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Relationship Between Digestive Tract Pathogens and HIV Infection in Onitsha Metropolis with Respect to Occupation

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Abstract: Four hundred and seventy eight (478) individual's who exhibited some manifestation of chronic and debilitating illness including persistent cough, skin cancer and dermatitis, multiple lymph adenitis, diarrhea and enteritis, genital sore, urethritis, vaginitis and weight loss, were examined to establish relationships between human immuno-deficiency virus infection (HIV) and digestive tract pathogens in Onitsha metropolis. There was a significant relationship between HIV positive individuals and digestive tract diseases with HIV positive recording more digestive tract diseases and professional drivers recording highest prevalence rate in total intestinal pathogens 27.66% prevalence rate.

Key words: HIV, Digestive tract pathogens, Occupation and Intestinal pathogens.

INTRODUCTION

The digestive tract is one of the most common sites for clinical expression of human immune-deficiency virus infection [1, 2 and 3]. Many studies show that victims express oral Candidiasis, approximately one third of patients develop peri-rectal lesions due to Herpes Simplex virus. 30%-80% experience chronic or intermittent diarrhea. Several gastro intestinal diseases appear to be prevalent in these patients including visceral Kaposis sarcoma. Microsporidiosis, Crytosporidiasis, Cytomegalovirus of the digestive tracts [4, 5, 6 and 7]. Many HIV infected patients show symptoms of persistent diarrhea, fever and gastritis [8].

The cause of gastroenteritis may be due to proliferation of intestinal opportunistic parasites e.g protozoa, bacteria especially *Salmonella* species, fungi and viruses resulting from lowered immunity [9, 10 and 11]. Persistent gastroenteritis with fever and other symptoms of AIDS including weight loss and dermatological manifestations should raise a warning signal to clinicians and other health workers. It is possible that persistent digestive tract disease may be indicative of HIV infection but the exact relationship between the two is not clear especially with regards to our Nigerian environment which may present different types of intestinal parasites from such types seen in caucatian HIV individuals. It was therefore necessary to evaluate the intestinal parasites in HIV positive and negative individuals to determine the extent and distribution of the parasites with respect to occupation [12 and 13].

MATERIALS AND METHODS

Sample Population: The study examined four hundred and seventy eight (478) individuals.

Collection of Samples: Information regarding their occupations was obtained and data recorded. About 1-2grams of freshly passed stool samples were collected from each patient in a universal specimen container and labeled. Uncoagulated blood samples were collected by bacterial venipuncture and serum samples obtained from the blood samples were used for HIV and Western blot analyses using Savyon Diagnostic Ashdod Israel kit and Biorad, Paris France kit.

Stool Routine Examination: The stool samples were examined macroscopically for appearance prevalence of blood mucus and texture. A simple normal saline mount was made by picking a little quantity of the stool taken from suspicious sites and mounted in normal saline on a clean glass slide.

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Stool Concentration Technique: All stool samples that yielded no cysts larva, ova or protozoa in the simple normal saline direct wet film examination were subjected to concentration technique as follows:

About 1-2grams of stool were placed in a 20ml glass tubes. About 10ml of 10% normal saline was introduced in each 20ml glass tubes and sieved into small beaker using guaze strainers. This was transferred into 10ml glass tubes *i.e.* 6ml of suspension and 3ml of ether added, mixed well and centrifuged at 3000 rotation per minute for 1 minute.

By means of clean pasture pipettes, a few drops of the deposits were taken and mounted on slides, covered with cover slip and examined under low and high power objective lens of the microscope (Ramnik, 1990) [9].

Isolation of Digestive Tract Pathogens from Stool

Bacteria: Portions of the stool were picked by means of sterile wire loop previously flamed over Bunsen burner and inoculated on Xylose lysine dextrose agar. The inocula were streaked out on the plates using the wire loop flamed in-between streaks to avoid crowded growths in the XLD agar. The plates were incubated at 37°C over night.

Fungus: Using a flamed wire loop, small portions of the stool samples were picked up and inoculated into Sabouraud agar plates, slants and slides in pairs and incubated for 14 days at 37°C also observing daily growths.

Identification of Digestive Tract Bacterial Pathogens: Yellow colonies growing on XLD agar with black centers were suspected Salmonella colonies. These suspected colonies were sub-cultured on Nutrient agar to stabilize the species and the pure growths subjected to biochemical identification as well as motility test by hanging drop method.

