

Children with suspected malarial infection admitted to Kolandoto Hospital, Tanzania

Exploring statistical methods

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Preface

This assignment is the result of nearly two months spent at Kolandoto Hospital in north western Tanzania.

In our fourth year of medical school we had clinical rotation at the Department of Infectious Diseases, Ullevål University Hospital in Oslo, Norway. There we met Dr. Eyrun Kjetland. Her enthusiasm for the field of Infectious Medicine and for the continent of Africa made our choice of obligatory assignment easy. We wanted to write an assignment based on a stay in a Tanzanian rural hospital, and dr. Kjetland agreed to be our supervisor.

Besides the opportunity to improve our medical skills and learning the process of scientific work, we would also get the chance to experience another continent with a different culture and life set.

Abstract

BACKGROUND: Severe malaria accounts for up to nearly 1 million deaths annually in under-fives in Africa. **AIMS:** To give a clinical and laboratory description of the under-fives admitted with suspected malarial infection in a rural Tanzanian hospital, and to learn the use of some statistical analyses and learn how to interpret our findings critically. **METHODS:** This study included 21 consecutive patients below the age of 5 years in whom malaria was suspected because of fever. Patients were clinically investigated and mothers questioned on related clinical aspects, and laboratory investigations on parasitaemia and anaemia were done. **RESULTS:** The study suggests that girls were underrepresented among the admitted patients and may indicate that guardians seeking help at this hospital waited longer with girls, than with boys. Furthermore, results indicate that guardians tried other care strategies for girls first. However, boys in this study seemed to have a more dramatic clinical picture on the time of admission. **CONCLUSIONS:** This study demonstrates a possible gender difference in health care seeking and malaria presentation. However, our study population was very small for quantitative analyses, and results must be interpreted with great caution, even when there are statistically significant associations. Further studies are required in order to reveal gender differences in health care seeking behavior in this area of Tanzania.

Introduction

Malaria is a protozoan infection of red blood cells transmitted by the bite of a blood feeding female anopheline mosquito [1]. The pathophysiology of malaria results from destruction of erythrocytes, the liberation of parasite and erythrocyte material into the circulation, and the host reaction to these events [1]. *P. falciparum* malaria-infected erythrocytes are also sequestered in the microcirculation of vital organs, interfering with microcirculatory flow and host tissue metabolism [1]. The most common symptom is fever, although malaria may present with general malaise, headache, vomiting, or diarrhoea [2]. Severe disease, often with high parasitaemia, may cause cerebral affection with diminished consciousness and convulsions, often progressing to coma and death [2].

Malaria is a major health problem in the world, and especially in Africa. Between 350 and 500 million people develop malaria each year [3]. More than one million Africans die each year, and most of these are children under five years of age [3]. Febrile malarial illnesses are a major source of hospital admissions in sub-Saharan Africa. The transmission of malaria occurs in most parts of the Tanzania [4]. Seasonal variations in transmission occur. Total mortality rate for under-fives is 165 per 1000 live births [5, 6]. The number of reported malaria cases, the admitted malaria cases and malaria deaths are not recorded by the Ministry of Health [4]. However, the Roll Back Malaria Baseline Survey in Selected Districts [7] shows that 38% of deaths among the under-fives are attributed to malaria. Furthermore, a study reports that only 53% of under-fives with malaria are managed in accordance with WHO guidelines in health facilities, and only 11% of under-fives with fever/malaria receive correct treatment within 24 hours of onset of fever [7].

The aim of this study was two-fold. Firstly; over a short time span to give a clinical and laboratory description of the under-fives admitted with suspected malarial infection in a rural Tanzanian Hospital. Secondly; to learn the use of some statistical analyses and learn how to interpret our findings critically.

