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RESEARCH ARTICLE

PREVALENCE AND HAEMATOLOGICAL INDICES OF GARDIASIS AND MALARIA AMONGST PUPILS OF PAEDIATRIC AGE (0-15) IN OWERRI WEST IMO STATE, NIGERIA

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Abstract

This study investigated the prevalence and haematological parameters of gardiasis and malaria amongst pupils of paediatric age (0-15)in 20 randomly selected primary schools was carried out in Owerri West Local Government Area of Imo State, Nigeria. Observations were made of the age, sex, socio-economic status of parents and the schools' locations to determine the secondary outcomes measures to these two diseases. Both blood and faecal samples were collected from each of the pupils between the hours of 8.00 am and 11.00 am using 19cc needle guage and 10 ml syringes into EDTA bottles for the blood; and sterile applicator sticks employed for the faeces, into wide-mouthed screwed, non-greasy universal bottles. Exclusion procedures were also carried out to eliminate other possible protozoal parasites including administration of combantrin tablets to the 150 volunteers for deworming. Blood parasitaemia due to malaria parasites was determined using the giemsa stained thick and thin smears, while giardiasis was determined using the faecal wet preparation technique involving the Lugol's iodine faecal smears for the presence of trophozoites, cysts or casts. Determinations of other haematological parameters were carried out using histochemical techniques. Results showed out of 150 samples, 78(52.0%) were infected with either giardiasis, 20(25.64%) or other malaria parasite forms 30(38.46%), while 28(35.89%) had mixed infections of both malaria and giardiasis. A total parasite count for both infections was 1958 represented as 37% and 62.92% for giardiasis and malaria respectively. Forty-two (42) samples were discarded due to indeterminate results. RBC counts ($X10^{12}$/L) for control: scarification (5.6±2.0), unscarification (2.7±0.3); and for test (infected): malaria (4.1±3.2), giardiasis (2.9±1.3), and malaria+giardiasis (1.4±0.02) showed marked significant difference ($t=3.7$, $p<0.002$); and similarly between both infection categories ($t=1.8$, $p<0.007$). Recorded values for PCV showed control: scarification (42.5±3.3), unscarification (26.2±2.2); and for test (infected): malaria (30.4±4.2), giardiasis (28.4±1.0) are indirectly proportional to intensity of infection establishing significant correlation between parasite count (PC) and PCV ($r=0.45$, $p<0.002$), PC and RBC ($r=0.36$, $p<0.002$), PC and reticulocyte ($r=0.08$, $p<0.02$). MCV values did not show clear correlation pattern with PC ($r= -0.07$, $p>0.02$). Mean proteins from the test (infected) groups: total protein (malaria,

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6.13±0.01 & giardiasis, 4.50±0.10); albumin – (malaria, 5.52±0.30 & giardiasis, 7.41±2.2); globulin- (malaria, 6.40±0.15 & giardiasis, 8.15±0.22) reduced significantly ($p<0.01$) compared with each of the control groups and showed significant correlation ($r=0.01$, $p<0.003$) with PC: malaria ($104.4\pm3.3x10^5$), and giardiasis ($76.2\pm5.8x10^5$).Giardia lamblia and malaria parasite species are naturally prevalent protozoan parasites. Notable significant difference in prevalence of these protozoan parasites infestations among children abound.

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Introduction:-

Parasitic infections and in particular those caused by protozoa, are a major public health problem worldwide. They are among the most widespread human infections in developing countries, with children being the most vulnerable population (Harhay et al., 2010). Giardia lambia is the most common human enteropathogenic protozoan that can cause acute and chronic diarrhoea (Garba and Mbofung, 2010). Giardia lamblia is highly infectious protozoan parasite capable of causing gastrointestinal illness in both humans and animals (Suman et al., 2011). G. lamblia as reported by Suman et al., (2011) is most common in areas where sanitation and hygiene are poor. Increased prevalence in human as well as in some of the surrounding animals offers an emerging concern about the role played by some animals in human giardiasis. Each individual eliminates up to 900 million cysts per day. Higher prevalence is found in tropical and subtropical areas, in urban than in rural where Giardia labmlia affects up to 30% of the population (Minvielle et al., 2004).

