Defining malaria burden from morbidity and mortality records, self treatment practices and serological data in Magugu, Babati District, northern Tanzania

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Abstract: Malaria morbidity and mortality data from clinical records provide essential information towards defining disease burden in the area and for planning control strategies, but should be augmented with data on transmission intensity and serological data as measures for exposure to malaria. The objective of this study was to estimate the malaria burden based on serological data and prevalence of malaria, and compare it with existing self-treatment practices in Magugu in Babati District of northern Tanzania. Prospectively, 470 individuals were selected for the study. Both microscopy and Rapid Diagnostic Test (RDT) were used for malaria diagnosis. Seroprevalence of antibodies to merozoite surface proteins (MSP-1_{19}) and apical membrane antigen (AMA-1) was performed and the entomological inoculation rate (EIR) was estimated. To complement this information, retrospective data on treatment history, prescriptions by physicians and use of bed nets were collected. Malaria prevalence in the area was 6.8% (32/470). Of 130 individuals treated with artemisinin combination therapy (ACT), 22.3 % (29/130) were slide confirmed while 75.3% (98/130) of them were blood smear negative. Three of the slides confirmed individuals were not treated with ACT. Fever was reported in 38.2% of individuals, of whom 48.8 % (88/180) were given ACT. Forty-two (32.3%) of those who received ACT had no history of fever. About half (51.1%) of those treated with ACT were children <10 years old. Immunoglobulin against MSP-1_{19} was positive in 16.9% (74/437) while against AMA-1 was positive in 29.8 % (130/436). Transmission intensity was estimated at <0.2 infectious bites per person per year. The RDT was highly specific (96.3%) but with low sensitivity (15.6%). In conclusion, Magugu is a low endemic area. There is substantial over diagnosis, over treatment and self treatment in the community. The burden of malaria based on medical records is over estimated as was mostly presumptive. The low sensitivity of RDT reflects the low number of immune individuals as well as the low parasite density.

Keywords: malaria, morbidity, mortality, diagnosis, treatment, Tanzania

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Introduction

Every year an estimated 300-600 million cases of malaria occur globally, resulting in 1 million deaths. Approximately 90% of malaria cases and more than 85% of deaths occur in Sub-Saharan Africa, mostly among children under the age of five (Crawley *et al*., 2010). Over much of the Sub-Saharan Africa, malaria transmission intensity varies from one area to another. Repeated exposure to malaria parasites and transmission intensity govern the development of acquired immunity, which controls the erythrocytic cycle of malaria, thereby reducing clinical symptoms and life-threatening complications. Merozoite antibodies play an important role in protecting erythrocyte invasion and opsonising merozoites for phagocytoses (Murhandarwati *et al*., 2008). Where malaria is endemic, individuals get continuous exposure, thus clinical manifestations in adults are low grade though these individuals may have parasitaemia (Andersen *et al*,. 1997; Bruce-Chwatt, 1952; Warsame *et al*., 1997). In non-endemic areas progressive lowering of acquired immunity is experienced (Luxemburger *et al*., 1997) but even then serological markers are maintained. These markers (Craig *et al*., 1999) are not influenced by the seasonal fluctuation in malaria incidences and are a potential tool for defining the malaria transmission intensity of the area (Bousema *et al*., 2010).

A number of programmes have been implemented to fight malaria in SSA, in three different or combined approaches: use of antimalarial drugs, vector control (elimination of mosquito breeding sites, indoor residual spraying and use of insecticide treated nets - ITN) and mass drug administration (MDA). These interventions resulted in a decline of malaria in many areas in Sub-Saharan Africa including Tanzania (Bhattarai *et al*., 2007; Barnes *et al*., 2009).