Materials & Methods

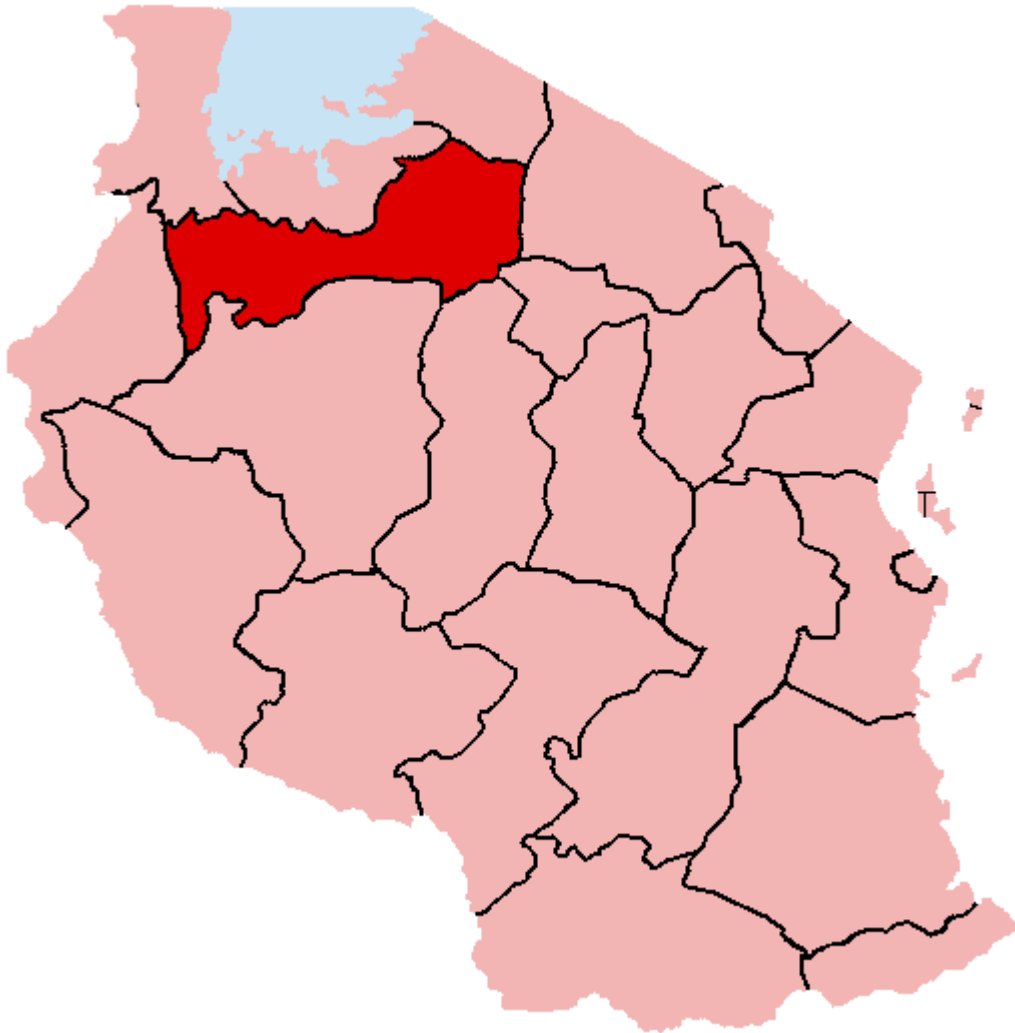
Study Area & Population

Our study was done in northwestern Tanzania at Kolandoto Hospital which is a missionary hospital in Shinyanga region. Shinyanga region, south of Lake Victoria has a dry climate. The hospital is surrounded by bush and light woods. There are two rainy seasons per year; “long rains” from March to May, and “short rains” in November and December. The predominant tribes of the Shinyanga region are the Sukuma, Nyamwezi and Sumbwa tribes. Traditional agriculture is maize, cotton, and rice production [8]. There are also some diamond mines in the area that employ part of the working stock, both men and women.