Biochemical Identification of Digestive Tract Pathogens: Sterile wire stabs were used to pick the suspected colonies and introduced into Durham fermentation tubes of glucose, lactose, sucrose and manitol. Also, the wire stabs of suspected whoever was pierced through Triple Sugar Iron Agar (T.S.I.A) and sub-cultured onto Mackonkey agar to ensure purity. The fermentation tubes, TSIA tubes and plates were incubated at 37°C over night. All colonies of suspected bacteria that were motile, fermented glucose and manitol with acid and gas, lactose and sucrose not fermented, TSIA slant with alkaline (pink) and acid with gas butt were identified as *Salmonella typhimurium*. The fermentation tubes with motile organisms that fermented glucose, did not ferment lactose and sucrose, acid manitol and orange yellow acid (only) butt of TSIA were identified as *Salmonella typhi.*

Serological Identification of Digestive Tract Bacterial Isolates: A drop of saline was applied on clean glass slide and the bacteria colonies under test on Nutrient agar plates were collected with a sterile wire loop and emulsified on the slide to form moderate suspensions. Monovalent Salmonella type specific antisera (Oxford England) were added and steered slightly and observed for characteristic agglutination reaction.

Identification of Digestive Tract Fungal Isolates: The Sabourated agar slants and plates that grew suspected creamy colonies were tested as follows: The colonies were picked up with the tip of sterile capillary pipette and gently transferred and emulsified in 0.5ml sterile serum in a small test tube. Pooled human sera were used.

The above was repeated in a second tube using a known *Candida albicans* culture as positive control. The tubes were incubated at 37°C for $2^{1/2}$ hours. The sera were mixed and transferred by means of pipette to a slide covered with coverslip. The slides were examined under high power and low powers of the microscope for the presence of short, lateral hyphal filaments (Green tube) formed by the yeast cells (Ramnik 1990) [9].

Identification of Digestive Tract Parasites: The X10 and X40 objectives of the microscopes were used to examine the slides that were mounted with emulsified stools and parasites identified pictorially continuously changing roundish structures with more than four nuclei identified as *Entamoeba histolytica.*

Roundish and occasionally oblong/oval flagellated highly motile cells were identified as *Trichomonas hominis*. By means of Pasteur pipette, drops of Lugol's iodine were applied to the slides to highlight and confirm the nuclear of Enta-amoeba histolytica (Ramnik 1990) [9].

Antibiotic Sensitivity Test: Each of the isolates *S. typhimurium* and *S. typhi* were subjected to antibiotic sensitivity as follows: relevant antibiotic mentioned discs e.g *Gentamycin, Amoxycillin, Perflacin, Chloramphenicol, Ampicillin, Tetracyclin, Oflocin, Ciproxin, Sporidex Ciproval* and *Ceporex* were placed on each Mueller-Hinton sensitivity solid agar plates that

has been previously seeded evenly with the test organisms diluted to $1x10^{4.5}$organisms/ml, disc diffusion method and incubated over night at 37°C (Tilton and Howard, 1987).

After incubation, the zones of inhibition surrounding the antibiotics sensitivity discs were taken as a measure of the inhibitory and bactericidal power of the drug against the particular test organisms and denoted as sensitive(s). The discs having the test organisms still growing around the discs were adjudged resistant.

Statistical comparison between digestive tract pathogens in HIV positive and negative individuals were carried out and results noted.

RESULTS

HIV Positive: The thirty-four cases of Digestive tract diseases yielded eighty two (82) isolates distributed as follows:

Clerical officers recorded highest prevalence rate of *Trichomonas hominis* 7.69%, followed by traders 7.5%, next was farmers 7.14%, next was professional drivers 6.38%, next was Technicians 4.49%, next was students 3.26%, next was professionals 2.70%, least was senior executive officers zero. Total twenty-five (25)

Professional drivers recorded highest prevalence of *Enta-Amoeba histolytica* 6.38%, followed by Clerical officers 6.15%, next was professionals 3.40%, next was farmers 4.76%, next was senior executive officers 3.70% next was Technicians 3.37%, least was students 3.26%.

Professional drivers recorded highest prevalence 8.51% of Candida albicians, followed by Traders 6.33%, next was professionals 5.40%, next was farmers 4.76%, next Clerical officers 4.62%, next was Technicians 4.49%, least was senior executives 3.70%.

Highest in prevalence of *Salmaneolla typhi* was professional drivers 4.26%, followed by farmers 2.38%, next was 1.27% *i.e.* traders, next was technicians 1.12%, next was students 1.09%, least were senior executives, professionals and clerical officers scoring zero. Total was six (6).

Farmers scored highest prevalence of *Salmonella typhimirium* 2.38%, followed by professional drivers 2.13%, next was traders 1.27%, least were senior executive officers, professionals, clerical officers all scoring zero. Total was four (4).

Professional drivers recorded the highest prevalence of total pathogens 21.42%.

