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Prevalence of Gastrointestinal Parasites among Sickle Cell Patients in Delta State, Nigeria

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ABSTRACT

Sickle cell anemia crisis and death in the under-developed nations constitute one of the most challenging global public health problems. It is estimated that each year out of the three hundred thousand babies born worldwide with severe forms of hemoglobin disorders, sickle cell disease constitute more than half of this figure. Sickle cell disorder is an abnormal hemoglobin condition caused by the inheritance of abnormal hemoglobin (Hbs) genes from both parents. This study was aimed at determining the prevalence of gastrointestinal parasites among sickle cell subjects in Delta State. Stool samples were collected from a total of 320 subjects, 160 each from Delta South (Warri) and Delta North (Agbor). Questionnaires and informed consent forms were given to the subjects that are of age and to the parents of the minors before sample collection. The stool samples were examined both macroscopically and microscopically for gastrointestinal parasites .InWarri, 40% (64/160) of SCA subjects infected with either gastrointestinal heamoparisites were male, while 48.1% were females. In Agbor, 47.5% were males, while 43.8% were females. This was statistically significant ($p< 0.05$). The greatest frequency of infection was observed in SCA subjects within the age bracket of 1-10. (42.5%) in warri and 56.2%atAgbor. In Warri *A.lumbricoides*, hookworm, *T.trichuria, E coli*and malaria parasites were observed, 18.1%, 5.6%, 2.5%, 1.3% and 60.6% respectively among sickle cell subjects infected with either gastrointestinal haemoparasites. No microfilaria was isolated among subjects in warri, but was isolated at Agbor (0.6%).Also at Agbor, *A. lumbricoides*, hookworm, *T.trichuria, E. coli*,and malaria parasites had prevalence of16.8%, 2.5%, 1.3%, 1.9% and 68.1%respectively. In Warri, SCA subjects without parasitic infection have a mean PCV of 23.27% as against those with parasitic infection having a mean PCV of 12.88%. At Agbor, those without infection have a mean PCV of 22.84% as against 19.72% among those with parasitic infection. Among SCA subjects with either gastrointestinal or haemoparasites in warri, 24.4%, 50.0% 13.7% resides in rural, urban and riverine Communities respectively. At Agbor 60%, 31.3% resides in rural & urban communities respectively. The prevalence of infection among those who deworm always, occasionally only and those who never deworm were 32.5%, 53.1% and 2.5% respectively in Warri and, 10%, 60% & 21.3% respectively in Agbor. All sampled SCA subjects received blood transfusion always or occasionally as 70.6% and 17.8% respectively in Warri, as 85.0% and 6.3% respectively in Agbor. Prevalence of infection among subjects who use water closet and latrine were 18.7% and 6.9% respectively. Subjects who never wash their hands before and after eating have prevalence of 7.2% while those who wash their hands before and after eating always were 18.4%. The prevalence of malaria parasites 60.6% and 66.8%, Warri and Agbor respectively. Overall, the prevalence of parasitic infections among SCA subjects in Warri was 88.1%. Of this 48.1% were females.SCA subjects between the ages of 1-10 were mostly infected. *A. lumbricoides* (18.1%) was the gastrointestinal parasite

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mostly isolated. This study concludes that prevalence of gastrointestinal and haemoparasite among sickle cell subjects is very high in Delta State. Therefore, the need for all sickle cell anemic subjects to strictly use insecticide treated nets is highly advocated.

KEYWORDS: Gastrointestinal, prevalence, sickle cell, anaemia and malaria.

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INTRODUCTON

The goal of reducing and managing sickle cell anemia crisis and deaths especially in the under developed nation including Nigeria is one of the most challenging global health problems [1].It is estimated that each year over 300,000 babies are born worldwide with severe forms of hemoglobin disorders, sickle cell diseases and thalasamia with sickle cell disease forming more than 200,000 of the above figure. The majority of subjects with sickle cell anaemia live in the under developed nations where endemic parasitic diseases are prevalent and this may exacerbate the severity of steady-state anaemia in infected subjects. Previous study of the impact of intestinal parasites on haemotological parameters of sickle cell anaemia subjects (SCA) aged 18-35 years in Kano, Nigeria revealed a prevalence of 27% parasitic infections (Ahmed *et al.*,2011).A breakdown of the 27% prevalence of the intestinal parasitic infections among sickle cell subjects, *Ascaris Lumbricoides* had 37% (10), *Ancylostoma duodenale* 22.2% (6), *Trichuris trichuria* 11.1 % (3) *Strongyloides stercoralis* is 7.4% (2) *Entamoeba histolytica* 25% (7) *Entamoeba coli* 18.5% (5) and *Giadia lamblia* 7.4% [2]. The sickle cell gene is widespread in Africa, middle east, and Asia as a result of population movement, in the Caribbean, North America and Northern Europe (Agi and Ebenezer 2016). The frequency of sickle cell carriers (HbAs) is up to 20%-25% in West Africa including Nigeria. The mean values of haematocrit level of SCA subjects with gastro intestinal parasites is lower than those without intestinal parasites. Also the mean leukocyte count and mean platelet count of SCA subjects with intestinal parasites are higher than those without intestinal parasitic infections [2]. In Nigeria and across the globe there is paucity of information on the prevalence of impact of gastro intestinal parasites among sickle cell subjects. Meanwhile the prevalence of gastro intestinal parasites is very high in school children in Nigeria, with over 50% in rural communities [3].