Kolandoto Hospital shares encatchment area with Shinyanga Regional Hospital and Mwadui Hospital, both within 15 km of Kolandoto. According to the 2002 Tanzanian National Census the population of Shinyanga Region is 2,796,630. The regional capital, Shinyanga town is situated 15 km south of Kolandoto Hospital, and has approximately 135,000 citizens. The average household size in the region is 6.3 persons [9].

Figure 1

Map of Tanzania with Shinyanga region outlined



The total bed number at the hospital is 186, with the following wards: Out Patient, Male, Female, Maternity, Paediatric, Private, Intensive Care, Leprosarium and Ophthalmology. The total number of workers is 115. There are 5 doctors, 4 clinical officers and 68 nurses. In the laboratory there is one laboratory attendant, one technologist, two laboratory technicians and one laboratory assistant. Attached to the hospital is a school for nurses and laboratory assistants. The students participate actively in the daily work at the hospital. In 2004 5602 patients were admitted to the hospital [10]. Of these admissions, 1277 (23 %) were in the Paediatric Ward [10]. Malaria, anaemia and cataract were the three most common diseases in in-patients departments [10].

Criteria for Inclusion

All patients admitted to the Paediatric Ward at Kolandoto Hospital with temperature above 38.0 degrees Centigrade were included if parents were willing to allow a clinical investigation and to be interviewed. Patients at this ward were all below 5 years of age. The inclusion period was from May 25th to June 8th 2005.

Data collection and clinical investigation

There were 3 means of collecting information; the interview, from the case journal and by clinical investigation. Parents of all included patients were interviewed and examined on admission. A parent (usually the mother accompanied the child) was asked about the duration of fever, co-existing symptoms and whether the children had received any treatment before admission and in that case what kind of treatment. The information was mostly collected with the assistance from a Clinical Officer who also interpreted from the local language.

The clinical examination included measurement of axillary temperature, abdominal palpation and percussion for hepatomegaly and splenomegaly, inspection for jaundice and auscultation for lung crepitations. We also registered whether the children received anti-malarial treatment on admission. However, further development of symptoms, signs, laboratory results and treatment after admission were not recorded.



Laboratory methods

Blood samples were obtained prior to treatment. The test results were available the same day, or the next. Results were recorded in a laboratory book, and registered in the patient's record.

The parasitaemia (parasites / μL (microliter)) was determined on a modified Giemsa (Field-stained [®] thick film, details in Appendix 1) Parasites per 200 leukocytes was counted, and density per μL calculated assuming a leukocyte count of 8000/ μL .

The haemoglobin level was determined using a colorimeter (Jenway 6030 [®], details in Appendix 2). Anaemia was defined as a level of haemoglobin <11 g/dL

(gramme per deciliter), and severe anaemia as <6 g/dL; this was also the hospital's cut off-value for blood transfusion.

Ethical considerations

Permission was granted by the Medical Doctor in Charge of Kolandoto Hospital. The investigations were conducted under the supervision of the ward's paediatrician. Patients were included in the study following their guardians' free and informed oral consent to clinical and laboratory investigations, and the guardians' free and informed oral consent to be interviewed.

Statistical Investigations

The statistical analysis was computed using Statistical Package for the Social Sciences (SPSS) version 13.0. Fischer's Exact test (Chi-square), Mann Whitney and Spearman Rank tests were applied, with statistical significance designated as $p < 0.05$.

Results

This is a small study, and we will interpret the results with caution. The weaknesses of the study and sources of error will be discussed later.