Contradicting reports still indicate high malaria incidences in Sub-Saharan Africa (Besnard *et al*., 2006; WHO, 2009), especially in rural areas where adequate diagnosis and prompt treatment continue to present major difficulties. Presumptive treatment in a malaria declining era (Gosling *et al*., 2008), has limited application. Over-diagnosis (Elgayoum *et al*., 2009) and over-treatment of malaria (Chandler *et al*., 2008) may lead to missing other causes of febrile illnesses. This may result in overuse of antimalarial drugs (Mwanziva *et al*., 2008) and increases the chance of developing drug resistance (Maude *et al*., 2009; White, 2010). Furthermore, self-treatment (Nsimba & Rimoy, 2005; Hodel *et al*., 2009) and mistreatment of febrile illnesses in health facilities results into increasing hospitalization and deaths due to diseases other than malaria. The objective of this study was to estimate the malaria burden based on serological data and prevalence of malaria, and compare it with existing self-treatment practices in the community and in medical practice in Magugu, a malaria endemic area of northern Tanzania.

Materials and Methods

Study site

Magugu ward (S3099' S4001'; E35070' E35077') in Babati District is located in northern Tanzania along the rift valley, at approximately 900m to 1200m. The ward has a total population of 26,131 people residing in seven villages. There is one government owned health centre which serves all the seven villages. Babati District is one of the eight sentinel sites in Tanzania for monitoring anti-malarial drug efficacy. Livestock and crop agriculture (mainly rice, maize, sugar cane and

vegetables) are the main occupations of the population. Average annual rainfall is about 650mm. There are two rainy seasons, short rainy season from October to December and long rainy season from mid March to May, followed by a cool and dry season from June to mid August, and a hot dry period from mid August to October.

Data collection

Retrospectively, medical records covering 2005-2007 from Babati Hospital and Magugu Health Centre were reviewed. The record comprised overall record of malaria morbidity of all patients attending the two facilities. Prospectively, individuals attending the facilities from June to August 2009 were asked to participate in the study regardless of their main complaint. A structured questionnaire was administered to record participant's information including age, gender, date of attendance and use of mosquito bed nets. Information was also collected on type of medication (antimalarials and other medicines) used in the previous two weeks. Body temperature, request for blood slide, rapid diagnostic test (RDT) and their results were recorded. Clinical diagnosis and treatment given before leaving the health facility was documented.

Three drops of blood were collected from a single finger prick and examined for malaria infection. The first drop was used for microscopic parasite detection; both for a thin and thick blood smear, and stained with 10% Giemsa. Parasite density was estimated by counting the number of asexual parasites per 200 white blood cells (WBC) and the number of sexual forms per 500 WBC. A slide was judged negative if no parasites were seen after inspection of 100 high power fields. For quality control, a second microscopist, who was unaware of the results from the first reading, re-read all the slides. The second drop of blood was used to determine the presence of malaria parasites by RDT (Paracheck-Pf®, Orchid Biomedical Systems).

A third drop of blood was collected on Whatman's 3mm filter paper (Whitman, Maidstone, UK) for serological tests. Serum was eluted from filter papers as described by Corran *et al*. (2008). Immunoglobulin G antibodies against blood stage antigens were determined by indirect enzyme-linked immunosorbent assay (indirect ELISA), as previously described (Drakeley *et al*., 2005) using recombinant MSP-1_{19} (Wellcome Genotype), produced as previously described by Franks et al. (2003) or recombinant AMA-1 (3D7). Briefly, flat bottom 96-well microtitre plates (Nunc, Roskilde, Denmark) were coated overnight with 50 µl of 0.5 mg/ml dilution of the specific antigen. After washing with PBS-0.05% Tween [(PBS-T), 200 µL of blocking buffer (1% skimmed milk [Marvel, Spalding, Lincs, UK] in PBS-T) was added for 3 hours at room temperature. After washing, 50 µL sera with a dilution of 1:1000 for MSP-119, and 1:2000 for AMA-1, were added to the plate in duplicate and incubated for 3 hours at room temperature. Antigen-specific antibodies were detected using rabbit anti-human IgG (DAKO A/S Denmark) in a 1:5000 dilution and incubated for 20 minutes at room temperature. Plates were developed with o-phenyline-diamine (OPD) [Sigma]- H2O2 and the reaction was stopped with 50µl H_2SO4. Plates were read at a wave length of 490 nm and the Optical Density (OD) values recorded.

Data analysis

Statistical analysis was carried out using SPSS 16.0 for windows (SPSS Inc, Chicago, USA). Proportions for categorical data were reported, and the Pearson Chi-squared test was used to assess the relationship between groups. Fisher 's exact test was used in cases of less than 5 counts.

