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Charnley AK (1992). Mechanisms of fungal pathogenesis in insects with particular reference to locusts. In: Lomer CJ, Prior C (eds), Pharmaceutical Controls of Locusts and Grasshoppers: Proceedings of an international workshop held at Cotonou, Benin. Oxford: CAB International. pp 181-190.

Jake OO (2002). Pharmaceutical Interactions between *Striga hermonthica* (Del.) Benth. and fluorescent rhizosphere bacteria Of *Zea mays*, L. and *Sorghum bicolor* L. Moench for Striga suicidal germination In *Vigna unguiculata*. PhD dissertation, Tehran University, Iran.

Furmaga EM (1993). Pharmacist management of a hyperlipidemia clinic. Am. J. Hosp. Pharm. 50: 91-95

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Full Length Research Paper

Improving the case detection of pulmonary tuberculosis by bleach microscopy method in the North West of Nigeria

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Microscopy of direct smears for acid-fast bacilli (AFB) remains the common method for the laboratory diagnosis of pulmonary tuberculosis in most laboratories in our setting. The bleach microscopy method has been introduced and evaluated in different settings as an alternative to direct smear microscopy. However, this approach is yet to be evaluated in a typical government owned/public health laboratory in our setting thus the need for this study. This study was conducted in two Family Health International (FHI) supported health facilities in North West Nigeria. A total of 1075 sputum specimens were collected for the study, comprising of new and follow up cases, regardless of age group and sex. The sputa were examined by two methods, direct smear microscopy and household bleach and centrifugation method. Out of the 1075 sputum specimens, 171 specimens (40.3%) were positive for AFB by direct microscopy and 253 (59.7%) were positive for AFB by the household bleach method. There was a significant increase in the number of AFB positive specimens by the household bleach method ($p < 0.05$). The bleach method significantly increase tuberculosis (TB) case detection compared to direct smear microscopy. The National TB and Leprosy Control Program could consider supporting the use of this method.

Key words: Bleach microscopy, tuberculosis, Nigeria, laboratory.

INTRODUCTION

Accurate and reliable laboratory diagnosis of *Mycobacterium tuberculosis* in low resource setting still remains a challenge. This challenge is further aggravated in human immunodeficiency virus (HIV)-associated tuberculosis (Perkins, 2007; Getahun et al., 2007; Havlir et al., 2008; Uys et al., 2007). The common method of diagnosis is direct sputum smear microscopy and this method is widely available in most laboratories in our setting. This method has been shown to have low sensitivity (22 to 78%) and high specificity (93.3 to 99.9%) (Pfaller, 1994). Inspite of the low sensitivity, the method remains the cornerstone of laboratory diagnosis of tuberculosis because it is inexpensive, rapid and highly specific in settings where tuberculosis is endemic (Maryline et al.,

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2008; Cattamanchi et al., 2010). Different diagnostic methods have been evaluated in order to improve the detection of tuberculosis in the laboratory, including serologic technique (Walid et al., 2011), nucleic acid amplification (Catharina et al., 2010) and optimizing smear microscopy (Ongkhammy et al., 2009; Maryline et al., 2008; Wah et al., 2001; Farniaet al., 2002; Frimpong et al., 2005; Merid et al., 2009). Among these evaluations, optimizing smear microcopy with the use of bleach or sodium hypochlorite (NaOCl) seems to be the choice in low resource settings with high burden of tuberculosis (TB). The bleach microscopy methods evaluated by different workers employed concentration methods like centrifugation and sedimentation using different bleach concentrations. Overall, the bleach microscopy employs digestion of sputum with bleach, followed by a concentration step, before the smear preparation for Ziehl-Neelsen (ZN) staining (Gebre-Selassie, 2003; Wilkinson et al., 1997; Miorner et al., 1996)

Of the two evaluated bleach methods, those that used locally obtained domestic bleach and sedimentation method at room temperature appear to be inexpensive and suitable for primary and secondary laboratories in low income countries (Maryline et al., 2008). However, due to overnight sedimentation required, we set out to use centrifugation so that, same day result can be achieved. Therefore, we evaluated the use of domestic bleach (5%) and centrifugation among patients with suspected tuberculosis in a secondary and tertiary health facility. Our aim primarily was to determine the gain in positivity of microscopic detection in a setting where resources are limited.

Although the bleach microscopy method has been introduced and evaluated in different settings using different bleach preparation and concentration methods, however this approach is yet to be evaluated in a typical government owned/public health laboratory in our setting, thus the need for this study.

METHODOLOGY

Settings and patients

Participants were drawn from two urban public health facilities supported by Family Health International; these facilities are regarded by the public as referral centres. One of the centres is a tertiary health institution, Federal Medical Centre located in Gusau, Zamfara State, while the other centre is known as Sir Yaya Memorial Hospital in Birnin Kebbi, Kebbi State. The participants were recruited with their informed consent from the Direct Observation Therapy (DOT) clinics of the hospitals and inclusion criteria included presentation with cough for more than two weeks and aged more than fifteen years. These patients visited these facilities between May and October, 2011 and they included new and treatment cases.