5.1. Characteristics of the study population

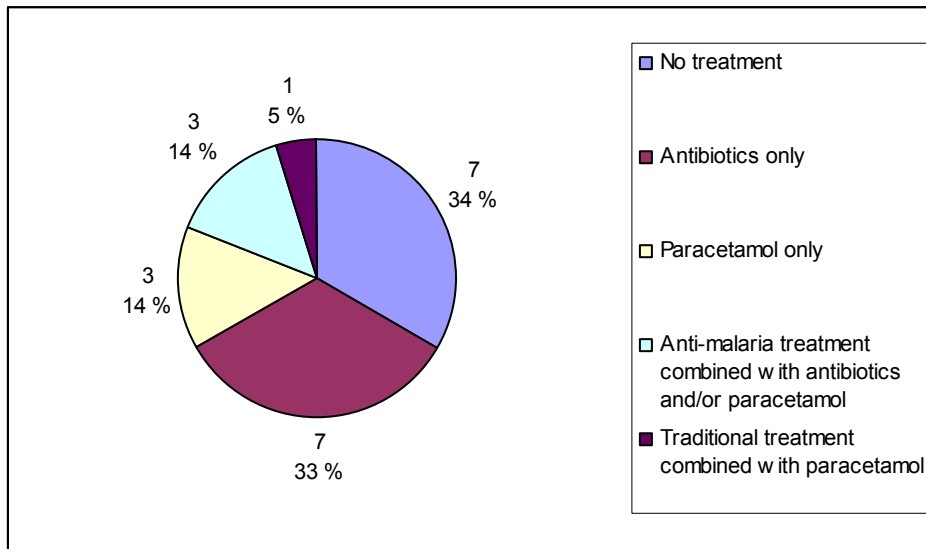
This study included 21 consecutive patients below the age of five years. Of these 38% (8/21) were girls; 95% (20/21) of the patients were younger than 3 years of age. Temperature on admission ranged from 38.0 to 40.7 degrees Centigrade, with a median of 39.0 degrees Centigrade. The duration of fever before admission ranged from 1 to 14 days, with a median duration of 3 days. All the 21 guardians who were approached agreed to participate in the study. No treatment was delayed for the sake of the study. All included patients were given quinine on admission. None of the study population passed away in the study period.

In this study population the two most common co-symptoms besides fever prior to arrival at the hospital were cough (62%, 13/21) and vomiting (38%, 8/21). Nineteen percent (4/21) had a history of convulsions, and 10% (2/21) had had diarrhea. On clinical examination 19% (4/21) had splenomegaly, 10% (2/21) had lung crepitations, 5% (1/21) had jaundice, and none had hepatomegaly.

Treatment given outside of the hospital prior to admission was registered. In total 67 % (14/21) had received some kind of treatment. Figure 2 shows the distribution of treatment types.

All patients but one had parasitaemia on admission. The parasite density ranged from 40 to 20000 parasites per μL , with a median of 400 parasites per μL . All patients were anaemic, with a mean haemoglobin level of 5.9 g/dL (Standard deviation of 1.77). Forty eight percent (10/21) had grave anaemia.

Figure 2



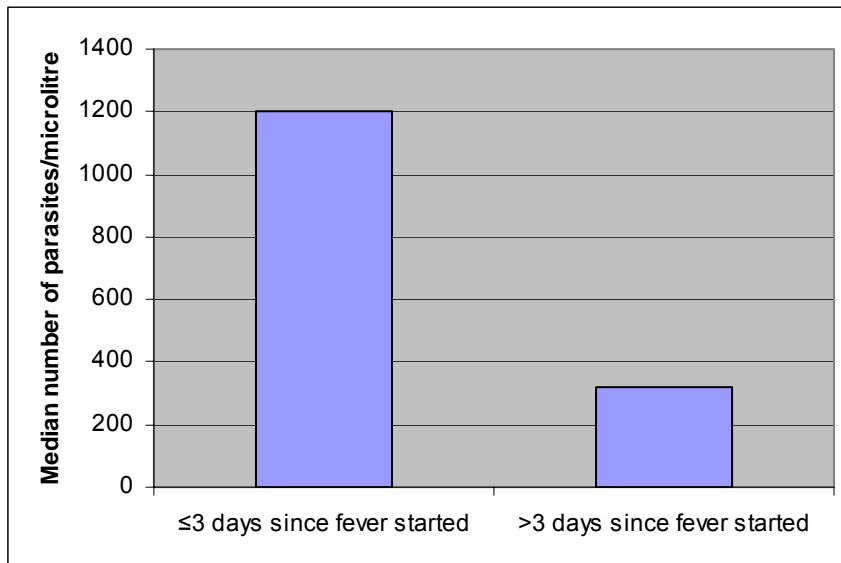
5.2. Factors associated with high parasite load

As indicated in Figure 3 there was a strong significant correlation between high parasite load and short time since fever started ($p=0.001$). Patients who had not received anti-malaria treatment prior to admission had a non-significant tendency of higher parasite load ($p=0.14$).

