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ORIGINAL ARTICLE

Diagnostic Accuracy of Thin Film in Detection of Scanty Parasitaemia Malaria Taking Thick Film as Gold Standard

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ABSTRACT

Background: Analyzing a blood film is the most accurate approach to determine the number of parasites present. People question which blood film and counting method is the most accurate and can be used again and over again.

Objective: To see if thin peripheral blood smear films may identify malaria with little parasitaemia, thick peripheral blood smear films were used as the gold standard.

Material and Methods: The study was conducted by the National Institute of Child Health in Karachi's Department of Paediatric Medicine and was a cross-sectional one. 177 people were admitted to the hospital. A vein yielded about 5 milliliters of blood for the experiment. As long as there was a single control band and two testing bands, the test result was positive for both P. falciparum and P. viridans. When the control band and the test band were found to be present, the test verified the presence of P. falciparum. Results were deemed negative if neither the test nor control bands appeared. Comparison of thin peripheral blood smear accuracy to the gold standard of thick peripheral blood smear accuracy was used to assess the thin blood smear diagnostic accuracy.

Results: Sixty-one percent of the patients were men, and only 39 percent were women. The average age was 30 years and 15 months. The P.falciparum was 66.0%, the P.vivax was 22.7%, and the P.ovale was 11.3%. With a thick peripheral blood smear film, 52.5 percent were found to have scanty parasitaemia malaria, and 48 percent were found to have it with a thin peripheral blood smear film. The accuracy, specificity, and sensitivity were 95.3%, 87.5%, and 90.9%, respectively.

Conclusion: The results of the study showed that thin blood smear films are accurate diagnostic tools 90.9% of the time. It could be used as a second way to confirm a diagnosis of malaria.

Keywords: Diagnostic Accuracy, Thin Peripheral Blood Smear Film, Parasitaemia Malaria, Thick Peripheral Blood Smear Film

INTRODUCTION

All around the world, malaria is a major problem. One of the greatest causes of death and illness in individuals of all ages, including children, it is found in more than a hundred countries. Infants and children under the age of two are particularly vulnerable to the disease's effects in tropical climates. Around 216 million people were infected with malaria in 2010. [1]

174 million (113–239 million) of the total cases were reported from the African Region, which accounted for almost 81% of the total. An additional 13% of the total came from the South-East Asian region.[2] Falciparum malaria is found in 42,5 of every 1000 feverish children worldwide, despite the fact that P. vivax malaria is the more common type. 59 percent of the world's clinical malaria cases are found in Africa; 38 percent are found in Asia, and 3 percent in the United States. [2]

More than a million people die every year as a result of malaria, which affects an estimated 300 million to 500 million people annually. As a result, malaria is the most important problem for those living in poverty today.

[3] In 2013, it was estimated that 103 countries and territories throughout the world had a malaria risk. There are four of them. A substantial increase in funding is still required, however, to meet the WHO's ambitious targets of decreasing the number of people infected by malaria and eliminating the illness completely. The WHO Global Technical Strategy for Malaria 2016–2030 [6]

P. falciparum, P. vivax, P. malariae, and P. ovale are all parasites that can cause malaria in humans. There are two main types of malaria parasites: P. falciparum and P. vivax, with the former being the deadlier. Prognosis is generally good for youngsters, but those who are still young when they receive a diagnosis may have more difficulty dealing with it. [7] Researchers rarely study the frequency of malaria among children. Some areas of Sindh and Balochistan are estimated to have a higher prevalence of P. falciparum than other areas of Pakistan. [8]

Depending on whether you live in a "high risk" or a "low risk" area, the criteria under IMCI for treating malaria are different. The World Health Organization sets these guidelines. As a result of conducting Pakistani prevalence research, the process of drafting plans will be streamlined. [8] A diagnosis based on parasites should be used in all cases when malaria is suspected, according to the World Health Organization (WHO). [9] An correct diagnosis is essential for both patient management and surveillance in the fight against malaria. However, there are a lot of different ways to find parasites, and these approaches vary in their ease of use, reliability, and cost. [10]

Even though it is time consuming and necessitates the knowledge of trained specialists, looking at blood smears stained with Giemsa under a microscope is the most accurate method of determining whether or not a person has malaria.