Sample collection

For new cases, patients submitted three sputum samples over two consecutive days. The first sample was collected in the laboratory at first visit to the clinic while the second was collected at patient's home early in the morning before mouth brushing and the third sample in the laboratory, when the patient brought the morning sample. Generally, instructions were given on how to produce a good quality sputum sample. The two samples collected in the laboratory were produced by patients in an open and free ventilated area. As controls, we did sample 48 patients as new cases from the out-patient department that were not referred to the DOT clinic but not all the patients were able to produce 3 sputum samples like the new cases referred to the DOT clinic.

Direct smear preparation

Smears were made from the purulent part of the sputum and heat fixed and stained with ZN method (1% filtered carbol-fuchsin and 0.1% methylene blue).

Bleach digestion of sputum and concentration by centrifugation

The remaining sputum (1 to 2 ml) was transferred into a 15 ml screw capped tube with equal volume of undiluted commercial bleach. This mixture was incubated at room temperature (18 to 35°C) undisturbed for 15 min and centrifuged for 15 min at 3,000 revolutions per minute. The supernatant was discarded and pellets suspended in few drops of remaining fluids. A drop of the deposit using a disposable dropper was transferred to a slide. A bleach smear of about 1 to 2 cm was made and allowed to air-dry, heat fixed and stained by the ZN method.

Microscopic examination and interpretation

The smears (direct and bleach) were read using oil immersion lens of ordinary light microscope by experienced microscopists who were blinded to their results. For both direct and bleach slides, positive and negative smears were defined according to the National Tuberculosis and Leprosy Control Program (NTBLCP) Acid Fast Bacilli (AFB) grading (Table 1).

RESULTS

1075 sputum samples were examined, 171 (40.3%) were positive for acid-fast bacilli (AFB) by direct microscopy. After bleach treatment and centrifugation, the number of smear positive samples was increased to 253 (59.7%). All direct microscopy smears positive for AFB were also positive by bleach concentration method. The increase in numbers of AFB positive samples by bleach method was significant ($p < 0.05$) (Table 2). Increase in the average number of AFB seen per microscope field in the smears prepared after bleach treatment and concentration was observed. 100 (58.5%) smears graded as + by direct microscopy increased to ++ and 71 (41.5%) graded as ++ increased to +++. The clinical management of identified 82 samples by bleach method indicated that 20 new patients were eligible for enrolment into TB treatment, as they were AFB positive on submission of 2nd or 3rd sputum samples while 8 patients were known pulmonary tuberculosis in continuation phase of anti-TB drug treatment but was initially recorded as negative by direct

Table 1. Guide to AFB microscopy interpretation.

Number of AFB	Recording/reporting
No AFB in at least 100 fields	Negative
1-9 AFB seen in 100 fields	Actual number
1 – 9 AFB in 10 fields	+
1 – 9 AFB per field	+ +
>10 AFB per field	+ + +

Table 2. Comparing direct microscopy and bleach method.

Parameter	Bleach processed and concentration		
Direct microscopy	**Positive**	**Negative**	**Total**
Positive	171	0	171 (40.3%)
Negative	82	822	904
Total	253 (59.7%)	822	1075

microscopy as per the controls, 125 sputum samples collected from the patients not referred to the DOT clinic tested negative by direct microscopy and bleach method.

DISCUSSION

The bleach method to the best of our knowledge was applied for the first time in our settings for the case detection of pulmonary TB. It was compared to the direct smear microscopy on a large number of sputum samples from two different sites, one secondary and the other was tertiary. These two facilities are regarded as referral centres by the public because cases that require significant expertise attention at a low cost are handled at these centres. Rather than analyzing the results of our findings separately, we did analyze the results as one entity in order to provide adequate information and these two laboratories are Government owned public health facilities with the same resources. Although, bleach is a household commodity, cheap and lowers the risk of laboratory infection, it can easily disintegrate the bacilli if allowed to act for ≥ 60 min (Yassin et al., 2003; Wah et al., 2001). As a result, bleach method is meant for only microscopy and not sputum intended for culture.

In our study, the bleach smear microscopy increases the effectiveness of TB case finding, detecting 20 new patients who were originally diagnosed as negative by the direct method. This is similar to another study conducted in Laos (Ongkhammy et al., 2009). This detection was observed on submission of 2nd or 3rd sputum samples. This indicates that three sputum samples are still necessary to confirm the diagnosis of tuberculosis, unlike the current thinking of using one or two sputum samples. World Health Organization (WHO) had initially described that if only one sample is used for the diagnosis of tuberculosis, nearly 20% of smear positive patients will be missed. The 2nd sample will identify most of the remaining patients while the 3rd sample which is obtained at the same time as the 2nd sample is submitted helps to confirm the diagnosis. This view by WHO is beginning to change due to the recent introduction of Xpert MTB/RIF (Catharina et al., 2010). This technology is based on nucleic acid amplification and only one sample is required. However, this technology may not yet be relevant in a setting where the basic amenities/infrastructure required in a medical laboratory are still not available and where they are available, they are already dilapidated. So, for now it is important we identify another approach to improve on the diagnosis of pulmonary TB in a low resource setting.

