BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, June 2012. Vol. **9**(1), 249-252

Malaria Parasite Density

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(Received: 10 March 2012; accepted: 29 April 2012)

Malaria parasite density is also called parasite count and is a malariometric index which focuses on assessing the severity of the burden of malaria infection .(kakkilaya ,2004)

This measure of parasitaemia is used in the study of further threshold and estimation of fever risk s, assessing the efficacy of the therapeutic interventions and in stratifying resistance to antimalaria treatment (cheesbrough, 2006) (O'meara *et al.,* 2005)(Montari *et al.,* 2001)

Calculating parasite density is also important for estimating herd immunity to malaria and for determining end point for interventions such as malaria vaccines, impregnated bed nets and chemosuppression (O'meary *et al.,* 2005); (Montanari *et al.,*2001); (Petersen *et al.,* 1996); (Schellenberg *et al.,*1994)

Visual inspection of blood smears by light microscopy is the method available for quantifying malaria parasite density ,doing so by comparing the ratio of counted parasites within a given number of microscopic fields against either counted white blood cells (WBC) or counted red blood cells (RBC) within these same fields and then multiplying the ratio by either the measured or estimated density of WBCs or RBCs in the patients' blood (O'meara *et al.,* 2005).

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The different methods for calculating parasites density described in literature can be as follows

- Expressing parasiteamia in a thick film as +,++, +++ based on the scheme
- 1-10 parasite/100 high powerfields(hpf) ————————+
- 11-100 parasites/100 high power fields (hpf) ++
- 1- 10 parasites /every hpf ----- +++
- >10 parasites/every hpf ————————————++++

- Counting parasites /WBC in a thick film until the high power field (hpf) with the 200th WBC then multiplying with a ratio of the total WBC count /uL. (cheesbrough, 2006); (O'meara *et al.,* 2005); (Kakkalaya, 2004).
- Counting parasitized RBCs in a thin film in 100 hpfs or until 1000th RBC and multiplying with a ratio of total RBC count /uL. (O'meara *et al.,* 2006); (Cheesbrough, 2006); (Kakkilaya, 2004).
- Examining 100hpf and if they all contain at least one parasite, then counting the number/ field in 10-100 fields depending on density and multiplying with aratio. A correction factor 2(-in(1-P) is applied . (Petersen *et al.,* 1996)
- Finding the average number of parasites per high power field (hpf) after counting the parasites in 10-50 hpfs, then multiplying by 500.

- This last method proposed by greenwood and Armstrong was found by them to be more accurate and quicker than counting parasitesagainst white blood cells (WBCs) as described above. Using the count of parasitized RBC gave the most accurate assessments, however it is more time consuming and required much technical expertise.(Cheesebrough,2006)(O'meara 2005)(Greenwood and Armstrong ,1991).

The thick film is preferred to the thin film in estimating parasite density it is 30 times more sensitive than the thin film and has been known to detect as low 5 parasites/uL .(Cheesebrough,2006) . the rapid diagnostic tests including parasight , malaquick, ICT malaria pf are quick and single to carry out with no technical apparatus. The sensitivity is 90-95 % when parasiteamia is more than 100 parasite/UL, thus low parasiteamia is often missed . So a thick film is a more sensitive assay than rapid diagnostic tests

Method which can be used to concentrate the parasites in a sample thus increasing malaria parasite density include

a) Centrifuging EDTA –anticoagulated venous blood in a capillary and using the blood just below the buffy coat layer. (quarantine buffy coat method). This gives excellent concentration especially of plasmodium vivax.(cheesbrough, 2006)
b) Using a magnetic field to concentrate malaria parasites. This is based on the paramagnetic property of haemozoin , a method called magnetic deposition microscopy (Zimmerman *et al.*, 2006)
c) Magnetic deposition microscopy also concentrate gametocytes (Karl *et al.*, ,2008)
d) Density gradient centrifugation and removal of WBCs using cellulose powder column or filtration unit, thereby concentrating parasitized RBCs (Trang *et al.*, 2004)

Studies on malaria microscopy has often documented the frequency of false positive or negative results which increase with lower parasite density. (O'meara *et al.*, 2005); (Mackenzie *et al.*, 2003); (Durrheim *et al.*, 1997); (Beljaer *et al.*, 1985)

These findings make the concentration of parasites very desirable when initial count shows a low density. However, identical consistent techniques may be more important than increasing the number of fields read when precision among readers is desired, moreso, sequential samples taken from each patient in the course of a study stand to potentially improve the sensitivity and accuracy of estimates (O'meara *et al.*, 2005); (O'meara *et al.*, 2006).