HIV Negative: The twenty-three (23) cases of digestive tract disease yielded thirty-nine isolates distributed as seen in table 2.

There was statistical difference at P=0.05 in HIV positive and negative individuals for DTP in HIV positive and negative.

Bacterial Pathogens from Digestive Tract Infection and Antibiogram

Salmonella Typhi: Gentamycin recorded the highest frequency for sensitivity by being sensitive to all the *S. typhi*-specie isolated from HIV positive individuals (6 in number) and HIV negative individuals (2 in number) totaling eight (8) *S. typhi* isolates.

Ciproxin was the next antibiotic in terms of performance *i.e.* seven sensitive out of eight isolates. The following antibiotics tried in terms of performance viz *Oflacin, Amoxyl, Ciproval, Perflacin, Sporidex* and *Chloramphenicol* came next with six (6) sensitivities out of the eight.

Distribution of Digestive Tract Pathogens (DTP) in Onitsha Metropolis and Their Relationship with Infection with Respect to Occupation

Table 1: HIV Positive

	HIV Positive									
	S. Ex	Prof.	Tech.	Traders	Cler. Off.	Prof. drivers	Farmers	Stu.	Total Iso	Total HIV
A	-	2.70	4.49	7.59	7.69	6.38	7.14	3.26	25	
B	3.70	5.40	3.37	3.80	6.15	6.38	4.76	3.26	21	
C	3.70	5.40	4.49	6.33	4.62	8.51	4.76	4.35	26	
D	-	-	1.12	1.27	-	4.26	2.38	1.09	6	
E	-	-	-	1.27	-	2.13	2.38	1.09	4	
F	7.4	13.50	13.47	20.26	18.46	27.66	21.42	13.05	82	169
G	No sig. X^2=0	No sig. X^2=2.84	No sig. X^2=0	Sig. X^2=8.24	Sig. X^2=6.10	No sig. X^2=1.54	No sig. X^2=3.50	No sig. X^2=0.46		

There was a significant difference in occurrence of DTD in HIV positives and negatives.

Key: A=*Trichomonas hominis*, B=*Enta-Amoeba histolytica*, C=*Candida albicans*, D= *Salmonella typhi*, E= *Salmonella typhimurium*, F= Total no. of Pathogens in occupation and G=Sig. diff. at P=0.05 FOR DTP in HIV Positive and Negative for each occupation. S. Ex = Senior executive officers, Prof= professional drivers, Tech= Technicians, Cler. Off. = Clerical officers, Stu.=Students and Total Iso=Total isolates.

Table 2: HIV Negative

	HIV Negative											
	S.Ex	Prof.	Tech.	Traders	Cler. Off.	Prof. drivers	Farmers	Stu.	Total Iso	Total HIV +ve and –ve Isolates	Total HIV Negative	Total HIV Positive and negative
A	3.70	-	4.49	1.27	-	2.13	-	1.09	8			
B	3.07	2.76	3.37	1.27	1.57	4.26	2.38	1.09	11			
C	-	2.70	1.12	-	3.08	6.38	4.76	5.43	16			
D	-	-	-	-	-	2.13	-	1.09	2			
E	-	-	-	-	-	2.13	-	1.09	2			
F	7.40	5.40	8.98	2.54	41.12	15.03	7.14	9.79	39	121	309	478

There was a significant difference in occurrence of DTD in HIV positives and negatives.

Key: A=*Trichomonas hominis*, B=Enta-Amoeba histolytica, C=Candida albicans, D= Salmonella typhi, E= Salmonella typhimurium and F= Total no. of Pathogens in occupation for each occupation.. S. Ex = Senior executive officers, Prof= professional drivers, Tech= Technicians, Cler. Off. = Clerical officers, Stu.=Students and Total Iso=Total isolates.

The least were Septrin, Ampicillin and Tetracycline each recorded five out of eight.

Salmonella Typhimurium: Antibiogram for the *S. typhimurium* recorded *gentamycin* again as the highest in sensitivity frequency along with *Oflaxin* and *Perflacin*. The three organisms isolated recorded zones of inhibition *i.e.* three out of three. *Ciproxin, Ceporex, Sporidex* and *Chloramphenicol* recorded two out of three each. The least were *Amoxyl, Ciproval, Septrin, Ampicillin* and *Tetracycline*, one out of three.

DISCUSSION

Professional drivers recorded the highest prevalence of total number of pathogens isolated in HIV positive 27.66%. Professional drivers are very mobile and already known for their tendency to socialize as they move from one station to the other.

Only traders and clerical officers showed significant difference between DTP in HIV negative individuals with more DTP in HIV positive. Other occupational group showed no significant difference in DTP for HIV positive and negative.

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