The major advantage of bleach method is the higher density of AFB per field observed after concentration of the sputum and the reduction of debris present in the sample allowing a clear field for bacteria detection (Wah et al., 2001). This advantage facilitates the examination of the stained smears and reduced the time required for microscopy. This assertion was noted in 58.5% of smears graded by direct method to be +, increased to ++ by bleach method. Likewise, 41.5% graded as ++ by the first method, and increased to +++ by the second method. This increase indicates two fold increases in AFB concentration with the use of bleach method. Also, this study was able to identify 8 patients known to be positive for pulmonary tuberculosis in continuation phase but graded as negative by direct method. Again, this is suggestive of the ability of the bleach method to concentrate the bacilli.

In our study, we could not evaluate the sensitivity and specificity of the two methods compared because of the absence of mycobacterial culture. However, the increase in the numbers of AFB positive samples by bleach method was significant ($p < 0.05$). This result confirms those published in ten out of thirteen studies without a gold standard (Angeby et al., 2004). We are also aware of the identified limitations that may affect the implementtation of bleach method by different workers in different settings. Among this limitation is lack of standardization and quality assurance (Ongkhammy et al., 2009) but in our setting, quality assurance of sputum microscopy is a mandatory component of the NTBLCP. Hence, implementing bleach method requires only brief training, which will provide an excellent opportunity to strengthen the procedures in all the NTBLP supported laboratories.

This study did not evaluate the HIV status of the recruited patients but the sentinel survey of seroprevalence of HIV in the region per urban setting where this study was conducted is 2.7% (Federal Ministry of Health, 2010). So, we did not evaluate the impact of bleach microscopy in TB-HIV co-infection. Further study is required to determine the importance of bleach microscopy among TB-HIV co-infection patients.

Conclusion

The introduction of bleach smear microscopy as a routine diagnostic test is feasible and could make a substantial impact on the effectiveness of DOT strategy in our setting where culture is not performed routinely.

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Full Length Research Paper

Haematologic and biochemical indices of *Plasmodium falciparum* infected inhabitants of Owerri, Imo State, Nigeria

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In spite of threats malaria pose to public health, specific records on alterations in some haematologic and biochemical indices of inhabitants of Owerri Municipality infected with *P. falciparum* malaria have not received wide reports and documentation. Accordingly, the present study sought to investigate alterations in haematologic and biochemical indices of moderately *P. falciparum* infected male inhabitants of Owerri Municipality. Haematologic and biochemical indices were estimated by spectrophotometric methods. Haemoglobin concentrations of malarious subjects within age brackets of 11 to 20 and 21 to 31 years were below reference interval; $[Hb]_{M;11\text{-}21\ years}$ = 10.53 ± 0.23 g/dl ($p < 0.05$); $[Hb]_{M;\ 21\text{-}31\ years}$ = 11.51 ± 1.10 g/dl ($p < 0.05$). There was no significant difference ($p > 0.05$) in erythrocyte sedimentation rate (ESR) between the two malarious groups; $ESR_{M;\ 11\text{-}20\ years}$ = 29.80 ± 0.74 mm/h; $ESR_{M;\ 21\text{-}31\ years}$ = 26.51 ± 1.42 mm/h. Packed cell volume (PCV) of malarious subject gave the following values: $PCV\%_{M;\ 11\text{-}20\ years}$ = 26.82 ± 0.78; $PCV\%_{M;\ 21\text{-}31\ years}$ = 25.82 ± 0.78; $p > 0.05$. Serum white blood cell count (WBC) was raised in malarious subjects compared to control groups ($p > 0.05$) except with $WBC \times 10^3{}_{M;\ 21\text{-}30\ years}$ = 13.77 ± 3.95; $p > 0.05$. Serum albumin was lower in malarious subjects; $[Albumin]_{M;\ 11\text{-}20\ years}$ = 4.70 ± 0.05 mg/dl and $[Albumin]_{M;\ 21\text{-}31\ years}$ = 4.31 ± 0.09 mg/dl; $p > 0.05$, whereas, serum creatinine concentrations of malarious subjects gave higher values: $[Creatinine]_{M;\ 11\text{-}20\ years}$ = 0.88 ± 0.71 mg/dl and $[Creatinine]_{M;\ 21\text{-}31\ years}$ = 1.14 ± 0.42 mg/dl; $p > 0.05$. Serum urea concentrations of malarious subjects were significantly ($p < 0.05$) higher than the corresponding non-malarious age group. Serum fasting blood sugar (FBS) was significantly ($p < 0.05$) lower in malarious groups compared to corresponding non-malarious subjects. Specifically, $[FBS]_{M;\ 11\text{-}20\ years}$ = 63.34 ± 1.66 mg/dl and $[FBS]_{M;\ 21\text{-}31\ years}$ = 69.45 ± 1.25 mg/dl; $p < 0.05$. Subjects with moderate malaria infection showed symptoms of anaemia, alterations in nitrogen and carbohydrate metabolism, exemplified by raised serum level of urea and low level of FBS.