However the prevalence of intestinal parasites has been shown to decrease with increasing age and is generally lower in older children and adults [4].. Gastro intestinal parasitic infections are predominantly due to soil transmitted helminthes and protozoan. The infections are strongly associated with poverty and poor personal and environmental hygiene. The aim of the study is to investigate the prevalence of gastrointestinal and haemoparasites among sickle cell subjects in Warri and Agbor Communities of Delta State, Nigeria.

MATERIALS AND METHODS

STUDY AREA

This research was carried out at the central Hospital Warri and Central Hospital Agbor; comprising of sickle cell subjects attending the sickle cell clinic/club of both hospitals in Delta State.

STUDY POPULATION

A total of 320 of Sickle Cell Subjects were been used in this research comprising of 160 each from both the Central Hospital Agbor and Central Hospital Warri being SCA subjects attending the Sickle Cell Clinic of both Hospitals.

SAMPLE SIZE

The sample size (N) was calculated using prevalence from previous studies of pattern and prevalence of sickle cell disease among children in tertiary and non tertiary institutions in South Eastern Nigeria 5%. The sample size of this study was obtained using the formula described by (Kelvin *et al.,* 2016).

Minimum sample size, $N = \frac{Z^2 Pq}{d}$, where

N = Desired Minimum Sample size if population is more than 10,000

Z = The confidence level, (95%) set at (1.96)

P = Estimated proportion of the attribute present in the population (i.e) prevalence rate, here pattern and prevalence of Sickle Cell Disease in South Eastern Nigeria is 5%(Kelvin*et al.,* 2016) = 0.05

Q = (1-P) = (1-0.05) = 0.95

d = degree of accuracy desired: set at (0.05).

Substituting the values into the above equation,

$$N = \frac{Z^2 Pq}{d^2} = \frac{(1.96)^2 X\ 0.05\ X\ 0.95}{0.05^2} = \frac{0.0931}{0.0025} = 37.24$$

Minimum sample size = 37.24

For this study, because of an in-depth and all inclusive analysis, the sample size was increased to 320 to allow for better analysis.

INCLUSION AND EXCLUSION CRITERIA

Inclusion Criteria

The SCA subjects from the age of 2 years and above confirmed by haemoglobin electrophoresis were included in the study.

Exclusion Criteria

Any subject not confirmed positive for Hb-SS by Haemoglobin Electrophoresis was excluded.Blood and stool samples of Children of one year and below were also excluded.

ADMINISTRATION OF QUESTIONNAIRE/ INFORMED CONSENT FORMS

A structured questionnaire bothering on bio-data and socio economic information was administered on the 320 SCA subjects. The information sought included age, sex, type of community of residence, use of mosquito treated nets, rate of blood transfusion, level of hygiene observed etc. The questionnaire was numbered against the container given to the SCA subjects for the purpose of sorting. An informed consent form written in English Language was also given alongside for the parents of the children and the adults to approve before collecting sample from the SCA subjects.

ETHICAL APPROVAL

Ethical approval/permission was obtained from the ethical committee of Central Hospital Warri and Central Hospital Agbor respectively.

SAMPLE COLLECTION

Stool samples were collected from 320 SCA subjects into clean plastic or glass bottles (wide mouth) containing about 5ml of 10% formal saline. The containers were appropriately labeled with the numbers, age and sex of subjects.

Two milliliters of blood samples were collected from the same subjects into ethylene diamine tetra acetic acid (EDTA) containers for haemoparasite.

Bloody urine samples from some subjects passing blood in urine were also collected into sterile urine universal containers to screen for *Schistosoma haematobium* ova. All samples were sent to the laboratory for immediate analysis.