Standard deviation (SD) was used to describe the spread around the mean. A p-value < 0.05 was considered statistically significant. To generate an Optical Density (OD) cut-off value above which samples were deemed antibody positive, the distribution of OD values was fitted as the sum of two Gaussian distributions (assuming a narrow distribution of seronegatives and a broader distribution of seropositives) using maximum likelihood methods. The mean OD of the Gaussian distribution corresponding with the seronegative population plus 3SD was used as the cut-off point (Corran *et al*., 2008). The OD values were expressed as the percentage of the positive control. The seroconversion rate (SCR or λ) was estimated by fitting a simple reversible catalytic model to the measured seroprevalence, stratified into yearly age groups, using maximum likelihood methods. For these models only individuals aged one year and above were included to remove the effects of the maternally derived antibodies in infants. The $\boldsymbol{\lambda}$ values were converted to EIR equivalents using a log regression based on previously collected EIR values from areas of different transmission intensity in Tanzania (Drakeley *et al.*, 2005; Corran *et al*., 2008).

Ethical considerations

Informed consent was obtained from all participants participating in prospective data collection (questionnaire and blood sampling). Only consenting individuals were included. The treatment practice by the medical personnel was not interfered with by the research activity. All results, including those of the RDT were accessible by the medical personnel as our study was observational.

Results

Data collected retrospectively at the sentinel site at Babati Hospital for the years 2005, 2006 and 2007 showed a small increase in malaria prevalence from 2005 (33.9%) to the years 2006-2007 (41.5% and 40.5%, respectively). Records from Magugu HC (Figure 1) show a similar trend with increase of the burden of malaria during 2006 and 2007 in the area. Ten deaths were reported in 2006 at the Magugu health facility, 3 were children under the age of five. In 2007, 13 deaths were reported, of which 3 were children under the age of five. On both occasions cause of death was associated with malaria.

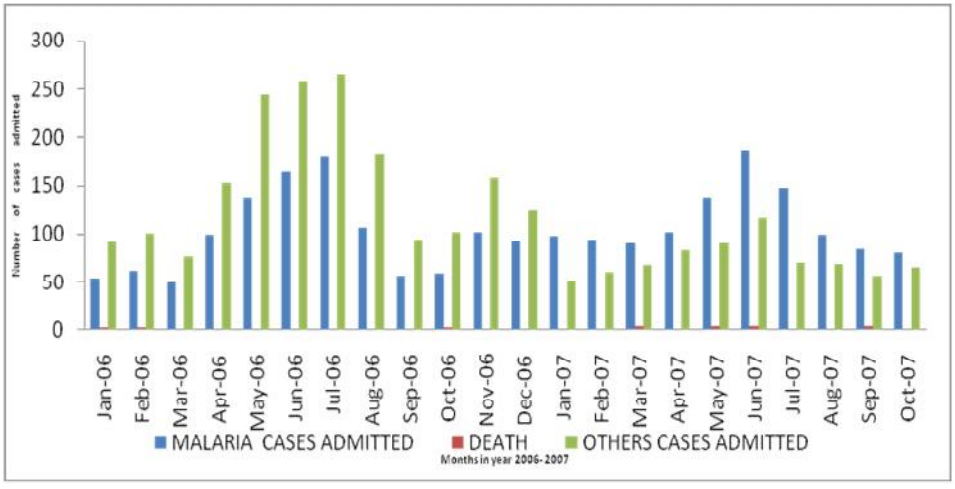

Figure 1: Cases admitted at Magugu HC in all age groups during 2006 – 2007

Prospectively, during 2009 a total of 470 individuals (mean age= 17.9 years) were included in the study (Table 1). Of the <5 years old children 42.9% (104/242) were reported to have fever (≥37.5^{0}C). Out of 180 children presenting with fever, 48.8% (88/180) were given antimalarial treatment, while 27.2% (49/190) were given antibiotics. Thirty two percent (42/130) of the individuals who received antimalarials had no fever, while the majority of those with fever (67.7%) received antimalarials. Among individuals who presented with fever, 57.2% (103/180) were children under the age of five.

