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Full Length Research Paper

Assessment and evaluation of the capacity of clinical laboratories at South Kordofan State for the diagnosis of Malaria

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Abstract

Malaria is a major cause of death in tropical countries, it caused by different species of plasmodium, falciparum, vivax, malaria and ovale, even malaria diagnosis measures are setting properly, and miss-diagnosed cases are still causing death and major health complications. Causative agents for miss-diagnosis case could be due to protocol used, which include staining methods, trained personal and occupational work areas. So this study aimed to evaluate laboratory work for testing malaria infection by blood films, whether thick or thin, tools involved and so on. 50 members of laboratory workers, at Kordofan state were evaluated though their educational levels, training, protocol of laboratory work. Data obtained was analyzed using the statistical package of social science program. Most of laboratory workers were well educated with increased experience, conducting optimal malaria diagnostic tools, such as standard staining preparation was followed by 3%, timing of staining done by 83% and performing of thin and thick blood films were found among 89% of personal. Level of education and type of training involved workers presented with influence on results obtained, as when they were compared significant difference was obtained, p value 0.000 and 0.032 respectively, but experience duration did not affect the results obtained. So it recommended to follow the ideal protocol of routine malaria testing steps and it should include raise the qualification of personal and provided excellent tools for such reason.

Keywords: Plasmodium, protocol, miss-diagnosis.

INTRODUCTION

Malaria continues to be a major health problem in the tropical and temperate regions of the world1. An estimated 3.3 billion people approximately one-half of the world's population living in 109 countries are at risk of contracting this serious and often life threatening disease. Malaria accounts for ~250 million clinical cases and nearly 1 million deaths each year, the great majority of which occur in children younger than 5 years of age and in young, pregnant women. Malaria influences the social and economic well-being of societies in affected areas, draining scarce health and human resources, interfering with educational achievement, and causing persistent economic disadvantage. Malaria is caused by Plasmodium parasites that infect humans through the bites of an infected female mosquito of the genus Anopheles and destroys red blood cells.

There are four known human malaria parasites, Plasmodium falciparum, Plasmodium vivax, Plasmodium malaria and Plasmodium ovale. The first two species cause the most infections worldwide, (Goncalves *et al.,* 2012), but Plasmodium falciparum is the most pathogenic and frequently fatal if not promptly given treatment, (Azikiwe *et al.,* 2012). When Plasmodium parasites are transmitted by Anopheles mosquitoes into their

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mammalian host, they are confronted with extreme environmental changes as they move from the salivary gland of a cold-blooded insect host to the skin tissue of a warm-blooded mammalian host. Once injected into the skin, the motile sporozoites transmigrate several cells before eventually crossing endothelial cells to reach a blood vessel, (Frischknecht *et al.,* 2004; Vanderberg and Frevert, 2004; Amino *et al.,* 2006). Only a portion of the injected sporozoites (35%) enters a blood vessel and is carried by the bloodstream to the next destination, the liver, (Amino *et al.,* 2006).

The effort to eradicate malaria is based on comprehensive interventions, including strategies to diagnosis the disease, (Murphy *et al.,* 2013). Malaria, as a disease and public health challenge, reflects an extremely complex set of interactions between the parasite, the human host, and the vectors responsible for transmission.

Environmental, social, economic, and behavioral factors enable and foster these interactions and, thus, support perpetuation of malaria as long as the life cycle of the parasite remains intact. In principle, any intervention that achieves a complete blockage at any point in the life cycle of the parasite would effectively interrupt transmission and facilitate eradication efforts. To date, however, no single intervention with such complete activity has been identified, and until that occurs, multiple interventions operating at various points in the life cycle of the malaria parasite will be needed to maximally inhibit progression through the life cycle and prevent transmission, (Plowe, 2009).

World Health Organization (WHO) is trying to eliminate presumptive drug treatment of malaria and currently makes the tentative recommendation that parasite-based diagnosis should be used in all cases. All suspected malaria cases are confirmed with a parasite-based diagnostic test prior to therapy, (WHO, 2010).

The majority of malaria cases are found in countries where cost-effectiveness is an important factor and ease of diagnostic test performance and training of personnel are also major considerations, (Warhurst and Williams, 1996). Giemsa stained blood films are the accepted laboratory practice for the diagnosis of malaria with microscopic examination, (Warhurst and Williams, 1996).

The expected sensitivity of thick blood film examination can be achieved by an experienced microscopist is about 50 parasites/µl of blood (assuming a total RBC count of 5 × 106/µl of blood), which is equivalent to 0.001% of RBC infected, (Milne *et al.,* 1994), found that most routine diagnostic laboratories generally achieved a lower sensitivity of detection (average, 0.01% RBC infected, 500 parasites/µl) in an examination of results from British laboratories submitted to the Malaria reference Laboratory, (Warhurst and Williams, 1996).