LABORATORY ANALYSIS

Stool samples

Macroscopic Examination:

The stool samples were subjected firstly to macroscopic examinations for consistency, color and presence of constituents such as mucus, pus, blood, segments and adult worms. The consistency was a guide as to whether the egg or worm of the parasite is likely present. Samples containing blood and mucus were examined before the watery samples and then the hard from sample [2].

Microscopic Examination

Wet Preparation (Saline Mount)

Principle

When suspended in a fluid and examined microscopically, many parasites are seen to be motile, moving from one position to another.

This is termed true motility; this is different from Brownian movement (vibration caused by molecular bombardment) or convention currents [6].

Method

A drop of normal Saline was placed on a clean grease free slide. Small amount of the faecal samples were emulsified on the Saline with the aid of an applicator stick. Each of the preparations were covered with cover slips. They were examined under the microscope using X 10 and X 40 objective lenses using the condenser. Iris should be closed for good contrast and assist in the detection of eggs [7].

FORMAL ETHER CONCENTRATION TECHNIQUES

Principles

This is based on sendimentation techniques in which parasites are sedimented by gravity or centrifugal force.

The faeces are emulsified in formal saline water; the suspension is strained to remove large faecal particles. ether or ethyl acetate is added and the mixed suspension is centrifuged. Cysts, ova, eggs and larvae are fixed and sedimented and the faecal debris is separated in a layer between the ether and the formal saline water. Faecal fat is dissolved in the ether, [2].

Method

Using an applicator stick emulsification of approximately 1g of faeces in 4mls of volume for volume 10% formol saline was done. It was mixed by shaking and sieve in a beaker and added into a conical tube and add 3ml of *di ethyl ether*. Stopper the tube and mix for few seconds With a tissue wrapped around the top of the tube, loosen the lid Centrifuge the tube for 1 minutes at 3000pm Discard the ether faecal debris and formol saline leaving the sediment. Re-suspend the sediment and drop a little on a clean slide and cover with coverslip. Examine microscopically using X 10 objective to detect parasites and X 40 to assist in the confirmation of the eggs.

IODINE PREPARATION

Iodine preparations for both the microscopic wet preparations and formol ether concentration methods were done on a clean glass slide. This was done for the detection of cysts of protozoa seen as *Escherichia coli, Escherichia histolytica and Giadia* species.

Principles

This is based on the same principles of both the wet preparation (saline mount method) and the formol ether concentration techniques; except that the iodine preparation is added to assist in the identification of cysts of protozoa seen as *Escherichia coli; Escherichia histolytica* and Giadia species [8].

Method

A normal smear of samples was made with normal saline and one drop of lugus iodine. It was emulsified and cover with a cover slip and examined under the microscope using X 40 objective lense.

BLOOD EXAMINATION

Haemoglobin Electrophoresis

Sickle cell status of subjects was confirmed by the electrophoretic technique (genotype test method using tris-buffer PH (8.0).

Principle

This is based on the principle of Electrophoresis, where the blood samples (Haemoglobins) are separated due to the movement of charged particles through an electrolyte medium (buffer) under the influence of an electric current.

Based on the sharpness of the bands of the separation; the different types of haemoglobin genotypes are identified [4].

Method

Some of the tris buffer was placed in the trough of the genotype machine; allowing it to cover the electric wires, a filter paper placed into both sides of the trough where the cellulose acetate paper is to be attached. The acetate paper placed lightly on the surface of the tris' buffer in a shallow plastic tray or in the trough of the machine; allowing the buffer to impregnate the strip from below by capillary attraction before being submerged completely.

The impregnated strip is removed from the buffer and lightly blotted between two sheets of filter papers to remove excess moisture. With the aid of an applicator; the blood samples are spotted or applied at one end of the paper and positioned into the tank between the middle troughs. A known control sample of SS and AS are applied along side the test sample. Current voltage is then applied for 10-15 mins, to allow for separation. The separation is then read and compared with the control samples; AS or AC.

HAEMATOCRIT ANALYSIS (PCV)

The haematocrit level or PCV (packed cell volume) of all the blood samples were done to ascertain the percentages.

Principles:

This is based on the centrifugation of the blood samples to measure the relative mass of red cells present in a sample of whole blood;Using a micro-haematocrit centrifuge [2].

Method

From the blood already collected in the EDTA container mix properly and collect into Heparinized capillary tubes and one end of the tube using plasticine was sealed**. It was** placed in the Mirco-Hematocrit centrifuge and spin at 3,000 for 5mins. The PCV with a hematocrit reader was read and recorded,

DIRECT WET PREPARATION OF BLOOD SAMPLES

This was done on all the blood samples collected for the detection of microfilalia on trypanosome species.