Furthermore, there were no associations between high parasite load and any of the co-symptoms or clinical signs, besides a tendency towards significant association between jaundice and high parasite load ($p=0.13$). In this study we found no correlation between the parasite load and haemoglobin levels ($p= 0.59$).

Figure 3

Parasite load compared to time since fever started.



5.3. Gender differences in the study population

Table 1 show that female patients had more often been treated at home prior to hospital admission. They also had a significantly longer duration of symptoms before admission. Male patients had a significantly higher parasite load compared to female patients, and a tendency towards significantly lower haemoglobin values. Furthermore, female patients showed fewer clinical signs on admission compared to male patients. However, there was no significant gender difference in temperature on admission.

Table 1

Clinical and laboratory findings in relation to gender in children under the age of 5 years admitted to Kolandoto Hospital.

Parameter	Male	Female	P-value ^a
Mean age in years	1.5 (SD = 0.66)	1.4 (SD = 1.30)	0.39
Median duration of fever (days)	3 (1-7)	5 (3-14)	0.023
Median parasite load (parasites/microlitre blood)	400	160	0.045
Treatment prior to hospital admission	6/13 (46 %)	8/8 (100 %)	0.018
Anti-malarial treatment prior to hospital admission	0/13 (0 %)	3/8 (38 %)	0.042
Antibiotics prior to hospital admission	3/13 (23 %)	6/8 (75 %)	0.032
Paracetamol prior to hospital admission	3/13 (23 %)	3/8 (38 %)	0.63
Traditional treatment (herbs) prior to hospital admission	0/13 (0 %)	1/8 (13 %)	0.38
Mean temperature on admission (degrees Centigrade)	39.4	39	0.31
Cough	8/13 (62 %)	5/8 (63 %)	1
Vomitting	3/13 (23 %)	5/8 (63 %)	0.16
Diarrhea	1/13 (8 %)	1/8 (13 %)	1
Convulsions	3/13 (23 %)	1/8 (13 %)	1
Splenomegaly	4/13 (31 %)	0/8 (0 %)	0.13
Hepatomegaly	0/13 (0 %)	0/8 (0 %)	
Jaundice	1/13 (8 %)	0/8 (0 %)	1
Lung crepitations	1/13 (8 %)	1/8 (13 %)	1
Grave anaemia ^b	7/13 (54 %)	3/8 (38 %)	0.66
Mean haemoglobin value in g/dL	5.4 (SD = 1.76)	6.8 (SD = 1.47)	0.11