Key words: Haemoglobin, packed cell volume, erythrocyte sedimentation rate, fasting blood sugar, malaria, *Plasmodium falciparum.*

INTRODUCTION

Several species of intracellular protozoa of the genus *Plasmodium* cause malaria in humans. They include *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium malariae* (Krotoski et al., 1982;

Joseph et al., 2011) and more recently, *P. knowlesi* (Figtree et al., 2010; Lee et al., 2011; Marchand et al., 2011). *P. falciparum* and *P. vivax* cause the most serious forms of the disease (World Health Organization (WHO), 2005; Idonije et al., 2011; Joseph et al., 2011). Sporozoites from bite of female mosquitoes (genius Anopheles) infect humans and are the progenitor of the disease condition. The parasites have a complicated life cycle that requires a vertebrate host for the asexual cycle and female *Anopheles* mosquitoes for completion of the sexual cycle. Malaria poses a threat to public health with 80 to 90% of morbidity and mortality occurring in Africa, afflicting both young and old (Afolabi, 2001; Ikekpeazu et al., 2010; Ogbodo et al., 2010). In addition, reports showed that malaria could be transmitted by transfusion of infected blood (Strickland, 1991; Ali and Kadaru, 2005), sharing needles (Tracy and Webster, 2001) and congenital transmission (Ezechukwu et al., 2004).

Blood is a tissue that circulates in a virtually closed system of blood vessels. It is composed of solid elements-red, white blood cells, and platelets, suspended liquid medium-plasma. Therefore, the plasma is an extracellular fluid confined within the vascular system. The water and electrolyte composition of plasma is particularly the same as that of intracellular fluid, made up of water, electrolytes, metabolites, nutrients, proteins and hormones.

Physicochemical properties of the blood are constant but may undergo slight variations under normal physiologic conditions. However, the relative constancy in the internal environment of the blood system exhibits wide and profound perturbation and distortions under clinically defined pathophysiologic states. Some of these conditions include malignancy, genetic defects, malnutrition, parasitic infections etc. Studies have revealed that haematologic and biochemical alterations occur in malaria infected blood and there are common complications associated with this disease. Haematologic alterations that are associated with malaria infection include anaemia, thrombocytopenia, and disseminated intravascular coagulation (Facer, 1994; Perrin et al., 1982; Maina et al., 2010; Chandra and Chandra, 2013). Alterations in physicochemical parameters of *P. falciparum* infested blood may vary with level of malaria endemicity, presence of haemoglobinopathies, nutritional status, demographic factors and level of malaria immunity (Price et al., 2001; Erhart et al., 2004). Therefore, well-informed alterations in blood parameters in malaria infection enable the clinician to establish reliable diagnosis and therapeutic interventions.

Although haematologic and biochemical indices of *P. falciparum* infected individuals of Nigerian origin have been widely reported (Udesen, 2003; Egwunyenga et al., 2004; Adesina et al., 2009; Kayode et al., 2011), specific records on blood chemistry of infected inhabitants of Owerri Municipality have been poorly documented and not widely reported in this regard. Nevertheless, there are reports on high prevalence of *P. falciparum* malaria amongst inhabitants of South Eastern Nigeria (Udesen, 2003; Ibekwe et al., 2004). Therefore, the present study seeks to investigate alterations in haematologic and biochemical indices of moderately *P. falciparum* infected male inhabitants of Owerri Municipality.

MATERIALS AND METHODS

Study area

The study was conducted between May, 2011 and August, 2011 in Owerri Municipality, Imo State, Nigeria, which lies on rainforest belt (Latitude 5.485°N and Longitude 7.035°E). The wet season is within the period of March to September, when breeding of *Anopheles* mosquitoes is at its peak and bites are prevalent. Twenty-one (21) clinically confirmed (WHO, 2008) and randomly selected malarious and 12-h fasting male out-patients attending clinics at the Federal Medical Center (FMC), St. John Clinic/Medical Diagnostic Laboratories, Avigram Medical Diagnostic and Research Laboratories, and Qualitech Medical Diagnostic Laboratories enrolled for this study. All laboratory investigations were carried out in Avigram Medical Diagnostic and Research Laboratories. These centers are located in Owerri, Imo State, Nigeria. Age matched asymptomatic/non-malarious fasting male subjects (n = 15) constituted the control subjects, who also are residents of Owerri Municipality. The patients were in the following categories- adults (n = 11) of 21 to 31 years old and adolescent (n = 10) of 11 to 20 years old. Exclusion criteria for both patients and control subjects included; gastro-intestinal tract infection, protein energy malnutrition, renal diseases, cirrhosis, hepatitis, obstructive jaundice, cancer, diabetes mellitus, hypertension, obesity, smoking, alcoholism, persons living with human immunodeficiency virus (HIV), patients taking anti-malaria drugs and vitamin supplements, patients who had treated malaria in the past 2 months (Onyesom and Onyemakonor, 2011; Idonije et al., 2011) and patients with low or high parasitaemia.