It is possible that total parasite quantity present largely determines pathological and clinical manisfestation yet peripheral measurement of parasites density a subset and not total parasite load (Delley *et al.*, 2000); (Armstrong –schelenberg *et al.*, 1994). During the course of a single infection, parasites density has been noted to fluctuate daily or even hourly in response to fever, immune mechanisms ,sequestration ,intra-host competition, antigen swithching and other events. This can lead to up to 20% or more under estimation of parasite prevalence. (O'meara *et al.*, , 2007)(Delley *et al.*,2000)(Genton *et al.*, , 1995) (Kibikamusoki, 1967).

Malaria parasite density studies in Mali , determined from peripheral blood (finger prick), varied during the day with a consistently higher parasite density measured at 13.00hours (12.00-13.00hours) (Delley *et al.*, 2000). A previous study had suggested an "internal check" which resulted in synchronised explosion of schizonts around noon (Hawkings, 1990).

Above observations suggest that consistency of techniquesand time of blood collections around noon should give a fairly high and reliable estimation of malaria parasite burden . also studies which derive a correction factor for sequestered parasites could be added to give a fuller picture of malaria parasitaemia in an individual (Gravenor, Henbroek & Kwiatkwoski,1998); (David *et al.*, 1983).

Relating malaria parasite density to fever onset appears to be a major clinical implication of studies on malaria parasite density.

Malaria parasite density thresholds have been proposed for the development of fever due to malaria and irt appoearded to vary from study to study (Rogier *et al.*, 1996); (Velema *et al.*, 1991) (Bauden *et al.*, 1986); (Troupe *et al.*, 1985) (Miller, 1985).

Important to note that the fact that high parasite levels >100000/uL can exist in persons with no symptoms at al , this is apparently a result of

premonition. (Hogh,1996)(Hogh *et al.,* 1983). Parasite density needed to trigger fever strongly varies from person to person, reflecting the possible role of immune factors on fever threshold. (Delley *et al.,* 2000); (Roger *et al.,* 1996)

In a particular study maximum risk of fever occurred with parasite density >1000/uL especially >10,000/uL . the fact tended to occur 24-48hrs after peak of parasite density at which time measured parasiteamia may not be at peak anymore (Delley *et al.*, 2006). In the rainy season in Mali, the best prediction of fevers were in under age (<4yrs), enlarged spleen and high parasite density (Bouvier *et al.*, 1997).

An age dependent threshold effect of parasite density on occurrence of fever was described the threshold varied from a maximum of 2.45 trophozoites /leucocytes at 1 year of age.

A minimum of 0.5 trophozoites / leucocytes at 60 years of age When an individual parasite density crossed the threshold level corresponding to his/her age , the risk of fever multiplied by 44 (Rogier, Commanges and Trape, 1996).

The density found in children is explained to be higher than in adults in areas of high transmission and so is the risk of fever . the number of children <10yrs with parasite density ≥ 100.000/ uL increased progressively with axillary temperature (Ejezie and Ezedinachi, 1992) a regression model has been proposed describing the probability of being sick from malaria in relation to body temperature and parasite density it appears that the ability to control the disease and parasite density develops earlier than ability to prevent parasite infection (Hugh, 1996).

Several studies have estimated parasite density in the two most vulnerable subsets of human population –children and pregnant women . estimation of mean parasite density was more accurate when the individuals total WBC count was used instead of an assumed value which can lead to overestimation of parasite burden (Jeremiah and Uko 2007).

Symptomatic children in a hyperendemic area of Nigeria (Ekpoma ,edo state) showed a mean parasite density of 4707.6 ±995.53uL –showing moderately high parasiteamia (Nmorsi *et al.,* 2008) . parasite density was significantly higher in children with splenomegaly than in those with non palpable spleen ,(Adeyemo *et al.,* 1999) . it is also significantly lower in stable children with sickle cell trait and sickle cell disease (Awoha- efebo *et al.,* 2004)

In healthy pregnant women mean parasite density was lower than in children with a range of 600-800 parasite /uL. (Adefoye *et al.,* 2007)

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