Intestinal parasitic infections are among the most common infections Worldwide. The prevalence of intestinal parasitism in primary schools in Nigeria have been linked with poor socio-economic background, migration, poverty, wars, illiteracy, ignorance to medical awareness, poor sanitation practices, over population, poor disposal of refuse, and poor personal hygiene (Auta et al. 2014).

Malaria is caused by obligate intracellular parasites, which live in host erythrocytes and remodel these cells to provide optimally for their own needs. It is a major public health problem in tropical areas, and it is estimated that malaria is responsible for 1 to 3 million deaths and 300 to 500 million infections annually (Iwuafor et al., 2016). Malaria remains the most complex and overwhelming health problem, facing humanity in vast majority of tropical and sub-tropical regions of the world, with 300 to 500 million cases and 2 to 3 million deaths per year (WHO, 2017). About 90% of all malaria deaths in the world today occur in the sub-Saharan Africa and this is because majority of infections are caused by Plasmodium falciparum, the most dangerous of the four human malaria parasites accounting for an estimated 1.4 to 2.6 million deaths per year in this region (WHO, 2018). About 91 percent of malaria–related deaths are in Africa with 86 percent of victims being children aged under five (Mia et al., 2011).

Materials And Methods:-

Study Area

The study area, Owerri West Local Government Area (LGA), is one of the LGAs in old Owerri LGA. It is located between the geographical coordinates of longitude 0.5°28' to 05°29' and latitude 06°59' to 07°00'. The vegetation is essentially a rain forest type with flat topography. Some communities are provided with social services such as electricity, pipe-borne water, health centers and schools.

Sample Population

A total of twenty (20) primary schools were randomly selected to cover the entire local government area, covering urban, semi-urban and rural areas. One hundred and fifty (150) pupils whose ages range from 0 – 15 years old were enrolled for the study. The consent of the pupils and class teachers were verbally sought and obtained.

Sample Collection

Stool and blood samples from the selected primary school pupils from each of the 20 schools were collected by the pupils who were properly instructed on how and time of collection. Each pupil was given a sterile, wide-mouthed

screwed cap, non-greasy universal bottle container for stool collection. In each case, the bottles were properly labeled to contain basic information such as name of the pupil (optional), age and sex, class and name of school, etc. The stool samples were also serially numbered to avoid misplacement. Sterile applicator stick was used to collect the stool samples between the hours of 8.00 am and 11.00 am each day by the researcher. This was transported to the laboratory for Giardia analysis. About 15 ml of venous blood sample was collected by vain puncture from each of the pupils by a medical laboratory scientist using 19 cc needle gauge and 10 ml syringe into EDTA bottles.

Exclusion of other parasites than malaria parasites (Plasmodia species) and Gardia lamblia in blood and faecal samples respectively:
Plasmodia species from blood and Gardia lamblia from stool samples were confirmed by thick and thin smears of blood from finger prick stained with Giemsastain at pH 7.2 for the plasmodia species and together with faecal smear stained with Lugol's iodine, examined under x 40 magnification and x 100 objective lens under the light microscope. Victims of other blood parasites like trypanosomes, micro-filarial worms, Leishmania were excluded from the study. Conbantrin tablets (Neimeth Int. Pharm. Plc., NY.), were administered to some victims of intestinal parasites until they deworm completely. Gardia lamblia were examined by finding the adult/cysts in a wet smear direct 75% normal saline preparation of faecal samples.