Principles

This is based on the identification of the suspended parasites from blood due to the parasite from the blood covered with coverslip due to the true motility from one position to another and/or vibration caused by molecular bombardment (Brownian movement) or agitations of the cells of microfilaria or trypanosome species [4].

Method

A drop of the blood was placed on a clean slide and cover with a cover slip and examined for microfilaria or trypanosome species, using X 40 objective.

MAKING, FIXING AND STAINING BLOOD FILMS (Preparation of Blood Films)

Two (2) films each were made for every blood samples collected in EDTA (Thin & Thick films).

Thick Films

Principles

This is based on the lysis of the red cells during staining process allowing haemoparasites (malaria and others) and white cells to be seen in a much large volume of blood.In this technique, the blood is not fixed. It is about 30 times more sensitive than the thin films and more suitable for rapid detention of Haemoparasties [1].

Method

A drop of blood was placed on a clean slide to cover an area of about 1cm diameter and of sufficient thickness to allow bold print to be seen. It was allowed to dry thoroughly to prevent flaking off during staining and was covered with 1 in 10 dilution of giemsa PH (7.2) It was allowed to stain for 30mintues then drained and air-dry. It was examine microscopically with oil emersion.

Thin Films

Principles

This is directly the opposite of the thick films; it is based on the fixing of Red cells; enabling the Haemo parasites to be seen in the parisitized red cells. The red cells are not lysed but may enlarged oval shaped or stippled. This features help in identifying the species of the parasites. This thin film method also assists in the identification of mixed infections [1].

Method:

A drop of blood placed close to one end of a clean glass slide.

Using a clean smooth edged spreader, touching the blood and allowed to extend along the edge of the spreader at an angle of 45 degree the drop, and then spread to make a film of about 40-50mm (2/3 of the slide).The films were allowed to dry and protected from dusts and insects. When completely dried, it was fixed in absolute methanol for one minute and stained with 1 in 10 dilution of giemsa and allow to stand for 5-10mins and washed and rinsed with water It was air-dried and examined microscopically using X 100 objective lense.

SAPONIN LYSIS AND CENTRIFUGATION METHOD FOR CONCENTRATION OF MICROFILARIA

All the samples were subjected to the saponin lysis and centrifugation method. It is particularly important for the diagnosis and concentration of microfilaria.

Principles

Anticoagulated blood is centrifuged to provide preliminary concentration, coupled with the lysis of red cells by the saponin saline. The addition of nuclear blue stain helps in the identification of the species.

Method

Centrifuging of about 1ml of the EDTA blood samples in a glass tube was done for 5mins at 3,000rpm. The supernatant was discarded and 5mg saponin was added and allowed to stand for 10min and discard supernatant. A wet prep was made from the deposit and examine microscopically using X 40 objective lens. Also a dry smear of the deposit was stained with the 1 in 10 dilution of germsa stain and

examined with 100 objective lens after fixing with methanol.

STATISTICAL ANALYSIS

The results obtained were analyzed using the statistical package for social sciences (SPSS). The raw data obtained were analyzed using chi square (x^2) to compare the proportions of data. The odd ratio was calculated for potential risk factors

RESULTS

DEMOGRAPHIC DATA

Blood and stool samples were collected from a total of 320 subjects; 160 from Delta-South (Warri) and 160 from Delta-North (Agbor). In Warri, 45.4% (64/141) of sickle cell subjects infected with either gastrointestinal or haemo-parasites were male, while 54.6% (77/141) were females. In Agbor, 52.0% (76/146) were male while 48.0% (70/146) were female, this was statistically significant ($P < 0.05$) (Table 1).

Table 1:Demographic Data of Sickle Cell Subjects with and without Gastrointestinal and Haemo-Parasites in Warri and Agbor Communities of Delta State.