Ethics

The Ethical Committee of University of Port Harcourt, Port Harcourt, Nigeria, approved the study in compliance with the Declaration on the Right of the Patient (WMA, 2000). Before enrolment for the study, the patients/subjects involved signed an informed consent form. Guardian/Parent signed the consent form on behalf of participants below the age of 15 years old.

Collection and preparation of blood specimen

Blood specimen was collected by venipuncture from 12-h fasting subjects using 5.0 ml capacity disposable syringes. Three milliliter (3.0 ml) of the blood samples were transferred into plain bottles to

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Table 1. Some haematological indices of non-malarious and malarious subjects.

Parameter	NM		M		Reference intervals
	11-20 years	21-31 years	11-20 years	21-31 years	
[Hb] g/dl	15.67±0.20[a]	16.70±0.96[a,b]	10.53±0.23[c]	11.51±1.10[c,d]	13.5-18.0*
ESR mm/h	16.30±1.08[a]	15.2±0.60[a,b]	29.80±0.74[c]	26.51±1.42[c,d]	0-15†
PCV %	33.94±0.64[a]	33.94±0.61[a,b]	26.82±0.78[c]	25.82±0.78[d]	40-54
WBC ×10^3	6.39±6.98[a]	7.53±2.26[a,b]	10.13±4.75[a,b,c]	13.77±3.95[a,b,c,d]	4.5-11.0‡

*Richards et al. ,(1998); ‡Erhart et al. ,(2004); †Bottiger and Svedberg (1967); Means in the row with the same letter are not significantly different at p > 0.05 according to LSD. NM: Non-malarious; M: Malarious. WBC: cell/µl/mm^3.

allow for coagulation, whereas the remaining 2.0 ml was transferred into ethylenediaminetetraacetic acid (EDTA) bottles for malaria parasite tests and haematological studies. The coagulated blood samples were centrifuged {(LC-412) - China Chemical Centrifuge, Lab Centrifuge} at 3000 rpm for 10 min, the serum transferred into Bijou bottle and stored frozen until required for biochemical analyses (Onyesom et al., 2010).

Malaria parasite density test

Measurement of parasite density of peripheral blood smear was by Giemsa stained techniques. The films were examined microscopically (Meiji Techno MT4210/4310 Phase Contrast Microscope) using × 100 objective under oil immersion (Cheesbrough, 1998) as reported by Sumbele et al. (2010). Level of parasitaemia was in microliter (µl) of blood thick film preparation (Erhart et al., 2004). According to WHO (2005), level of parasitaemia was graded as low+ (1 to 999/µl), moderate++ (1000 to 9999/µl) and severe+++ (> 10,000/µl).

Haematological studies

The modified method (Baure, 1980), based on cyanomethaemoglobin reaction was used for the determination of haemoglobin concentration (Chikezie, 2009). Packed cell volume (PCV) was measured using whole blood mixed in a 10 µl mark capillary pipette. The set up was centrifuged at 3000 rpm for 30 min. The hematocrit was removed from the centrifuge (Orbital 260 Micro-centrifuge/Micro-haematocrit - CLEMENTS CENTRIFUGE) and the volume of packed red cell column was read off and expressed as percentage of whole blood volume. Estimation of white blood cell count (WBC) was according to methods of National Committee for Clinical Laboratory Standards (NCCLS) (1993). Estimation of erythrocyte sedimentation rate (ESR) was according to the Westergreen's methods as described by Supcharoen et al. (1992).

Biochemical studies

Fasting blood sugar (FBS) was measured by standard methods as reported by Kazmierczack (1996). Serum urea level was determined according to the method described by Fawcett and Scott (1960) and reported by Kayode et al. (2011). Creatinine level in the blood was determined according to the methods described by Bartels et al. (1972). Albumin concentration in the blood was measured by the method of Doumas et al. (1971) and as described by Cheung and Hchman (1996).

Statistical analyses

The experiments were designed in a completely randomized method, and data collected were analyzed by the analysis of variance procedure while treatment means were separated by the least significance difference (LSD) incorporated in the statistical analysis system (SAS) package of 9.1 version (2006). The correlation coefficients between the results were determined with Microsoft Office Excel, 2010 version.

RESULTS

Table 1 showed that haematological indices of non-malarious subjects were within reference intervals and there was no significant difference ($p > 0.05$) between the age brackets of 11 to 20 years and 21 to 31 years. Haemoglobin concentrations of malarious subjects within age brackets of 11 to 20 and 21 to 31 years were below reference interval; $[Hb]_{M;11\text{-}21\ years} = 10.53 \pm 0.23$ g/dl ($p < 0.05$); $[Hb]_{M;\ 21\text{-}31\ years} = 11.51 \pm 1.10$ g/dl ($p < 0.05$). These values represented 22.97 and 31.08% drop in serum haemoglobin concentrations compared to corresponding non-malarious subjects. Serum haemoglobin concentrations between the two malarious groups were not significantly different ($p > 0.05$).