Collection of blood sample and determination of malaria parasitaemia:
About 15 ml of venous blood sample was collected by venepuncture from the subjects (both test and control groups), whose consent was sought and the reason for the research was explained to their teachers and guardian/ parents. About 5 ml of the blood collected was transferred to a plastic universal container with heparin at 10 units per ml and this was mixed well gently. About 3 ml was drawn from the 5 ml placed in clean plain glass universal bottle till retraction for serum separation, while the remaining 2 ml was put into EDTA bottles. The determination of the level of malaria parasitaemia was done by thick and thin smears of finger prick blood.

Collection and processing of faecal matter for the presence of Gardia species/cysts:
Faecal sample was collected into universal containers. This was examined for colour, blood and presence of adult/cysts of Gardia species macroscopically and microscopically respectively. About 1 gm of stool was dissolved in 5 ml of normal 75% physiological saline from where 0.002 ml was smeared and stained with Lugol's iodine on a microscope slide. This was examined under objective lens of a light microscope at x10 and x40 magnification, for the presence of Gardia adult/cysts.

Determination of haematological parameters:
About 2 ml each of both faecal (from the 1 gm of faecal sample dissolved in 5 ml of normal physiological saline) and blood sample was placed in EDTA bottles for analysis in same laboratory above. These analyses and estimation were based on the methods of Dacie and Lewis, (1984) as indicated below:

Determination of total white blood cell (WBC) count:
Faecal sample:
The lugol's iodine stained faecal microscope slide was examined under x10 and x40 magnification objective lens for the presence of WBCs. The essence of identifying WBCs is to differentiate an inflammatory from non-inflammmatory causes of diarrhea.

Blood sample:
Dacie and Lewis (1984) were employed with modifications. About 20µl of blood was delivered into 0.38 ml of white blood cell diluting fluid. This was prepared by adding 3 drops of gentian violet solution into 200 ml of 2% glacial acetic acid. The suspension was allowed to stand for about 5 mins after thorough mixing to allow for the complete lysing of red blood cells in the diluting fluid and thus leaving the leucocytes and their nuclei stained violet black. The suspended cells were mixed again and were used to charge a Neubauer haemocytometer (BS 748, Weber, England) using capillary tube.

Determination of differential white blood cell count:
A thin smear of the gentian violet stained whole blood sample and lugol's iodine stained faecal sample was made each on clean degreased microscope slides. These were fixed in methyl alcohol for 3 minutes. A buffer solution of pH 6.8 was added on the slides and thoroughly mixed. The diluted stain was allowed to stand for 7 mins. The stain was washed off with buffer solution and this was allowed to dry in air before examination under an oil immersion of

a binocular microscope at x 100 magnification. The longitudinal counting method was employed to record the proportions of neutrophils, lymphocytes, monocytes, eosinophils basophils.

Determination of red blood cell (RBC) count:
About 0.1 ml of the 10 ml whole blood drawn with 10 ml disposable syringe and 19 cc needle from the vein was delivered into a 9.9 ml of the Hayems fluid. This was to make a 1 in 100 dilutions and mixed. The 0.1 ml of the diluted whole blood was delivered into a charged improved Neaubaur chamber (S 748, Weber, England). Red blood cells within central squares were counted microscopically at x 40 and x 100 magnification.

Determination of packed cell volume (PCV):
About ¾ volume of the microhaematocrit capillary pipette was filled with whole unclotted blood and one end of the microhaematocrit capillary pipette was plugged with plasticin. This pipette was spun using a microhaematocrit centrifuge (Model MBIEC, Boston, USA) at 1,500 rounds per minute (rpm) for 5 minutes. The haematocrit percentage of the PCV was determined using microhaematocrit reader (Hawksley, England).

Results/Discussion:-

Table 4.1 shows the distribution of the parasite forms amongst the pupils in the selected schools in Owerri West LGA of Imo State. Out of the total of 150 samples of both test (stool and blood) and control groups, seventy – eight (52.0%) samples were infected; 20 (25.64%) with gardia sp, and 30 (38.46%) with other various species of malaria parasites. Twenty - eight (28) samples representing (35.89%) had mixed infections of Giardia and Malaria species. Total parasite counts (1958) were 726 (37.07%) and 1232 (62.92%) for Giardia and Malaria species. The intensity of both infections was significant when compared at P<0.02. No giardiasis or malaria parasite form was observed with the control group of the 30 samples except helminthes.