MATERIAL AND METHOD

This study conducted as part of survey as malaria infections were tested to be diagnosed, among Kordofanian residents, targeted occupational working areas with well-constructed building with safety measures, personnel education or qualification (BSc of medical laboratory science with parasitology specialty or non-parasitology specialty or malaria technician bases as malaria microscopist) and working experience, blood collection methodology, tools used in malaria diagnosis; microscopes stains and types of blood films examined and kind of result presentation, whether it full report or not. In order to collect the data involved the academic qualification of the quality of the microscopes and the preparation of the dye according to the standards of the World Health Organization and the quality required and prepare the result writing, each variable was given a percentage to be expressed in relation to the study. The proportions were distributed as follows: Building and safety were profound, qualified personnel, standard microscopes, standard staining method, standard blood film and slide and standard result and report.

RESULT

Cross sectional study as evaluation pattern for local assessment of malaria diagnosis bases among Kordofanian residents was conducted involving 50 members of laboratory work, it turned out that personal qualification involved out BSc of medical laboratory science 8 (16%) with parasitology sub-specialty and 32 (64%) with non-parasitology sub-specialty, Diploma of medical laboratory science involved 6 (12%) and 4(8%) were malaria microscopist. Personal experience was divided to less than one year which involved 3 (6%) members, 1-2 years were 4 (8%) and more than 3 were 43 (86%) members. Out of the 50 laboratory workers, 26 (52%) with no training, 21 (42%) with basic training and with refreshing training were 3 (6%). Microscopic evaluation was conducted through availability and efficiency of parts and utilities. High scored mark was measured out of 25 as principle as in table 1.

Table-1: Frequencies and percentages of microscope quality score

Score	Frequency	%
15-20	12	22.2%
10-<15	33	68.5%
<10	5	9.3%
Total	50	100%

Staining evaluation revealed that all participants in the study used Giemsa's stain. The quality of the stain usually confirms by the technique used to prepare and the diluent for the stain, which it should be buffer with PH 7, 10 out of the 50 laboratory workers involved in this study were testing malaria with rabid test, as

Table 2: Frequencies and percentages of Report quality indicators

Indicator	Frequency	%
Report Species	38	95%
Report Stage	38	95%
Report parasite density	36	90%
Maintain a copy of the result	28	70%

Table 3. Independent T-test for Personnel Qualification and test result

Personnel Qualification	Test result		Total	*P-value*
	Good	Poor		
BSc MLSc-Parasitology	8 (100.0%)	0 (0.0%)	8 (100.0%)	0.000
BSc MLSc-Non Parasite	8 (25.0%)	24 (75.0%)	32 (10 0.0%)	
MLSc diploma	1 (16.7%)	5 (83.3%)	6 (100.0%)	
Malaria Microscopist	4 (100.0%)	0 (0.0%)	4 (100.0%)	
Total	21 (42.0%)	29 (58.0%)	50 (100.0%)	

Significant difference p value <0.05

Table 4. Independent T-test for personnel experience and test result

Personnel Experience	Test result		Total	*P-value*
	Good	Poor		
3 years or more	19 (44.2%)	24 (55.8%)	33(100.0%)	0.178
1-2 years	2 (50.0%)	2 (50.0%)	4 (100.0%)	
less than 1 year	0 (0.0%)	3 (100.0%)	3 (100.0%)	
Total	21 (42.0%)	29 (58.0%)	50 (100.0%)	
3 years or more	19 (44.2%)	24 (55.8%)	33(100.0%)	

Significant difference p value <0.05

Table 5. Independent T-test for Personnel in-service training and test result

Type of Training	Test result		Total	*P-value*
	Good	Poor		
Basic	10 (47.6%)	11 (52.4%)	21 (100.0%)	0.032
Refreshing	3 (100.0%)	0 (0.0%)	3 (100.0%)	
Non	8 (30.8%)	18 (69.2%)	26 (100.0%)	
Total	21 (42.0%)	29 (58.0%)	50 (100.0%)	

Significant difference p value <0.05

immunochromatogrophy test (ICT) and out of the 40 members, 2 (5%) of personal were using adjustment buffer, the rest used running tap water, 37 (93%) were followed working solution disposal after 30 minutes. Timing of satin showed that 38 (95%) were using time standard time for blood film which 10 minutes. Using stain control was found among 39 (98%), conducting thin and thick blood film for each patient also performed by 39 (98%) of the participants, out of the 50 laboratory workers, 47 (94%) were working through safety measures. Result outcome could be through full report for the film, which usually includes density, species and stage of the detected parasite. Out of the 40 laboratory workers, 38 (95%) were report species, 38 (95%) and 36 (90%) were reported parasite's density. Only 28 (70%) were maintained copies of the results as in table 2. Comparing educational and qualification of laboratory worker, who were involved in this study performing malaria diagnostic tests mainly blood film, bringing good and poor result, showed that significant difference, as p value obtained was 0.000 as in table 3. While comparing effect years of working experience on results, it did not give significant difference, as p value was 0.178 as in table 4. Basic knowledge and training has effect on obtained result, as comparing that gave significant difference with p value 0.032 as in table 5.