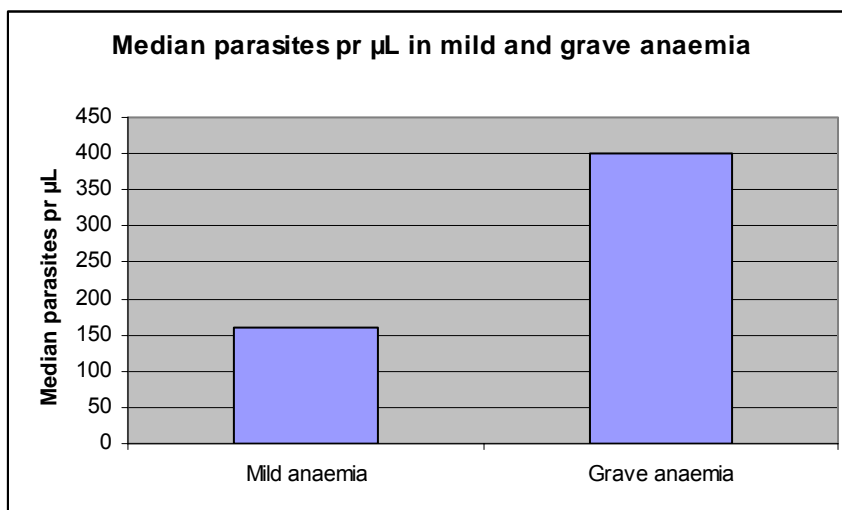
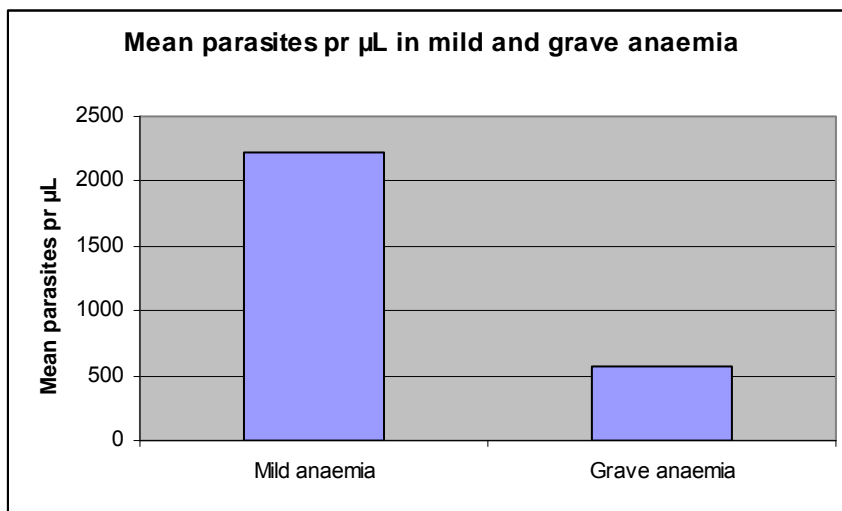
^a Continuous variables were tested using Mann Whitney. Dichotomous variables were tested using Chi-Square, in cases where a variable was <5 Fisher's Exact Test was applied.

^b Haemoglobin level below 6 g/dL

5.4. Mean versus median values

Figure 4 shows how the bars differ choosing mean or median values. These graphs are included in the report only to clarify our discussion of mean versus median values, and we found no significant association between parasite density and grave anaemia.

Figure 4



Discussion

Although the study shows a number of significant and interesting results, especially concerning gender differences, the study is small and results are prone to Type 1 and Type 2 errors. Hence the main emphasis of this chapter will be a discussion of the weaknesses of the study. But first, we will discuss the results, also in relations to other studies.

The study suggests that girls were underrepresented among the admitted patients and may indicate that guardians seeking help at this hospital waited longer with girls, than with boys. Furthermore, results indicate that guardians tried other care strategies for girls first. However, boys in this study seemed to have a more dramatic clinical picture on the time of admission. The current study is too small to make conclusions about gender differences in health care seeking or morbidity in children, however other studies partially confirm our findings. Tursz and Crost did an epidemiologic study of health care seeking behaviour of children under 5 years of age by sex in Algeria, Togo and Congo [11]. They found that girls were underrepresented among outpatients and had longer duration of the development of symptoms contacting the health services, leading to increased severity of symptoms. The latter was contrary to findings in our study where boys were more ill. Another study, from low-income communities in India, shows a lower rate of care-seeking for girls despite the fact that there was a higher mortality for girls than for than boys [12]. In contrast others have not found gender differences in care-seeking behaviour. A study done in southern Tanzania found no association between sex and any indicator of morbidity or care-seeking, suggesting that boys and girls were treated similarly by carers [13]. The different findings may well be caused by socio-cultural differences between the study regions. Hence, the study done in southern Tanzania may be more similar to the current study population although we cannot confirm this.