	Warri			**Agbor**		
Demograhic Data n = 19	Without infection n = 141(%)	With infection *P-value*		n = 14 Without infection n = 146	With infection *P-value*	
SEX						
Male	12 (63.2)	64 (45.4)	0.2205	1 (7.1)	76 (52.0)	0.0013*
Female	7 (36.8)	77 (54.6)		13 (92.9)	70 (48.0)	
Total	**19 (100.0)**	**141 (100.0)**		**14 (100.0)**	**146 (100.0)**	
AGE (years)						
1 – 10	11 (57.9)	68 (48.2)	0.4612	7 (50.0)	90 (61.7)	0.5462
11 – 20	3 (15.8)	45 (31.9)		3 (21.4)	31 (21.2)	
21 – 30	5 (26.3)	26 (18.5)		4 (28.6)	25 (17.1)	
31 and Above	0 (0.0)	2 (1.4)		0 (0.0)	0 (0.0)	
Total	***19 (100.0)***	***141 (100.0)***		***14 (100.0)***	***146 (100.0)***	

Statistical tool used; Chi-square X^2

**Significant at p< 0.05*

With or without infection = either gastro intestinal or haemoparasite infection

The greatest frequency of infection was observed in sickle cell anaemia subjects within the age bracket 1 – 10; (48.2%) in Warri, and (61.7%) in Agbor. There was no relationship between Age and the frequency of parasitic infection among the subjects (Table 1).

PREVALENCE OF GASTROINTESTINAL AND HAEMO-PARASITES PARASITES

In Warri, *A. lumbricoides*, hookworm, *T. trichiura*, *E. coli* and malaria parasite were observed in 20.6%, 6.4%, 2.8%, 1.4% and 68.8%, respectively among infected sickle cell subjects. No microfilaria was observed among the subjects in this area. However, in Agbor, microfilaria was observed in 0.7% (1/146) of the infected subjects with *A. lumbricoides*, hookworm, *T. trichiura*, *E. coli* and malaria parasite having prevalence of 19.9%, 2.7%,1.4%,2.0% and 73.3%, respectively (Table 2).

Table 2: Prevalence of Gastrointestinal and Haemo-Parasites Parasites among Sickle Cell AnaemiaSubjectsin War Communities of Delta State who are infected

Types of Parasite Identified **n = 141**	**Warri** **n = 146**	**Agbor**
	N(%)	N(%)
Ascarislumbricoides	29 (19.9)	29(19.9)

Hookworm	9 (6.4)	4 (2.7)
Trichuristrichiura	4 (2.8)	2 (1.4)
Entamoeba coli	2 (1.4)	3 (2.0)
Malaria	97 (68.8)	107 (73.3)
Microfilaria	0 (0.0)	1 (0.7)
Total	**141 (100.0)**	**146 (100.0)**

EFFECTS OF PARASITIC INFECTIONS ON PACKED CELL VOLUME (PCV)

In warri, sickle cell subjects without parasitic infection have mean PCV of 23%, while those with parasitic infections have mean PCV of 13%. Those without parasitic infection in Agbor have mean PCV of 23% as against 20% among those with parasitic infection. Both results were statistically significant ($P < 0.05$) (Table 3).

Table 3: Packed Cell Volume (PCV) of Sickle Cell Anaemia Subjects with and without Parasitic Infection (Gastro and Haemoparas

	Warri			**Agbor**		
	Without infection (n= 19)	With infection (n= 141)		Without infection (n= 14)	With infection (n= 146)	
	Mean (SD) (%)	Mean (SD) (%)	*P-value*	Mean (SD) (%)	Mean (SD) (%)	*P-value*
PCV	23 (2.77)	13 (0.28)	0.005*	23 (3.10)	20 (2.85)	<0.0001*

**Significant at p < 0.05*

Statistical tool used here is Chi-square X^2

Type of community of residence on Prevalence of Parasitic Infection(Gastro and Haemoparasites)

Among sickle cell anaemia subjects infected with either gastrointestinal or haemoparasite in Warri, 27.7%, 56.7% and 15.6% reside in rural, urban and riverine communities, respectively. In Agbor, the infected subjects reside only in rural (52.0%) and urban (48.0%) communities (Table 4).

Table 4: Prevalence of gastrointestinal and heamoparasitic infections among sickle cell subjects in relation to type of community of residence in Warri and Agbor Communities of Delta State.

	Warri			**Agbor**		
	Without infection No(%)	With infection No(%)	*P-value*	Without infection No (%)	With infection No (%)	*P-value*
COMMUNITY						
Rural	2(10.5)	39 (27.7)	0.0717	10 (7.1)	96 (52.0)	0.7744
Urban	16 (84.2)	80 (56.7)	4 (92.9)	50 (48.0)		
Riverine	1 (5.3)	22 (15.6)		0 (0.0)	0 (0.0)	
Rural-Riverine	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
Total	**19 (100.0)**	**141 (100.0)**		**14 (100.0)**	**146 (100.0)**	

**Significant at p < 0.05*

Statistical analysis used is chi-square (X^2)

Frequent Deworming Practice and Prevalence of Parasitic Infection

The prevalence of parasitic infection in those who deworm always, occasionally and those who never use antiparasitc drugs were 36.9%, 60.3% and 2.8%, respectively, in Warri, and 11.0%, 65.7% and 23.3%, respectively, in Agbor (Table 5).