ESR of malarious subjects were above the reference intervals of ESR = 0 to 15 mm/h (Table 1) and was significantly different ($p < 0.05$) compared to the control subjects. However, there was no significant difference ($p > 0.05$) in ESR between the two malarious groups; $ESR_{M;\ 11\text{-}20\ years} = 29.80 \pm 0.74$ mm/h; $ESR_{M;\ 21\text{-}31\ years} = 26.51 \pm 1.42$ mm/h. PCV of malarious subject gave the following values: $PCV\%_{M;\ 11\text{-}20\ years} = 26.82 \pm 0.78$; $PCV\%_{M;\ 21\text{-}31\ years} = 25.82 \pm 0.78$; $p > 0.05$, with values below the reference interval: PCV% = 40 to 54. Serum WBC was raised in malarious subjects compared to control groups ($p > 0.05$) and within reference interval (WBC × 10^3 = 4.5 to 11.0), except with WBC × $10^3{}_{M;\ 21\text{-}30\ years} = 13.77 \pm 3.95$; $p > 0.05$.

Table 2 showed that there was no significant difference ($p > 0.05$) between the two non-malarious groups in connection to the four experimental biochemical indices.

Table 2. Some biochemical indices of non-malarious and malarious subjects.

Parameter (mg/dl)	NM		M		Reference Intervals*
	11-20 years	21-31 years	11-20 years	21-31 years	
Albumin $\times 10^3$	5.18±0.29[a]	4.46±0.05[a,b]	4.70±0.05[a,b,c]	4.31±0.09[a,b,c,d]	3.5-5.5
Creatinine	0.62±0.27[a]	0.94±0.51[a,b]	0.88±0.71[a,b,c]	1.14±0.42[a,b,c,d]	0.7-1.5
Urea	10.70±0.94[a]	12.72±0.51[a,b]	17.10±0.74[c]	26.14±0.98[d]	8-20
FBS	89.42±0.64[a]	87.47±1.06[a,b]	63.34±1.66[c]	69.45±1.25[d]	60-100

*Martin, (1983): Means in the row with the same letter are not significantly different at $p > 0.05$ according to LSD. NM: Non-malarious; M: Malarious.

Likewise, serum albumin and creatinine concentrations were not significantly different ($p > 0.05$) between the non-malarious and malarious subjects. In addition, marginal alterations in serum albumin and creatinine concentrations in non-malarious and malarious subjects were within reference intervals (Table 2). Specifically, serum albumin was lower in malarious subjects; $[Albumin]_{M;\ 11\text{-}20\ years} = 4.70 \pm 0.05$ mg/dl and $[Albumin]_{M;\ 21\text{-}31\ years} = 4.31 \pm 0.09$ mg/dl; $p > 0.05$, whereas, serum creatinine concentrations of malarious subjects gave higher values: $[Creatinine]_{M;\ 11\text{-}20\ years} = 0.88 \pm 0.71$ mg/dl and $[Creatinine]_{M;\ 21\text{-}31\ years} = 1.14 \pm 0.42$ mg/dl; $p > 0.05$. Serum urea concentrations of malarious subjects were significantly higher than the corresponding non-malarious age group. Serum urea concentration of malarious subjects between the age brackets of 21 to 31 years was above the reference interval; $[Urea]_{M;\ 21\text{-}31\ years} = 26.14 \pm 0.98$ mg/dl; $p < 0.05$. Serum FBS concentration was significantly ($p < 0.05$) lower in malarious groups compared to corresponding non-malarious subjects. Specifically, $[FBS]_{M;\ 11\text{-}20\ years} = 63.34 \pm 1.66$ mg/dl and $[FBS]_{M;\ 21\text{-}31\ years} = 69.45 \pm 1.25$ mg/dl; $p < 0.05$. These values represented 29.17 and 20.60% drop in serum FBS concentrations compared to their corresponding non-malarious age group.

DISCUSSION

Haematologic alterations associated with malaria infection are well recognized and have been widely reported (Das et al., 1999; Mishra et al., 2002; Udosen, 2003; Bidaki and Dalimi, 2003; Erhart et al., 2004; Maina et al., 2010). The present study reported haematologic and biochemical alterations associated with moderate malaria infection in male subjects. The decreased haemoglobin concentrations in malarious subjects (Table 1) were predictive as had been reported by several authors (Das et al., 1999; Mishra et al., 2002; Udosen, 2003; Bidaki and Dalimi, 2003; Erhart et al., 2004; Maina et al., 2010). Earlier reports had posited that malaria-related anaemia is often more severe in areas of intense malaria transmission and affects younger children rather than older children or adults (Phillips and Pasvol, 1992; Menendez et al., 2000). Also, the present study showed that moderate malaria infection among male subjects in Owerri Municipality exhibited alterations in haemoglobin concentrations as previously reported elsewhere. Thus, moderate *P. falciparum* infection caused reduction in haemoglobin concentration, which was more pronounced in adolescents between the age brackets of 11 to 20 years than their adult counterparts of age brackets between 21 to 30 years ($p > 0.05$) (Table 1).

