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Definitive Diagnosis of *Plasmodium* Species and Treatment of Malaria, Using Parasitological and Immunodiagnostic Techniques among Households in Idah Local Government Area (LGA), Kogi State, Nigeria

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Abstract: A survey was conducted to determine the prevalence and intensity of the four species of plasmodium known to affect humans.The research was carried out between June, 2012 and May, 2013. Both parasitological and immunodiagnostic (Immuno-Chromatographic-Test: ICT-Australia) tests were carried out to bring about definitive diagnosis of the species of plasmodium. The study area is Idah Local Government Area (LGA). This was divided into Idah Metropolis (IM) and Idah Suburbs (IS). The necessary biodata information such as sex, literacy level was collected from each participant through structured questionnaire. A total of 573 male and female Literate (L) and Illiterate (IL) subjects were examined for malaria parasites. Of this total (573), 313 (54.6%) infected subjects was recorded as the overall prevalence of infection. Statistical analysis revealed that there was no significant difference ($p>0.05$) between the rate of male, L and IL infection ($\chi^2_{cal.} = 3.251$; df = 9). Similarly, infection rate between female L and IL did not differ significantly ($p>0.05$) ($\chi^2_{cal = 14.551\ DF = 9}$). However, comparison of the four factors, viz. sexes (male and female), L and IL involved in the rate of parasite infection revealed significant difference ($p<0.05$) ($\chi^2_{cal.} = 56.287$; df = 27) with the prevalent rate being higher in female than in their male counterparts. In this study, the overall Geometric Mean Intensity (GMI) recorded was 774.8 per μL of blood. Also, significant difference was observed ($p<0.05$) in prevalence of malaria infection between IM and IS, being higher in the latter than the former ($\chi^2_{cal} = 21.552$; df = 3). The GMI recorded for subjects in the Idah suburbs was clearly significant ($p<0.05$) than that of Idah metropolis ($\chi^2_{cal} = 45.431$; df = 3). Generally, GMI was recorded for Idah metropolis and Idah suburbs under heavy infections with the exemption of Polytechnic community where both heavy and moderate intensity of infection was recorded. This finding corroborates the fact that the species, *Plasmodium falciparum* was encountered throughout the study. The treatment regimens of this study were inconclusive because the 34 subjects who were divided into two groups of 17 each failed to turn up for re-examination after the initial treatment with artesunate and fansidar, respectively.

Key words: Definitive diagnosis, Idah LGA, intensity, *Plasmodium* species, prevalence, treatment

INTRODUCTION

Malaria, a disease caused by four species of the genus Plasmodium, places a huge burden on human life. The disease ranks first on the list of parasitic infections responsible for high morbidity and mortality rates with individuals in all countries being potentially at risk. Each year, at least 30 million clinical malaria cases and about 1.5-2.7 million deaths occur worldwide. Malaria presents a diagnostic challenge to laboratories in most countries due to its endemicity, population movement and contraction of the disease where little expertise is available. In most cases the laboratory scientists stop at sighting any malaria parasite in blood film without distinguishing the species and assigning plus sign +, ++ or +++, depending on level of parasitaemia and the doctor simply prescribes any of the antimalarial drugs.

Definitive diagnosis which is cost effective and sure way of managing malaria parasite infection is hardly carried out in most laboratories. Within the tropics and subtropics of the world malaria is endemic and the disease presents a diagnostic challenge to laboratories. This has been attributed to the endemicity of the disease and population movement. Majority of malaria is found in countries where cost-effectiveness is an importantfactor and ease of performance and training is a major consideration (Barker *et al*., 1992). Although, highly sensitive and species-specific assays have developed to

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improve diagnosis of the four human plasmodium, the microscopic examination of blood smears (parasitological technique) remains the "gold standard" in the diagnosis.

Individuals in all countries arepotentially at risk but the greatest suffering falls to people in tropical countries. The degree of endemicity varies between countries and even between different areas in the same country (Miller *et al*., 1994). The pattern of pathology also differs with change in the degree of endemicity. Seasonal variations in the endemicity and frequency of asymptomatic malaria parasitaemia in a rural and urban school children (aged 6-12 years) has been reported by Salako *et al*. (1990).

Malaria is a leading cause of morbidity and mortality in tropical countries of the world, especially in sub-Saharan African. Each year, at least 30 million clinical malaria cases and about 1.5-2.7 million deaths occur worldwide (WHO., 1997; Peter and Anatoli, 1998). This situation is worsened by the widespread and increasing resistance of plasmodium falciparurn to commonly used antimalaria and especially chloroquine the drug that has been the mainstay of malaria treatment for decades (White *et al*., 1992; Newton *et al*., 1994). It is a public health problem in some 90 countries, inhabited by nearly 40% of the world population that is over 2 billion people (Rietveld and Kouznetsov, 1997).

