clean bill of a histology report following biopsy of one of the lesions. The lesions were benign. His weight had increased to 60 kg by this time and he was looking much better. The CD<sub>4</sub> cell count was then 400 cells/mm<sup>3</sup>.

Physical examination revealed: large perianal condyloma acuminata above and below the anal orifice. Other smaller lesions were seen at the buttocks, urethral meatus and penile coronal areas (figures 1 and 2). They were soft, fleshy, slightly mobile but not tender. Normal skin colour was observed in some of them while others were hypopigmented. Much smaller ones were flat, discrete and fleshy. His treatment was 25% podophyllin hydrochloride applied thrice a week. After two months most lesions were gone without scars.

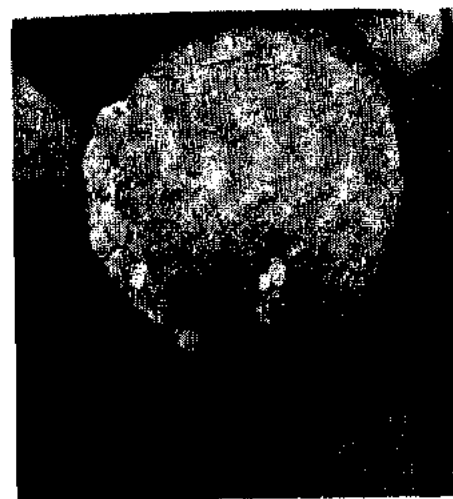


**Fig 1: Verucose Warts in the anal region and buttocks**

## DISCUSSION

Mucosal Human Papilloma Viruses (HPVs) infect primarily the anogenital tract epithelium, but these HPV types can also be found in the oral mucosa,

conjunctiva, and respiratory tract. Genital tract HPV infection is thought to be the most common viral sexually transmitted disease (STD) in the United States of America (5). Such data do not exist in Nigeria but current evidence suggests that over 50% of sexually active adults have been infected with an HPV associated with genital infection (6). Our case is a young adult of African descent who was HIV positive, already on therapy (*HAART*) but was still active with homosexual activity inspite of his serostatus. The reason why the warts became florid after months of antihIV-treatment is unknown to us. This has occurred inspite of the fact that the young man had appreciated in weight, CD<sub>4</sub> cell count, physical and psychological well being. The prevalence of HPV infection in the genital tract is not well defined. Estimates have varied with the diagnostic method used. What is clear is that as with other



**Fig 2: Verucose Warts on the Urethral Meatus and Coronal Sulcus**

sexually transmitted diseases, evidence of current infection is most frequently detected among sexually active young people (7, 8). In the early 1990s, it was estimated that

about 1% of men and women in the United States between 15 and 49 years of age had clinically evident genital warts.

Our case report is a single case out of the 605 patients that were attending the clinic since 2003. Florid cases like this are uncommon by our experience. Clinicians are therefore encouraged to watch out for such cases. Local 25% podophyllin application was able to virtually clear all the lesions before the patient got lost to follow up.

This should be encouraged instead of radical surgical interventions. Recurrence is common even with surgical excision. However, healing may be slow. With podophyllin, those prolonged healing time in a HIV seropositive person, scars that may result, pain and financial pressures on the patient may be reduced substantially.

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