Table 1: Characteristics of the study population (n=470)

Variable	Measure
Female (% (n/N)	56. 4 % (265/470)
Age in years, mean ± SD	17.9 ±22.28
< 5 years	51.4 % (241/469)
5 – 15 years	9.2 % (43/469)
> 15 years	39.4 % (185/469)
Bed net use	90.5 % (418/461)
Fever reported (≥ 37.5^{0}C) % (n/N)	38.2 % (180/470)
Residence in Magugu	75.9 % (356/469)

SD = Standard Deviation

Majority (82.3 %) of the patients who came to the Magugu HC had a history of having taken at least a medicament at home. Use of antimalarials prior to reporting at the health facility was reported by 160 of 470 (34%) of individuals. A total of 122 individuals (25.9%) took antibiotics, while 105 (22.3%) took other treatments. Only 10.6% (50/ 470) reported having not taken any medicine. Thirty three participants (7%) could not remember having taken any medication prior visit to health facility.

Table 2: Blood smear results in relation to presence of fever, positive RDT and malaria clinical diagnosis among study participants

Variable	**Blood slide**		**P-Value**
	Positive	**Negative**	
Fever	27	153	< 0.001
RDT positive	5	14	0.072
Malaria clinical diagnosis	29	307	0.156

Out of 469 individuals who visited the health facilities, 373 (79.5%) were clinically diagnosed as malaria. Of those who had history of fever, 88.8% (160/180) were clinically diagnosed as malaria cases. Of the 289 who had no fever, 213 patients (73.7 %) were also clinically diagnosed as malaria cases. Medication history of the patients had an influence on the clinical diagnosis, since of those who reported taking antimalarial drugs prior to visiting the health facility, 40.2% (150/373) were clinically diagnosed as malaria cases. Malaria diagnosis based on microscopy was the gold

standard, and 7.8% (32/406) had a blood smear positive result. Only 16.1 % (29/180) of the individuals presenting with fever had blood smear positive for malaria parasites. RDT was requested among 409 individuals, and 4.6% (19/409) were positive. Twenty six per cent (5/19) of the RDT positive were also blood smear positive, while 6.6% (26/390) of the RDT negative were blood smear positive (Table 2). Using blood smear results as a gold standard, sensitivity of RDT was 16.1 % and specificity of 96.2 %. The positive predictive value (PPV) was 26.3 % and negative predictive value (NPV) 93.3 %.