For hundreds of millions of children in Africa, malaria is a constant threat during their early years. Although, only a small proportion of infections endanger life, so, prevalent is malaria that this small proportion is numerically massive (Greenwood *et al*., 1991). In endemic area for malaria, about 25-50% deaths occur in children aged <5 years (Alonso *et al*., 1991). It has been established that plasmodium falciparum is the only species responsible for severe/cerebral malaria and many of the underlying processes leading to it are probably common to all patient groups but there are important differences between non-immune adults and African children (Marsh *et al*., 1996). Marsh *et al*. (1995) recorded 85% cases of severe malaria among African children below the age of 5 years, pregnant women and non-immune adults.

The research of Oyedeji *et al*. (2005) in Ibadan, South Western Nigeria has shown that *P. falciparurn* is the predominant species and transmission is uniformly intense through most of the year (Salako *et al*. 1990). In the same vein, the research of Ejima *et al*. (2013), revealed that *P. falciparum* was the only species encountered and was responsible for malignant/severe malaria in Minna Metropolis, Niger State, Ngeria.

Although, it is well known that some patients may die with low parasite counts while others may be mildly ill with parasitaemia that are orders of magnitude higher there is a loose positive correlation between parasitaenmia and prognosis in falciparum malaria (Field, 1949). Malaria disease and deaths resulting from it in other areas of the world occur mainly among individuals who lack immunity and are infected with *P. falciparum* in areas where appropriate diagnosis and treatment are not available (Peter and Anatoli, 1998). Concomitant immunity, a resistance to an infection, develops in an actively infected host in an endemic area to withstand subsequent challenge infection (Davis, 1996). Once in hospital, many children die despite treatment and 80% of these deaths occur within 24 h of admission (Molyneux *et al*., 1989; Brewster *et al*., 1990; Marsh *et al*., 1995).

It is a common practice in virtually all the health centres/hospitals in Nigeria and other developing countries to place patients on presumptive medication by medical practitioners upon oral interview granted by the patients. Even when laboratory test is recommended, the laboratory technologist hardly gets to the root of the problem due to lack of appropriate facilities. Hence, the patients continue on presumptive medication to the point of lethal dose. More so, this practice of trial and error (drug abuse) has led to development of resistance by pathogens to some efficacious drugs. This is evident in *P. falciparum* infection which is currently resistant to most antimalarial drugs (Newton *et al*., 1994).

The present research was designed to diagnose malaria parasites to species level, i.e., *plasmodium vivax*, *P. ovale*, *P. malariae* and *P. falciparum* which are the causative agents of malaria in humans. The latter is responsible for severe/cerebral/malignantmalaria. The following orderof magnitude (parasitaemia level) of merozoitesortissue schizonts released into the blood stream from the liver at end of the life cycle by the four species: *Plasmodium falciparum* 40,000 (59.7%)> *P. ovale* 15,000 (22.4%)> *P. vivax* 10,000 (14.9%)> *P. malariae* 2,000 (3.0%) (Garnham, 1966). In this way, themost prevalent speciesin this region would be established. It was alsoaimed at treating the positive cases with appropriate drugs, so as to evaluate curative rates of such drugs. Structured questionnaires would be administered to participants to gather information about the knowledge of causative agents of malaria vis-a-vis their malariological activities that predispose them to malarialinfection. Hence, the researchers would be in position to advise the relevant agencies to upgrade the health facilities to ensure efficient service delivery to the citizenry under the current National Health Insurance Scheme (NHIS) in Nigeria. Definitive diagnosis will forestall the problem of presumptive treatment which lacks cost effectiveness and will also ensure efficacy of drugs, since, the drug of

choice for each species diagnosed would be administered. Again, any standard health facilities with proven diagnostic procedures and effective treatment would form a veritable source of revenue to the organization. It would, no doubt, rekindle the hope of civil servants and they will feel secured under the scheme (NHIS).

MATERIALS AND METHODS

Collection and analysis of blood samples: A total of 573 patients with undignosed fever and those with recent history of fever were included in the study. This number was randomly selected and examined from Idah Metropolis (IM) and Idah Suburbs (IS) of Idah LGA. The study area is Idah Local Government Area (LGA). Idah is the headquaters of Idah LGA and is situated in the Savanna region at latitude 7°5"N and 6°45"E. The necessary bio-data information such as sex, literacy level was collected from each participant through structured questionnaire. Blood samples were collected from the participants, using health facilities available namely: general hospital, Idah, Fed Poly Idah (FPI) medical centre and Primary Health Care (PHC)within the Idah suburbs of Idah LGA. About 1mL blood was obtained from each patient in EDTA/heparin through vein puncture at the upper arm using a disposable syringe.