Table 5: The Influence of Frequent Deworming Practice on the Prevalence of Gastrointestinal AndHaemo-Parasites Parasites Among Sickle Cell Subjects In Delta State

	Warri			Agbor		
	Without infection	With infection		Without infection	With infection	
No (%)No (%)	*P-value*	No (%)	No (%)	*P-value*		
RESPONSE						
Always	13 (68.4)	52 (36.9)	0.0291*	7 (50.0)	16 (11.0)	0.0003*
Occasionally	6 (31.6)	85 (60.3)		6 (42.9)	96 (65.7)	
Never	0 (0.0)	4 (2.8)		1 (7.1)	34 (23.3)	
Total	**19 (100.0)**	**141 (100.0)**		**14 (100.0)**	**146 (100.0)**	

**Significant at p< 0.05*

Statistical analysis used is chi-square (X^2)

Frequent Blood Transfusion and Prevalence of Parasitic Infection

All sickle cell subjects received blood transfusion always or occasionally. The prevalence of parasitic infection in those who are transfused always and occasionally were 80.1% and 19.9%, respectively, in Warri, and 93.1% and 6.9%, respectively, in Agbor (Table 6).

Table 6: The Influence of Frequent Blood Transfusion and Major Sickle Cell Crises on the Prevalence of Gastrointestinal and Haemo-Parasites Parasites among Sickle Cell Subjects in Warri and Agbor Communities of Delta State.

Warri	**Agbor**					
	Without infection	With infection		Without infection	With infection	
	No (%)No (%)	*P-value*		No (%) No (%)	*P-value*	
BLOOD TRANSFUSION						
Always	16 (84.2)	113 (80.1)	1.0000	136 (93.1)	0.0903	11 (78.6)
Occasionally	3 (15.8)	28 (19.9)		10 (6.9)		3 (21.4)
Never	0 (0.0)	0 (0.0)		0 (0.0)		0 (0.0)
Total	**19 (100.0)**	**141 (100.0)**		**146 (100.0)**		**14 (100.0)**
SICKLE CELL CRISES (Response from questionnaire)						
Always(1–2 times every month)	7 (36.8)	138 (97.9)	144 (98.6)	0.0000*	0.0000*	8 (57.1)
Occasionally(1 every 3 months)	12 (63.2)	3 (2.1)	2 (1.4)			6 (42.9)
Never	0 (0.0)	0 (0.0)	0 (0.0)			0 (0.0)

Total	**19 (100.0)**	**141 (100.0)**	**146 (100.0)**
14 (100.0)			

**Significant at p< 0.0*

Statistical analysis used is chi-square (X^2)

Parasitic Infections and Sickle Cell Crisis

All sampled subjects have history of sickle cell crisis. The prevalence of parasitic infection in those who have sickle cell crisis always and occasionally were 97.9% and 2.1%, respectively, in Warri, and 98.6% and 1.4%, respectively, in Agbor (Table 7). both results were statistically significant ($P < 0.05$).

Table 7: The Prevalence of Gastrointestinal Parasites as Related To Water Source, Toilet and Hand Washing Before and After Eating.

Without Infection No (%)	**With Infection** No (%)	P-value
SOURCE OF WATER		
Borehole	213 (89.5)	71 (86.6) 0.5431
Well	25 (10.5)	11 (13.4)
Stream	0 (0.0)	0 (0.0)
River	0 (0.0)	0 (0.0)
Total **238 (100.0)**	**82 (100.0)**	
TOILET TYPE		
Water Closet	206 (86.6)	60 (73.2) 0.0096*
Pit	0 (0.0)	0 (0.0)
Latrine	32 (13.4)	22 (26.8)
Bush	0 (0.0)	0 (0.0)
Total **238 (100.0)**	**82 (100.0)**	
HANDWASHING BEFORE AND AFTER EATING		
Always	213 (89.5)	59 (72.0) 0.0003*
Occasionally	25 (10.5)	0 (0.0)
Never	0 (0.0)	23 (28.0)
Total **238 (100.0)**	**82 (100.0)**	

**Significant at p< 0.05*

Statistical analysis used is chi-square (X^2)

Source of Water and Personal Hygiene and Prevalence of Gastrointestinal Parasites.

All sampled subjects either have borehole or well as their main source of water supply. From the two sampled areas (Warri and Agbor) 86.6% of infected subjects have access to borehole and 13.4% to well water .