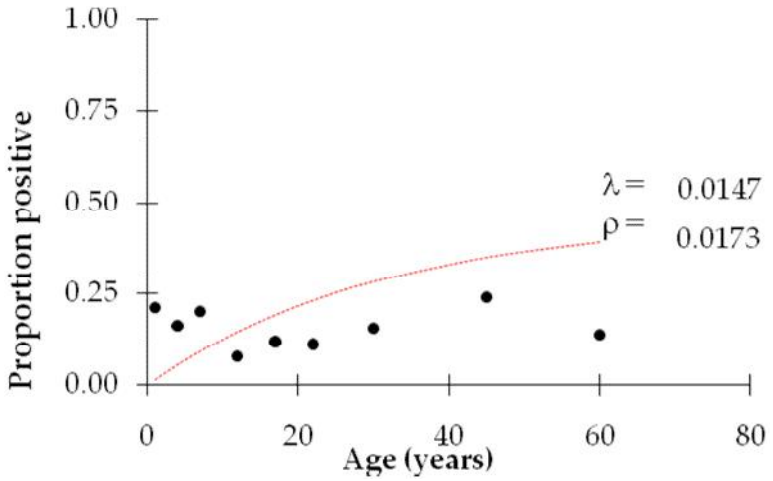

Figure 2: Seroprevalence of MSP-1$_{19}$ fitted into the model

A visit to the health facility ensured medication to be given to the individual since 94.3% (443/470) received any medicament. Some 42.8% of those who treated themselves with other treatments prior to the consultation were given antimalarial treatment. However, 20.6% (33/160) of individuals received again antimalarials. Of 130 individuals who received antimalarial treatment, 98 (75.3%) had negative blood smears. This treatment was prescribed based on laboratory results. Bed-net use among participants was high at 90.5% (418/462; Table 1). The majority 92.9%(223/ 240) of children below five years slept under a bed net, and all those who were blood smear positive for malaria owned a bed net. The overall seroprevalence for MSP-1$_{19}$ was 16.9% and that for AMA-1 was 29.8% (Figure 2, 3). The seroprevalence showed a mild trend of increase with age in both antigens (NS). The pooled age group into three categories showed an increasing trend of seroprevalence and a fall in the older age groups. The seroprevalence for MSP-1$_{19}$ by age category were as follows: <5years 18.5% (42/227), 5 to 15 years 15.4% (8/39) and > 15 years 15.2 % (26/171). For AMA-1 no clear gradient increase with age was observed: < 5 years 31.7% (72/227), 5 to 15 years 28.9% (11/38) and > 15 years 27.5% (47/171). The EIR calculation using seroprevalence by AMA-1 (EIR 1.19) was higher than that of MSP-1$_{19}$ (EIR 0.20). The lambda (**λ**) expressed MSP-1$_{19}$ was 0.015 while that expressed by AMA-1 was 0.033.

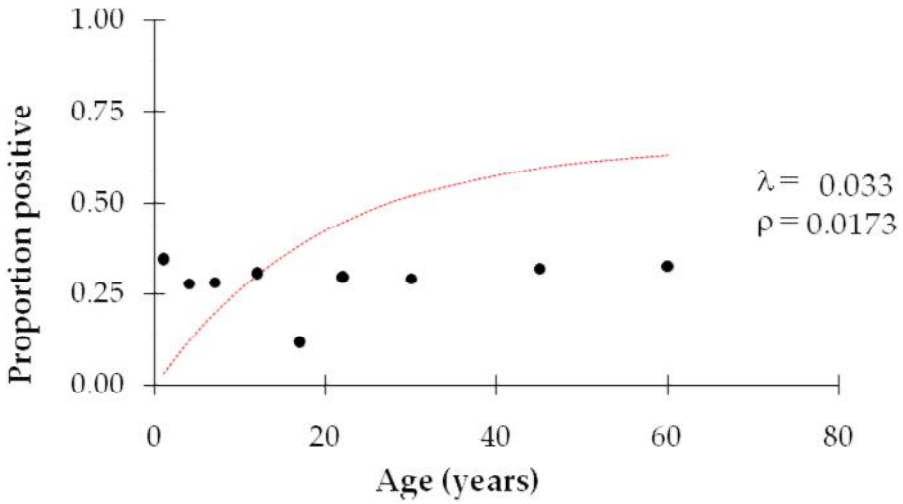

Figure 3: Seroprevalence of AMA-1 fitted into the seroprevalence model

Discussion

We found that only 7.6 % of the malaria cases were confirmed slide positive, while 5.6% of the treated presumptive malaria cases were true malaria cases. This implies a significant overuse of ACT in the community as previously reported in another study in Tanzania (Nicastri *et al.,* 2009). This irrational use of antimalarials puts further pressure on constrained resources and may promote development of malaria drug resistance. The majority of the patients who attended the health facility had a history of having taken a drug, mostly an antimalarial drug.

Attendance to the health facility could signify a poor response to treatment given at home. Awareness of malaria by the people in this area is very high, as reflected by practicing protective measures against malaria, including the use of bed nets and self medication against malaria in case of fever. Although the quality of the bed nets was not clear (whether treated or untreated), yet the majority claimed to be sleeping under a mosquito net. Similar results of recognizing fever and linking it with malaria among the caregivers/parents has been reported from elsewhere in Tanzania, Malawi and Kenya (Kaatano *et al*., 2006; Chibwana *et al*., 2009; Ye *et al*., 2009). The consequences of interpreting all fevers as being malaria are that other causes of febrile illness in the community are not recognised, resulting in delay of treatment (Rowe *et al.,* 2006). Also it provides wrong statistics for malaria in terms of malaria morbidity and mortality.