Parasitological method (microscopy): A few drops of the blood obtained was used to prepare thick film which is nearly 10 times more sensitive for diagnosis of malaria (Houwen, 2002) on a clean, properly labelled glass slide. The thick blood films were stained with Giemsa's stain at pH values of 6.8-7.0 which revealed pinkish appearance of the red blood cells, well stained trophozoites of malaria parasites and prominent Schuffner's stippling. Each of the prepared slides was viewed with a compound microscope (CX 31 Olympus), under 100× oil immersion objective. At least 100 fields of the thick smears were examined for each subject before confirming a slide negative.

Quantitation of parasitaemia: The level of parasitaemia per microlitre (μL) of blood in thick film was determined by enumerating the number of parasites in relation to a standard (8000 leucocytes). This can be accomplished in positive cases with identified species of Plasmodium by:

- Using two hand tally counters; one counting leucocytes and the other for parasites; then the following procedures was followed
- If, after counting 200 leucocytes, 10 or more parasites are found, record the result on the data sheet in terms of parasites/200 leucocytes
- If, after counting 200 leucocytes, the number of parasites is just 9 or lower, continue counting until 500 leucocytes are enumerated and then record the parasites/500 leucocytes
- If the countingof leucocytes goes beyond 500 before sighting *Plasmodium* species is categorized as light infection

Mathematically, number of parasites per microlitre (μL) of leucocytes is given by the expression: parasite counted ×8000/Leukocyte counted (i.e., 200 or 500). The above method was employed as it is the normal practice to count all asexual forms of *P. falciparum* present. This method has an advantage over the simple 'plus system' as the former enables proper monitoring of the response to schizonticidal drugs which would not be expected to have effects on gametocytes.

Immunodiagnostic method (reagent strip): The remaining blood from each patient in the EDTA/Heparin container was used to conduct Immuno-Chromatographic Test (ICT). This test will be carried out by adhering strictly to the manufacturer's instruction (ICT-diagnostics australia). About 10 μL of whole blood was added to the sample pad where lysis occurs and pfHRP2 antigen, if present, binds to colloidal gold-labelled antibody. The appearance of a pink line indicates presence of *Plasmodium falciarum* antigen.

Treatment of diagnosed positive patients: A fraction of the positive malaria patients who initially agreed to participate in treatment regiments were enrolled for treatment with appropriate antimalarial drugs (Artesunate and Fansidar). The 34 subjects who were divided into two groups (blind folded) of 17 each wereasked to come back a week after treatment for re-examination. Prior to treatment, the body weight of each patient was measured by scaling. This would guide the dispense of chemotherapy per body weight. Through an enlightenment campaign the communities would adopt the necessary preventive measures such as the use of Insecticide Treated Nets (ITNs), regular, definitive and prompt diagnosis and treatment with appropriate antimalarial drugs.

Statistical analysis: The significance of differences in prevalence of malaria infection between sexes was assessed using Chi-square while the mean cure rate procured by the various antimalaria drugs shall be tested with t-test. Values of $p<0.05$ are considered significant.

Geometric Mean Intensity (GMI) was preferably used to evaluate the degree of intensity of parasitaemia level

per μL of blood between male and female Literate (L) and Illiterate (IL), respectively and between Idah Metropolis (IM) and Idah Suburbs (IS):

$$GMI = \text{antilog}\{^{\log x}_{n}$$

Where:

{ligx = Summation of logx and x is noof parasite's neggs/cysts/merozoites/and.isolated

n = Number positive only (Ejima and Odaibo, 2010)

RESULTS AND DISCUSSION

Table 1 showed the prevalence (%) and Geometric Mean Intensity (GMI) of parasitaemia level of *Plasmodium* species among households in Idah LGA. The results revealed that of the total subjects examined, 593 (54.6) was recorded as the overall prevalence of infection whereas 774.8 GMI per microlitre (μL) of blood was the overall intensity recorded. Of the suburbs of Idah (IS) examined, Okpachala had the highest (100.0%) prevalence of malaria parasite infection. This is followed by Okenya where prevalence and intensity of 85.7% and 1,132.4 were recorded, respectively. The least of prevalence and intensity, 16.7% and 258.5/μL of blood were recorded for others. Comprison of infection rate between male Literate (L) and Illiterate (IL) revealed no significant difference ($p>0.05$). Similarly, the female counterpart showed no significant difference ($p>0.05$) when female (L IL) were compared. However, comparison of the factors viz. male, female and literate, Illiterate with reference to prevalence rate revealed significant difference ($p<0.05$) (χ^2_{cal}, df = 27 = 56.287). Parasitaemia level (GMI) of subjects within Idah Metropolis (IM) compared with those in Idah Suburbs (IS) differ significantly ($p<0.05$) (χ^2_{cal} = 45.431 df = 3).