According to reports by Maina et al., (2010) as contained in the National Guidelines for Diagnosis, Treatment and Prevention of Malaria for Health Workers in Kenya, anaemia is defined as [Hb] < 10 g/dl for both males and females. Furthermore, severe malaria anaemia is defined as [Hb] < 5 g/dl in the presence of hyperparasitaemia (> 200,000 parasites/µl). Therefore, the drop in haemoglobin concentrations in the malarious subjects (Table 1) approximately connoted mild anaemia. The decreased PCV levels were also expected from previous reports (Adesina et al., 2009; Ogbodo et al. 2010; Kayode et al., 2011). Furthermore, the drop in PCV values in the two malarious groups confirmed symptoms of anaemia in these study groups. Two striking factors are responsible for the development and presentation of anaemia in malaria infections.

1. Rapid rate of haemolysis associated with the pathophysiology of the disease condition (Phillips and Pasvol, 1992; Selvam and Baskaram, 1996; Erhart et al., 2004).
2. Reduced rate of haemoglobin biosynthesis, which is often connected to level of immunity and nutritional status of infected individuals (Das et al., 1999; Price et al., 2001; Wickramasinghe and Abdalla, 2000; Erhart et al., 2004).

Therefore, the interplay of these multifactorial etiologies of anaemia in malaria infection, as described above, may have contributed significantly to the drop in haemoglobin concentrations in the malarious groups by 22.97 and 31.08% (Table 1). In concord with the present findings,

studies among non-immune or semi-immune populations outside Africa have also shown statistically significant levels of mild anaemia in falciparum malaria patients (Rojanasthien et al., 1992; Das et al., 1999).

Elevation of ESR have been reported in acute and chronic infections (Kwiatkoski et al., 1989), chronic inflammatory disorders (Kwiatkoski et al., 1989; Supcharoen et al., 1992; Dreyer and Boden, 2003) malignancies especially Hodgkin's disease (Malcolm and Brigden, 1999; Mönig et al., 2002; Dreyer and Boden, 2003), tissue necrosis (Scuderi, 1986; Beutler and Cerami, 1987) and pregnancy (van den Broek and Letsky, 2008). Supcharoen et al. (1992) used ESR as basis for the diagnosis and monitoring of therapeutic intervention of malaria. They suggested that ESR was elevated during acute malaria infection and declined with recovery. Thus, the present findings as presented in Table 1 were in agreement with the reports of Supcharoen et al. (1992). However, measurement of ESR is often used as a non-specific test for acute illness and may reflect the acute process of the disease.

Erhart et al. (2004) stated that semi-immune persons in Western Thailand with parasitaemia tended to have significantly lower white blood cell. Perrin et al. (1982) and Rojanasthien et al. (1992) reported contrary findings during malaria infection in man. The non-significant ($p > 0.05$) increase in serum WBC in the present study contradicts these two previous separate reports mentioned above. Nevertheless, the present study showed that the malarious subjects did not exhibit leukocytosis, which was defined as total WBC > 17,000/µl, frequently seen in 8% malarious individuals as against 3% non-malarious children living in Western Kenya (Maina et al., 2010).

In another study, Kayode et al. (2011) indicated significant increase ($p > 0.05$) in WBC of malaria and malaria typhoid co-infected patients, which they posited could have been elicited by increased production of leukocytes at the onset of the infection to wade off malaria parasite and typhoid pathogens. Similarly, increase in WBC in pregnant and non-pregnant malaria patients has been reported by Adesina et al. (2009) and Sumbele et al. (2010). However, the works of Ali et al. (2009) noted both increased and decreased WBC in the blood of typhoid patients examined in Dubai. From these indications, the use of serum level of WBC as an index for diagnosis may not be very reliable. Therefore, WBC should always be thoroughly re-evaluated for malaria for reproducibility and reliability.

Studies carried out by Amah et al. (2011) showed significant reduction in serum levels of albumin in malaria patients in endemic regions of Calabar, Nigeria. Many authors have proposed the use of serum albumin levels as a reliable biochemical marker for establishing severe pathologic conditions such as malnutrition and infectious diseases (Das et al., 1997; Kwena et al., 2012). Malaria infections are accompanied with significant decrease in plasma albumin concentrations (Kwena et al., 2012) as well as in malnutrition and pregnancy. However, the prevailing plasma albumin concentration in malaria infection is dependent on the nutritional status of the affected individual and hepatic functionality (Crawly, 2004; Ogbodo et al., 2010). Probably, based on the nutritional and hepatic status of the study subjects/participants in the study groups, the report presented here showed non-significant ($p > 0.05$) reduction in serum albumin levels in malarious subjects compared to non-malarious groups (Table 2). Contrary to these observations, Ogbodo et al. (2010) showed that there was initial significant ($p < 0.05$) increase in serum levels of albumin in low and moderate malaria infections, but decreased as the malaria density increased. Based on these observations, they recommended the use of albumin infusion in place of other colloidal solutions as a good intervention in severe malaria.