The prevalence of gastrointestinal parasites among subjects who use water closet and latrine were 73.2% and 26.8%, respectively .

Subjects who never wash their hands before eating have prevalence of 28.0%, while that of those who wash theirs before and after eating always was 72.0% (Table .8).

Table 8: Degree of Malaria Parasitemia among Sickle Cell Subjects in Delta State

	Neg	Scanty	+	++	Total	P-value
	No (%)	No (%)	No (%)	No (%)	No (%)	
Warri	63 (39.4)	5 (3.1)	90 (56.3)	2 (1.2)	160 (100.0)	0.4574
Agbor	53 (33.1)	9 (5.6)	97 (60.7)	1 (0.6)	160 (100.0)	

Use of Mosquito Nets and Prevalence of Haemoparasites

Haemoparasites (malaria and microfilaria) have prevalence of 36.1%, 21.9% and 42.0% among sickle cell patient who use treated net, ordinary net and those who never use mosquito net, respectively (Figure 1).

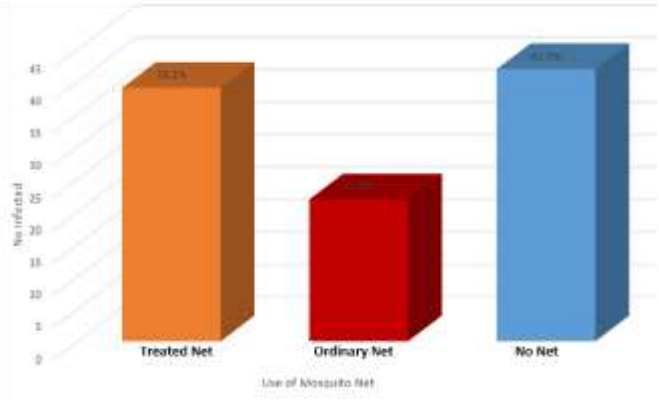

Fig: 1: Malaria Infectionin Relation to the Use of Mosquito Treated Net.

Degree of Malaria Parasitemia among Sickle Cell Subjects

In warri, out of 160 subjects tested for malaria parasites 63 (39.4%), 5 (3.1%), 90 (56.3%) and 2 (1.2%) gave negative, scanty, one plus (+) and two pluses (++) results, respectively. This was 53 (33.1%), 9 (5.6%), 97 (60.7%) and 1 (0.6%), respectively, inAgbor (Table 8).

Gastrointestinal and Haemoparasites Co-infections

Gastrointestinal and haemoparasites co-infections were observed in 18.1% in warri and 15.6% in Agbor among sickle cell subjects subjects (Figure 2).

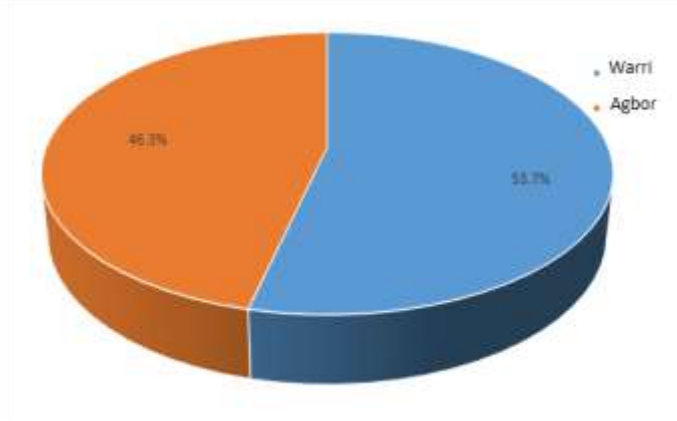

Fig. 2: Co-infection of Haemoparasite and Gastrointestinal Parasite among Sickle Cell Subjects in Delta State.

DISCUSSION

In sub-sahara Africa *Plasmodium falciparum* and intestinal parasites are capable of causing anaemia and are important factors triggering Sickle cell crisis with increase in morbidity and mortality among SCA subjects. In this present study, sampling was carried out in Central Hospital Delta-South (Warri) and Central Hospital Delta-North (Agbor).The prevalences of malaria parasites (60.6% and 66.8% - Warri and Agbor, respectively) are high compared to 3.3% reported in Tanzania. Although a prevalence of 13.8% was also reported in the same stud (Prevalence of *P. falciparium*) among sickle cell subjects [1]. when PCR instead of blood film was used. This prevalence is still low compared to our prevalence's in this study. This contrast may be due to difference in sample size. In Tanzania the sample size was 123 against 160 (each for Warri and Agbor) used in this study. [10].reported prevalence of 15.6% among sickle cell subjects in Kenya. This prevalence is low. Also, this malaria prevalence is close to the 30% reported in Yenegua Balyelsa State Nigeria [11].This disparity may be as a result of difference in environment.