Overall positive cases (%) of parasitaemia species infection by age, sex and status of literacy (L or IL) is shown in Table 2. For both maleand female, percentage occurrence peaked in the age group 50 and above; Male (25.7% L, 18.5% IL): Female (32.3%, L 17.3% IL) and this was followed by age group 25-29 years (male: 16.2% L; 13.8% IL Female: 19.2% L 18.7 IL).

Table1: Prevalence (%) and Geometric Mean Intensity (GMI) of Parastaemia level of *Plasmodium* species among households in Idah Local Government Area (LGA)

S/N/Communities and localities in Idah LGA	No. exam	No. positive (%)	Parasitaemia GMI per 200 Leucocytes (heavy)	Level per μL of blood: GIM per 500 leu (moderate)	Level per μL of blood: GMI per 500 leu and above (light)
Idah					
Metropolis Idah town (Ega and Sabon-gari)	221	123 (55.7)	634.60		
Igalogwa	24	18 (75.0)	1,036.60		
G.R.A	50	36 (72.0)	945.60		
Subtotal	295	177 (60.0)			
FPI community					
FPI-community	82	49 (59.8)	938.30	746.5	
Subtotal	82	49 (59.8)			
Idah-suburbs					
Okpachala	4	4 (100.0)	593.50		
Okenya	14	12 (85.7)	1,132.40		
Iyogbo	45	21 (46.7)	658.30		
Inachalo	18	11 (61.1)	853.40		
Ugwoda	94	36 (37.1)	917.60		
Others (Od/Ich)	18	3 (916.7)	258.50		
Subtotal	196	87 (44.4)			
G-total	573	313 (54.6)	77.48		

Table 2: Overall positive cases (%) of *Plasmodium* species infection by age, sex and status of literacy (Literate, L; Illiterate, IL)

Age group	Male L NO (%)	Male IL NO (%)	Female L NO (%)	Female IL NO (%)	G. Total
6 months	-	-	-	1 (1.3)	1 (0.3)
1-4	5 (6.8)	6 (9.2)	2 (2.0)	3 (4.0)	16 (5.1)
5-9	5 (6.8)	4 (6.2)	4 (4.0)	10 (13.3)	23 (7.3)
10-14	4 (5.4)	4 (6.2)	6 (6.1)	5 (6.7)	19 (6.1)
15-19	-	5 (7.7)	1 (1.0)	4 (5.3)	10 (3.2)
20-24	12 (16.2)	7 (10.8)	16 (16.2)	12 (16.0)	47 (15.0)
25-29	12 (16.2)	9 (13.8)	19 (19.2)	14 (18.7)	54 (17.3)
30-34	3 (4.1)	5 (7.7)	4 (4.0)	6 (8.0)	18 (5.8)
35-39	3 (4.1)	5 (7.7)	4 (4.0)	6 (8.0)	18 (5.8)
40-44	2 (2.7)	3 (4.6)	1 (1.0)	6 (8.0)	18 (5.8)
45-49	2 (2.7)	3 (4.6)	5 (5.1)	1 (1.3)	11 (3.5)
50 and above	19 (25.7)	12 (18.5)	32 (32.3)	13 (17.3)	76 (24.3)
Total	74 (100.0)	65 (100.0)	99 (100.0)	75 (100.0)	313 (100.0)

The fact that only *Plasmodium falciparum* was encountered in this study is quite in agreement with a host of other workers in their own regions. Marsh *et al.* (1996) established that only *Plasmodium falciparum* that was responsible for severe malaria and many of the underlying processes leading to it are probably common to all patients. This explains further why there were no significant differences in prevalence of the disease between male literate and illiterate subjects as well as female literate and illiterate subjects examined. Oyedeji *et al.* (2005), in Ibadan have shown that *P. falciparum* is the predominant species and transmission is is uniformly intense through most of the year (Salako *et al.*, 1990). Ejima and Odaibo (2010) also recorded only *P. falciparum* in their research in Minna Metropolis. Severe malaria caused by *P. falciparum* is to be expected because in the life cycle of the species 40.000 (59.7%) merozoites or tissue schizonts are released from the liver into the blood stream which ishigher than those of any of the other three species (Garnham, 1966). The higher prevalence ofthe parasite at older age (50 and above years) was probably because they might have acquired concomitant immunity and as such serve as potential reservoir hosts to younger age groups. Okpachala was one of the suburbs of Idah where 100% prevalence was recorded due to malariological activities of such rural setting.

CONCLUSION

The overall prevalence (54.6) and intensity (GMI: 774.8) of malaria due to *Plasmodium falciparum* recorded among households in Idah LGA were very high. This high rate calls for immediate concern by the government to bring about control measures, more especially as it is the species that is responsible for severe/cerebral malaria that is predominant in this region ofthe world.

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