Creatinine and urea are nitrogenous low threshold substances with immense clinical application in ascertaining renal function. Impairment of renal function during severe falciparum malaria is common (al-Yaman et al., 1997; Eiam-Omg and Sitprija, 1988; Gu¨nther et al., 2002; Mockenhaupt et al., 2004). Table 2 shows that the malarious groups presented marginal increases in serum creatinine concentration ($p > 0.05$). Paradoxically, serum levels of urea were significantly ($p < 0.05$) raised in the same malarious subjects under investigation. But elevation of serum urea concentration could also connote evidence of dehydration, consumption of proteinous meals and tissue catabolism. Nevertheless, this was an obvious indication that moderately malaria infected subjects exhibited alteration in nitrogen metabolism with underlying compromised renal function. According to Sitprija (1988), raised blood urea concentration reflected gradual progression towards renal dysfunction. Specifically, serum levels of urea had been observed to increase more rapidly than serum creatinine concentration in individuals with renal dysfunction (Emian-Ong, 2002).

Blood sugar levels in malaria infection have received the attention of several researchers. Studies by Kayode et al. (2011) indicated hypoglycemia in both malaria and typhoid co-infected patients. They posited that the level of hypoglycemia correlated with severity of infection, which was elicited by hyper-secretion of insulin. Their report corroborates the studies by Onyesom and Agho (2011), who noted the incidence of hypoglycemia in malaria patients in Edo-Delta state. The role of low serum insulin-like growth factor-1 (IGF-1) and low blood glucose levels in malaria infection was reported by Mizushima et al. (1994). They noted that *P. falciparum* infected children with low IGF-1 levels (< 50 ng/ml) presented hypoglycemia compared to other study groups. In another study,

Binh et al. (1997) reported the relative contribution of insulin-mediated and non-insulin-mediated plasma glucose levels in severe malaria. The report stated that there was a corresponding increase risk of hypoglycemia as infection progressed because host glucose production becomes insufficient for host/parasite demand. The study also revealed that basal plasma glucose increased in uncomplicated malaria because of peripheral insulin resistance. Moderate malaria infection caused significant reduction in serum FBS levels (Table 2) with blood sugar levels tending towards hypoglycemia ([FBS] < 60 mg/dl). Accordingly, the present report supports the findings of previous authors (Mizushima et al., 1994; Binh et al., 1997; Kayode et al., 2011; Onyesom and Agho 2011).

Finally, although these alterations in haematologic and biochemical indices in association with malaria infection are not novel, our findings have added more information, hitherto the limited knowledge and sparsely reports on alterations in blood profile of malaria infected individuals habitat in Owerri Municipality .

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UPCOMING CONFERENCES

13th National Congress of Medical Biology and Genetics, Aydin, Turkey, 29 Oct 2013

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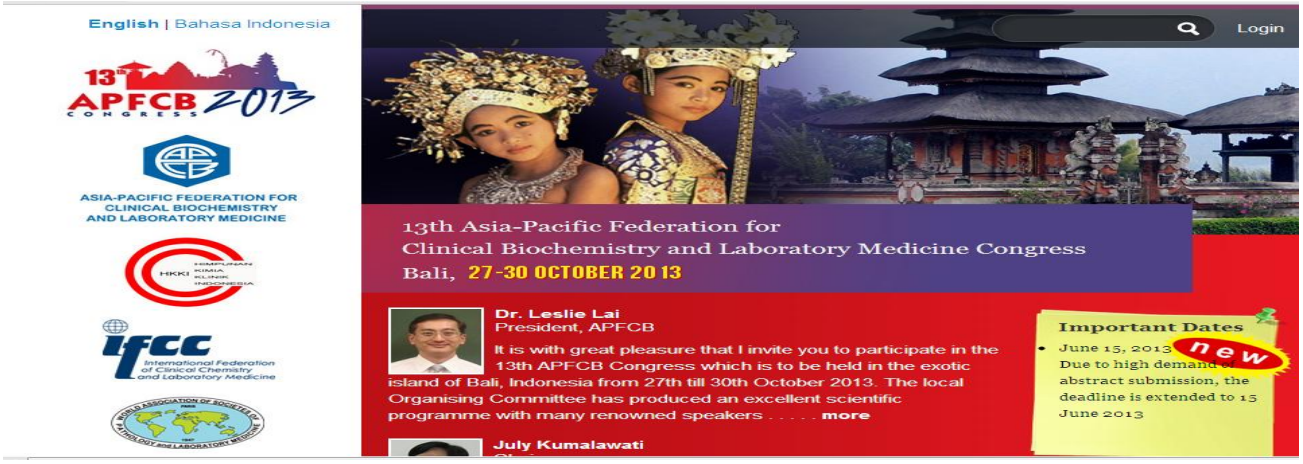

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