Table 4.1:- Nature and distribution of parasites (malaria and giardia) species in selected primary schools in Owerri West LGA.

No Examined	No Infected (%)	No. Infected with Giardia sp (%)	No Infected with Malaria sp (%)	No. Infected with both Giardia and Malaria sp (%)	Control
150	78 (52.0)	20 (25.64)	30 (38.46)	28 (35.89)	30 (20.0)
No. of Parasite forms	1,958	726 (37.07)	1,232 (62.92)	1,958 (100.0)	Nil

* 42 samples were discarded due to procedural errors

The nature and distribution of both protozoan parasitic infestionsamongst pupils in selected primary schools in Owerri West LGA, showed high prevalence rate (52.0%), close to 80.9% reported by Damenet al., (2011); who conducted similar research among the Almajiris in North-eastern Nigeria. This prevalence is higher when compared with previous findings of 28.0% in south-west Nigeria as reported by Salako, (2001); 55.2% in south-eastern Nigeria by Agwu, (2001); and 67.2% in south-south Nigeria by Meremikwu et al., (1995).

Table 4.2 presents the mean evaluation of some haematological indices like red blood cell (RBC), pack cell volume (PCV), reticulocyte proportion (RP), and mean cell volume (MCV), in the infected children. The mean RBC counts ($x10^{12}$/L) for control are 5.6±2.0 for scarification and 2.7±0.3 for unscarification; while the infected have 4.1±3.2 and 2.9± 1.3 for malaria and giardia, and 1.4±0.02 for malaria and giardia combined infections. There is a marked significant difference between control and infected (t=3.7, p<0.002) and also between the infection categories – malaria and giardia (t=1.8, p<0.007). There was a significant correlation between mean parasite count and RBC counted (r= 0.36, p<0.002). The mean PCV is indirectly proportional to intensity of infection. Recorded values for PCV are 42.5±3.3, 26.2±2.2 for scarification and unsarification(controls) and 30.4±4.2 and 28.4±1.0 for the malaria and giardia infected groups. There was a significant correlation between parasite count and PCV (r=0.45, p<0.002). The reticulocyte count increased with increase in intensity of infection (r=0.08, p<0.02). The MCV values did not show any correlation with intensity (r=0.07, p > 0.02), or any significant difference in values in two two infection categories.

Table 4.2:- Mean evaluation of some haematological indices.

Infection category	No studied	Mean Red blood cells (x 10^{12}/L)		Pack cell volume	Reticulocyte proportion	Mean cell volume	Mean parasite count (Intencity)
Control							
Uninfected	15	Scarification	5.6±2.0	42.5±3.3	0.72±0.36	82.6±5.1	-
Uninfected	15	Unscarification	2.7±0.3	26.2±2.2	0.653±0.25	80.2±7.2	-
Infected	30	Malaria	4.1±3.2	30.4±4.2	1.2±1.4	78.7±6.5	104.4±3.3x10^5
Infected	20	Giardia	2.9±1.3	28.4±1.0	1.4±2.2	72.1±1.3	76.2±5.8x10^5
Infected	28	Mal. + Giar	1.4±0.02	33.1±3.3	0.93±4.2	63.0±1.4	180.6±4.2x10^5

Table 4.3 shows the mean serum levels of total proteins, albumin and globulin. The mean value for total proteins reduced significantly ($p<0.01$) compared to the controls. The mean albumin and globulin levels increased compared to controls. However, marked correlation existed when comparing values for total protein (6.13±0.01 and 4.50±0.10), albumin (5.52±0.30 and 7.41±2.2) and globulin (6.40±0.15 and 8.15±0.22) for malaria and giardia respectively with intensity (104.4±3.3 x 10^5 and 76.2±5.8 x 10^5) $r = 0.01$, $p<0.003$

Table 4.3:- Mean serum levels of total proteins, albumin and globulin.