Treatment of patients with antimalarials at the health facility depended on the following; clinical presentation, result of blood smear, history of fever and age of the patient. The majority of those smears positive were treated with antimalarials. Similarly, half of those with history of fever were treated with antimalarials. The majority of those <5 years were given antimalarials regardless of laboratory results. Fever in our study had 83% diagnostic sensitivity and 68% specificity. These findings were similar to findings by Msellem *et al*. (2009). Despite a negative blood smear result, three-quarters of the patients received antimalarial treatment. Similar results have been reported in elsewhere (Joshi *et al*., 2008; Fehintola & Balogun, 2010). This practice calls for investigating other causes of fever (D'Acremont *et al*., 2010).

While RDT is expected to decrease workload, increase cost-effectiveness and ensure accurate diagnosis of malaria, this test may not be applicable in an area with very low parasites circulating in the community as in this study area. Our findings indicate only 5 of the 32 individuals who were positive by microscopy, were also RDT positive. RDT sensitivity becomes low when parasite densities are below the level of 200 parasites/ μL or less than 0.002% of the red blood cells infected (WHO, 2010). Low parasite density of malaria observed in this study is likely to have contributed to the low sensitivity of the rapid test (Ochola *et al.*, 2006). A few individuals were who were RDT positive were blood smear negative. This discordance between RDT and microscopy is due to the tendency of the RDT to remain positive weeks after parasite clearance (Hawkes & Kain, 2007) due to persistence of malaria antigen in the circulation. This is likely to have resulted in the low sensitivity and high specificity observed in this study.

Serological markers can be used to determine the malaria transmission indices as observed in this study. The development of antibodies (and of acquired immunity) depends on the degree of exposure to infected mosquitoes. Application of the validated model relating age-specific seroconversion rates to EIR (Corran *et al.,* 2007; Stewart *et al*., 2009), in our study area resulted into classifying Magugu area as a low transmission intensity area. This estimate correlates with the results of an entomological survey carried out in the nearby areas (Mwanziva *et al.*, 2011). The low EIR in the area reflects the low number of immune individuals in the community as well as the low number of asymptomatic individuals (Shekalaghe *et al.,* 2007), who are reservoirs of infection. Overall and age-wise, there was a difference in the observed seroprevalence of antibodies to MSP-1_{19} and AMA-1 antigens, the latter having the highest seroprevalence. Similar seroprevalence differences between the two antigens have been reported in other studies (Drakeley et al., 2005). At the age of 2-10 years there was increase in seroprevalence (results not shown) in both antigens used. Similar observations have been reported in Kenya (Williams et al., 2005) indicating that adaptive immunity in children is acquired between 2 and 10 years of age. Contrary to the findings of Steward *et al*. (2009) we did not observe a clear correlation between seroprevalence and age in this study. This is likely to be explained by the low transmission in the study area. As reported by Osier et al. (2008) a higher antibody response is observed among individuals with concurrent microscopic parasitaemia.

Also, bed net usage by the community, hence lack of constant exposure, plays a role (Targett, 1999). In addition, the small sample size may have lead to uneven distribution of individuals in different age categories. Whether migration of people into the study area who have not been recently or prior exposed to malaria, may have played a role in our findings could not be confirmed. It is likely that the serological results were not due to recent infection (Drakeley *et al.*, 2005; Bousema *et al*., 2010). The low seroprevalence observed in the older age group could be explained by the slow sero-reversion rate of individuals of this age group (Drakeley et al., 2005). A similar observation has been reported by Stewart *et al.* (2009) in a low endemic area of northern Tanzania.

Since bed nets decrease human-vector contact, it has an effect on the infectivity rate of the mosquitoes and hence its chance to infect humans (Drame *et al.*, 2010). This results into slow development of protective immunity, and eventually into severe malaria. Several factors were associated with this phenomenon including changes in physical environment (like climatic

variation and land use) resulting into population displacement leading to importation of malaria in non-immune people (Suh, 2004).

In conclusion, Magugu area of northern Tanzania is of low malaria endemicity. However, there is substantial over-treatment, presumptive treatment, and self-treatment among the communities. Training of the medical personnel (Ssekabira *et al*., 2008) and community sensitization are necessary measures for the population to understand the burden of malaria in the area. Treatment of malaria should be based on microscopic confirmation, and where this is not possible, strictly designed clinical algorithms are needed so that treatment of malaria will become specific and precise. Use of RDT in areas where malaria endemicity is low, is of limited use.

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