In Warri, the prevalence of parasitic infections among the sickle cell patient was 88.1%. Of this, 48.1% of those infected were females. Similarly, in Agbor the prevalence was 91.3% and only 43.8% female were infected. Subjects between the age of 1 and 10 were more infected. This may be due to increase contact with contaminated soil during play.

A. lumbricoides (18.1%) was the gastrointestinal parasite often isolated among the sickle cell subjects. The prevalence in this study is contrary to 37% reported in a previous study in Kano State, Nigeria (Ahmed and Uraka, 2011). This contradiction might have resulted due to differences in cultural practices and socio-economic status, and also because the study was only on gastrointestinal parasites. Other intestistinal parasites isolated include Hookworm (Warri - 5.6%, Agbor - 2.5%),T. trichiura (Warri - 2.5%, Agbor- 1.3%)and Entamoeba coli (Warri - 1.3%, Agbor - 1.9%). This is also contrary to 60% prevalence case in Jos and Zaria as earlier observed by Daniel *et al.* (2009), (42.78%, 28.35%) observed in Edo and Delta States [12].., 75.6% case in Ibilo Edo State (Asemota *et al.,* 2011). However, this intestinal study report is closed to the 15.3% study in Benin City, Nigeria [8].

Microfilaria was seen in the blood of one of the subjects (0.6%) in Agbor. However no microfilaria was observed in

Warri. This is contrary to the 20% prevalent rate of malaria parasites and 10.5% prevalence for microfilaria reported in Yenegua Balyelsa State [11].This may be due to the fact that this study comprises of both gastrointestinal and haemoparasites even among SCA subjects only.

The mean Packed Cell Volume (PCV) of Subjects without parasitic infection (Warri - 23.27%, Agbor - 22.84%) was significantly greater than those with infection (Warri - 2.88%, Agbor - 19.72%). Sickle cell anaemia subjects with parasitic infection have severe anaemia when compared with those without parasitic infection. This report is similar to the study conducted in Kano State, Nigeria, with mean haematocrit of SCA subjects without intestinal parasite infections (0.27 L/L) significantly greater than those in infection (0.23 L/L) [2].There was no relationship between parasitic infections and source of water supply, frequent blood transfusion, as well as with type of community. However, frequency of parasitic infections was significantly high in SCA subjects who often has crisis. The effect of malaria parasites on infected RBC as well as the malabsorption and gastrointestinal bleeding caused by intestinal parasites explain the reason for the frequent Sickle cell crisis among the subjects.

Although significant relationship was observed between intestinal parasites infection and toilet type. The reason for this is not clear. Most of the SCA subjectsin this study use Water Closet 266(320) and always wash their hands before and after eating 272(320). This explained the high frequency of infection observed in these categories. This also explained the high prevalence of haemoparasite infection observed in SCA subjects using treated mosquito nets (23.1%) as most of the sampled subjects use treated mosquito nets 184(320).

In Warri, all SCA subjects who never deworm were infected. Similarly, in Agbor 34 out of 35 who never deworm were found to have the present of one or more intestinal parasites. Sickle cell disease increases susceptibility of subjects to infections with a concomitant increase in morbidity and mortality [6].Of the SCA subjects studied in Warri and Agbor, 56.3% and 60.7%, respectively, have malaria parasites in the degree of (++) in their peripheral blood film.

Intestinal and haemoparasite co-infections were observed in only 9.1% and 7.8% of the SCA subjects in Warri and Agbor, respectively. Intestinal parasite infections increase the susceptibility to Plasmodium infection by suppressing pro-inflammatory Th1 response that generates immunopathology in malaria infection resulting in an increased risk of complications, hepatosplenomegaly and anaemia (Salazar-Castañon *et al.,* 2014). However, some studies reported that intestinal parasite infections confer protection against malaria by reducing the parasite density [1].

CONCLUSION

This study revealed a high level of gastrointestinal and haemoparasitic infection.These Parasitic infections aggravate the anaemia in SCA subjects and can be an important factor triggering Sickle-cell crisis in these subjects. Despite prevailing campaigns on the importance of the use of mosquito treated nets especially among SCA subjects. This study showed that many of these subjects still don't use one either due to ignorance or unavailability.

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