		No studied	Total protein (g/100 ml)	Albumin (g/100 ml)	Globulin g/100 ml	Intensity
Control (uninfected)						
	Scarification	15	9.51±0.02	3.8±0.02	4.54±0.20	-
	Unscarification	15	7.35±0.62	4.7±0.01	5.3±3.1	-
Infected	Malaria	30	6.13±0.01	5.52±0.30	6.40±0.15	104.4±3.3x10^5
Infected	Giardia	20	4.50±0.10	7.41±2.2	8.15±0.22	76.2±5.8x10^5
Infected	Mal+Giard	28	1.4±2.3	8.30±1.10	8.6±2.4	180.6±4.2x10^5

Table 4.4 shows both mean total and differential white blood cell count (WBC). Mean total WBC for giardia and malaria of 7.0±1.0 and 7.3±0.4 showedsignificant correlation existed between WBC and number of parasite forms ($r = -0.25$, $p< 0.005$). Results showed more correlation with eosinophilia ($r= -0.5$, $p<0.02$) than other WBC types.

Table 4.4:- Mean total and differential white blood cell counts (x 10^9/L).

Infection category		No subjects	Parasite count	Total WBC	Neutrophils	lymphocytes	Eosinophils	Monocytes	Basophhils
Infected	Giardia	20	726	7.0±1.0	3.0±1.2	3.3±1.5	0.4±0.1	0.73±0.12	0.20±0.10
	Malaria	30	1232	7.3±0.4	2.8±2.0	2.1±1.0	0.1±0.01	0.12±0.2	0.1±0.2
	Mal.+Giard	28	1958	15.4±1.3	4.3±1.3	3.8±2.0	4.2±1.3	1.4±2.2	1.4±0.3

Control(uninfected)	Scarifications	15	-	9.9±1.2	4.2±1.0	4.0±1.7	0.9±0.4	0.82±0.4	0.30±0.2
	unscarification	-	-	4.2±1.5	2.2±1.1	2.0±0.4	0.14±0.1	0.22±0.1	0.13±0.1

WBC ($X10^9$/L) =r = - 0.25, p< 0.005
Neutrophils ($X10^9$/L) = r = 0.2, p> 0.02
Eosinophils ($X10^9$/L) = r = -0.5, p< 0.02
Monocytes ($X10^9$/L) = r = -0.7, p< 0.001
Basophils ($X10^9$/L) = r = -0.4, p< 0.002

The lack of provision of modern toilet facilities, unavailability of potable pipe-borne water and the lag in improved personal hygiene and sanitary practices among pupils contributed to this high prevalence. Results show the different levels of endemicity categorised into holoendemic and hypoendemic schools. The observed endmicities were informed by poor personal hygiene and sanitation which encouraged vegetation, perennial farming activities, gullies dug during road constructions and irrigation channels, shrubs and trees. All these harbor vectors, pathogens, and however, provide shade for human rest. In all, considering the fairly restricted geographical areas of the different schools, the distribution patterns of the infection are mainly regulated by the different environmental conditions thus exposing pupils to risk behaviours. From results, malaria has its presence more in areas limited to temporary rain-fed pools which underwent considerable diurnal temperature fluctuation. Ecological reasons have been adduced for the prevalence and dispersion of giardiasis. Klumpp and Chu (1977) reported of mosquitoes' densities fluctuate and their vectorial ability correlates with fluctuations in the level of breeding sites.

The numerical estimation of blood cells in malaria and giardia protozoan infections has not been fully elucidated. There was a great loss, when the estimation of haemoglobin as it relates to anaemia and physical fitness was considered in this study. These results have shown significant reduction in haematological indices like PCV, RBC and MCV during prolonged cases of malaria and diarrhea caused by giardiasis or both malaria and giardiasis. This study showed significant reduction in RBC where diarrhea was observed to be associated with blood in either of these two infections.

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