The results indicate a strong correlation between high parasite load and short duration of fever. We hypothesize that high parasite load gave more worrying symptoms impelling the guardians to seek hospital sooner. However this study reveals no correlation between high parasitaemia and the more prominent clinical

features. Other studies however have shown that symptoms and signs increase with increasing parasitaemia [14-17]. Our findings may represent a Type 2 error.

In severe disease anaemia may develop rapidly; haemolysis of non-infected erythrocytes being the major contributor to this [1]. However, in this study there was no correlation between the parasite load and haemoglobin levels, confirming that malarial anaemia has been found with both high and low parasitaemia [18, 19]. This is controversial and other studies show that haemoglobin decreases with increasing parasite density [20-23].

There are many weaknesses of our study that one must have in mind before drawing conclusions. The data were collected in the course of a few weeks; hence season-dependent factors probably influence our data. Furthermore, our study population was very small for quantitative analyses, and results must be interpreted with great caution, even when there are statistically significant associations.

It may be difficult and complex to clinically define and diagnose malaria, because symptoms are non-specific and because asymptomatic parasitaemia is common in endemic areas. In our study, we chose to include all admitted, febrile children, and defined clinical malaria as fever with any parasitaemia. This however, seems as an oversensitive strategy, and is one of the weaknesses of the study. Mwangi et al. [24] have applied logistic regression methods to derive case definitions of malaria. In children less than 1 year of age clinical malaria was defined as fever accompanied by any parasitaemia. In older children (1-15 years of age) the clinical diagnosis was defined as fever accompanied by a parasitaemia of ≥ 2500 parasites/ μL blood. If we were to use these definitions in our study, most of the cases perceived as clinical malaria would in fact not have been that.

One consequence of this being a small group is the potential difference in mean and median values because of single values being extreme compared to the other population of values. In our material we had two such extreme values. One patient had as many as 20000 parasites pr μL . The other 19 of the 20 patients with parasitaemia had parasites load in the range of 40-2000 parasites pr μL . The mean value was 1440 parasites pr μL , whereas the median value was 400 parasites pr μL .

Another patient had fever duration of 14 days, whereas the 20 others had fever duration of 1-8 days, most only a few days. Here there is also a difference in mean and median values, being 4 days and 3 days respectively. Our opinion is that the median values in these two parameters (parasite density and fever duration) give a much more realistic picture of the group, and therefore we have chosen to use the median instead of mean value when calculating.

Other weaknesses are as follows: Because we knew only a little Swahili and no Sukuma, we required an interpreter during the interviews. This made the interview situation more stressful, and increased the chance of misunderstandings. The fact that we were whites may also have contributed to this stress. We recorded the patients' age in years. Ideally, age should have been recorded in months to make the analysis more accurate. However, most admitted patients had no birth certificate, and guardians were usually uncertain of the birth date. We measured the axillary temperature on admission, and ideally, rectal temperature should have been measured. However, axillary temperature measurement was the standard procedure at the hospital, and we decided not to change this routine during the study period. Furthermore, most families had no thermometer at home, so the exact time since fever started was difficult to tell. Also, because the time perception proved to be different to ours, the presented duration of symptoms must be considered unreliable. Hence we cannot preclude that social factors made guardians reported a longer duration of symptoms in the girls before bringing them to the hospital. Furthermore, in order to draw conclusions about parasitaemia on admission, pre-admission treatment, dosage and effect of anti-malarial treatment should have been recorded. Parasite numbers and haemoglobin levels were run in the laboratory by local staff and we did not run an extra quality control, hence mistakes and technical difficulties cannot be ruled out.

Conclusions

No conclusions about causality can be made from the results of this study, and it provides at best descriptions of fever illness/malaria as a health problem for children in this area. Despite its limitations this study demonstrates a possible gender difference in health care seeking and malaria presentation. Literature searches reveal that few studies have addressed illness in African children by gender. More focus may be needed on this subject, and should be discussed in future surveys on malaria and health care seeking behaviour.