DISCUSSION

Malaria, sometimes called the "King of Diseases", is caused by protozoan parasites of the genus Plasmodium, it is the most important infectious disease in tropical and subtropical regions, and continues to be a major global

health problem, with over 40% of the world's population exposed to varying degrees of malaria risk in some 100 countries. It is estimated that over 500 million people suffer from malaria infections annually, resulting in about 1-2 million deaths, of whom 90% are children in sub-Saharan Africa. Microscopic diagnosis using blood smears plays an important role in malaria diagnosis as its ability to diagnose and differentiate each species of malaria, and so it is set the gold standard for any new detection tool or technique (Wongsri *et al.,* 2007; Tangpukdee *et al.,* 2009; Alam *et al.,* 2011; Ouattara *et al.,* 2011; Batwala *et al.,* 2011), but this method still suffers from drawbacks, such as requiring a visual or light microscope with 100× magnification and relying on skillful and well-trained microscopists. Microscopic diagnosis is a morphology based identification so that Plasmodium species with closely similar in shape or characteristics such as P. malaria is prone to fault diagnosis, even by an expert. However, the main cause of error that is due to a low parasite density was not resolved by this approach, (Díaz *et al.,* 2009; Tek *et al.,* 2009; Das *et al*., 2015).

This is due to the fact that the average ability of microscopic diagnosis to detect Plasmodium in iRBCs (infected red blood cells) has a threshold of around 10 parasites/μL for a research setting (McNamara *et al.,* 2006), and in the range of 50–100 parasites/μL for outside a research setting, or less sensitive in a limited resource setting. Fluctuations of parasite density over the course of infection contribute to detection-limit of microscopy-based diagnosis and all other direct detection approaches, (Hawkins *et al.,* 2014). In this study, 50 members of laboratory worker were involved in order to evaluate their outcome result under the influence of their educational status, experience period, tools provided in order to achieve their laboratory work, which included detection of malaria infection through blood smears, it turned out that personal qualification involved out BSc of medical laboratory science 8 (16%) with parasitology sub-specialty and 32 (64%) with non-parasitology sub-specialty, Diploma of medical laboratory science involved 6 (12%) and 4(8%) were malaria microscopist. Most of them have been working in laboratories for more than years, but minimum percentage with refreshing training for malaria detection by slide methods were involved, microscopes used these laboratories were 50, only 12 (24%) had high score of quality according to the WHO criteria, 33(66%) with medium quality and the rest 5 (10%) with less. 40 laboratory used Giemsa stain, only 5% of them followed the protocol of standard staining techniques, while the rest did not committed to whole protocol steps.

Timing for staining (10 minutes) was conducted by 95%, performing microscopic examination for each patient by means of thin and thick blood film conducted by 98% of lab worker, who were also using stain control for their work. 95% of workers were reporting parasite species, stages and density, 70 % were maintained copies of malaria results. Most of laboratory worker were qualified with BSc of medical laboratory science, less frequent with sub-specialty of parasitology and those brought poor outcome results. While malaria microscopist individuals had good outstanding performance of malaria detection under microscope, this in partial agreement of study conducted in Congo, as External Quality Assessment (EQA) assessed microscopy of blood parasites among diagnostic laboratories addressed 445 participants in 10 provinces, they were checked for slide, routine stain preparation, 89% were response for quality preparation and staining, only 11% had good performance, 30.6% reported malaria. Only 13.6% of routine slides returned were correctly prepared and stained.

The proportion of correct/acceptable scores for at least 4/5 slides was higher among EQA-experienced participants compared to first time participants (40.9% versus 22.4%, $p = 0.001$) and higher among those being trained < 2 years ago compared to those who were not (42.9% versus 26.3%, $p = 0.01$), (Mukadi *et al.,* 2016). Other study supported the fact that microscopic examination of malaria is considered the gold standard method, it conducted a comparison between manual slide detection and rapid methods even rapid diagnostic method have acceptable sensitivity and specificity, (Mukry *et al.,* 2017). Also in order to assess laboratory work for malaria detection though old tradition method, which is microscopic examination, a South African study in agreement of this study, as it revealed a deficiencies in the quality of malaria diagnosis in routine laboratories and it suggested that quality systems across the spectrum of diagnostic facilities in South Africa need strengthening, to ensure progress towards elimination as well, (Frean *et al.,* 2013). Other study demonstrated that microscopy, not always available or feasible at primary health services in limited resource settings due to cost, lack of skilled manpower, accessories and reagents required and recommended Rapid diagnostic tests (RDTs) to be potential tools for parasite-based diagnosis since the tests are accurate in detecting malaria infections and are easy to use, (Siahaan, 2018).

CONCLUSION

Although Kordofan state is considered lack of principles of health care and provided tools for laboratory work, assessment of laboratory workers showed that they need follow standard protocols and training skills to increase their capabilities to right achievement.

RECOMMENDATION

Quality assessment should be conducted routinely and it should include personal and set advance training

program and remote areas, where microscopic examination is not available, rabid test should be used.

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