This report reveals that our study is small and weak in many ways. However, this study could possibly be used as a pilot study for further surveys in this area.

On a personal level, our stay at this rural hospital in Tanzania has given us invaluable experiences that will help us in our careers as doctors.



Acknowledgements

Firstly, we thank our supervisor Dr. Eyrun Kjetland for invaluable goodwill, inspiration and guidance.

We express our gratitude to the children and parents who participated in this study and the many people that assisted with this project. The authors thank in particular Dr. Mwandu for his hospitality and encouragement in the field.

Financial support was provided by the Centre for Imported and Tropical Diseases at the Department of Infectious diseases, Ullevål University Hospital, Skipsreder Tom Wilhelmsens Stiftelse and the Norwegian State Educational Loan Fund.

In accordance with the regulations of our department our funders made it possible to contribute a digital projector to the hospital in Kolandoto. This is meant for use in teaching staff and students at the hospital. Furthermore the report will be sent to the study site and we are committed to using what we have learnt to the benefit of future patients.

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Appendices

A. 1. Field's staining method for thick blood films

Malaria parasites were detected by microscopy in all patients. The equipment used was distilled water and two solutions named Field's stain A and B ®. A thick film of capillary blood was placed on a slide and dried, either by air or in an oven. The slide was dipped in Field's stain A 5-10 times, and then dipped in distilled water. Thereafter dipped in Field's stain B 5-10 times, and again dipped in distilled water. This process made the erythrocytes lyse. The parasite load was graded, according to the local standards, by the number of parasites per 200 leukocytes seen at high-power [100 X with oil] magnification. Assuming a leukocyte density of 8000 per μL , the parasite density per μL was calculated.

Recipe for Field's Solution # 1

1. Dissolve 1,6 g of methylene blue in 1 litre of distilled water
2. Dissolve 2,6 g of Na_2HPO_4 [anhydrous] to the solution from step 1
3. Dissolve 1 g of Azure 1 in the solution from step 2
4. Dissolve 2,6 g of KH_2PO_4 in the solution from step 3
5. Place on mild heat with stirring or shaking for 45 minutes to 1 hour
6. Let stand at room temperature for 24 hours
7. Filter

Recipe for Field's Solution # 2

1. Dissolve 2 g of Eosin Y in 1 litre of distilled water
2. Dissolve 2,6 g of Na_2HPO_4 in the solution from step 1
3. Dissolve 2,6 g of KH_2PO_4 in the solution from step 2
4. Filter

A. 2. Measurement of haemoglobin

Haemoglobin was measured using a calibrated Jenway 6030 colorimeter. The colorimeter was calibrated against a fixed sample consisting of 5 ml 100% Drabkin solution before each procedure. Twenty mcg capillary blood was mixed in 5 ml Drabkin solution and stored for 5 minutes, where after optical reading was done. A value was given and interpreted using a standard table and thereby giving the haemoglobin value in grams per decilitre.

The Drabkin Solution contained 200mg potassium ferric cyanide, 50 mg potassium cyanide, 1 g sodium bicarbonate and 1 litre distilled water.

A.3. Certificates



Human Participant Protections Education for Research Teams

Completion Certificate

This is to certify that

Marte Eline Ween-Velken

has completed the **Human Participants Protection Education for Research Teams** online course, sponsored by the National Institutes of Health (NIH), on 04/17/2007.

This course included the following:

- key historical events and current issues that impact guidelines and legislation on human participant protection in research.
- ethical principles and guidelines that should assist in resolving the ethical issues inherent in the conduct of research with human participants.
- the use of key ethical principles and federal regulations to protect human participants at various stages in the research process.
- a description of guidelines for the protection of special populations in research.
- a definition of informed consent and components necessary for a valid consent.
- a description of the role of the IRB in the research process.
- the roles, responsibilities, and interactions of federal agencies, institutions, and researchers in conducting research with human participants.

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