[12, 11] In spite of advances in molecular diagnostics, the majority of clinical laboratories still rely on blood film examination to establish whether or not a patient has malaria and to determine how many parasites are present in the blood. This is due to the fact that only a blood film examination can tell you how many parasites are in your system. The question of which blood films and counting processes produce the most accurate numbers and which are the easiest to use repeatedly begs to be answered. [12]

Microscopy is used to assess if a person has malaria by looking at peripheral blood smears (PBS). This is the most common approach. It has been feasible to discern the several phases of the plasmodial parasite since thick PBS was introduced in 1903. Aside from these disadvantages, the approach is difficult, time-consuming, and requires the expertise of a microscopist to identify parasites at low levels of parasitemia accurately. [13]

However, when there were fewer than 500 parasites per microliter, the results from the thin film approaches were no longer valid since they did not account for parasite density. The thick film method, on the other hand, could be used even if the liquid sample contained less than 500 parasites.

Study found that thin smear was 66.22% sensitive and 100% specific in comparison to the gold standard, the thick smear. [14] 33.8 percent of cases of malaria were found in thin peripheral blood smears, while 35.0 percent of cases were confirmed by a thicker peripheral blood smear.

[15] More than two-thirds of instances of malaria parasites were found when thick blood smears were employed, whereas only one-fifth of those cases were found using thin peripheral blood smears. For the condition in issue, the thin smear had a specificity of 98 percent and a sensitivity of only 72 percent. [13]

Malaria makes it difficult for doctors all over the world to pinpoint the source of a patient's symptoms. The goal of this study is to discover how effective thin films are at detecting malaria in people with low parasite counts. We'll use the same patients for thick and thin blood smears, as well as an antigen detection test, to see how accurate thin films are as a malaria diagnostic tool. If peripheral blood smears with thick and thin films were equally successful at diagnosing malaria, we could create a strategy to instruct and assure that all children exhibiting symptoms of malaria get a blood smear test. Diagnoses might be made faster, allowing patients to obtain therapy faster and for less money.

METHODOLOGY

The Department of Paediatrics Medicine at the National Institute of Child Health in Karachi served as the study's site. The following formulas were used to arrive at the final sample size: A 9 percent margin of error for sensitivity and 2 percent for specificity resulted in a sensitivity and specificity of 72 and 98 percent, respectively, for thin films in malaria. For this study, the prevalence was estimated at 38 percent and the confidence level at 95 percent. There were a total of 177 participants in the study. Researchers used non-probability consecutive sampling as a method for conducting their study.

The investigation was able to get begun following authorisation from CPSP. If patients presented themselves to the National Institute of Child Health in Karachi's Department of Paediatrics Medicine and met the inclusion criteria, they were eligible for participation in the study. After hearing about the study's goals, participants gave their consent to participate. These people each had about 5 milliliters of blood taken from a vein and sent to the laboratory when they experienced the highest temperature. Both thick and thin blood smears were made using the same process. Leishman's stain was used to color the streaks. Approximately 80-100 oil immersion fields were examined for the purpose of reporting in 8-10 minutes. To make a diagnosis, all of the patients were given thin peripheral blood smears. Antigen detection tests were done on each of the smears to see how they performed. Plasmodium LDH was found using an antigen detection kit that may be purchased. Non-coagulated blood was utilized in the test.

For P. falciparum, P.vivax/malaria/ovale, and P. falciparum, the test was regarded positive if only one control band and two test bands were present; it was considered negative if only one control band and no test band were present. We employed thick peripheral blood smears as the gold standard for establishing the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of thin peripheral blood smears. The data was recorded on a pre-created proforma by a primary investigator.

The SPSS statistical tool, version 21, was used to gather and analyze the data. Qualitative factors such as gender, the detection of thin and thick peripheral blood smears for malaria, and the types of malaria were analyzed in terms of frequency and percentage (plasmodium Falciparum, P. Ovale, P. Vivax). Displayed were the mean and standard deviation of the quantitative data. It is important to note that these characteristics include age and how long an illness has been in existence. We employed thick peripheral blood smears as the gold standard for establishing the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of thin peripheral blood smears. Stratification was used to take into account parameters like gender, age, severity of malaria, and different kinds of malaria (plasmodium Falciparum, P. Ovale, and P. Vivax). Prior to stratification We employed thick peripheral blood smears as the gold standard for establishing the sensitivity, specificity, positive predictive value, negative predictive value, and overall diagnostic accuracy of thin peripheral blood smears.

RESULTS

For the study, 177 children aged from one month to five years old had to meet certain criteria in order to be eligible for the study. Researchers conducted the study to see if thin peripheral blood smear films could diagnose sparse parasitaemia malaria as reliably as thick peripheral blood smear films, which are the gold standard in the field. A statistical application developed for use in the social sciences, SPSS 21, was used to collect and analyze data. When calculating quantitative data, the mean and standard deviation were used, whereas frequency and percentage were used for the qualitative variables. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy of thin peripheral blood smear film were calculated using thick peripheral blood smear film as the gold standard.

There were a total of 177 children, with 61% being male and 39% female. The average age of the youngsters was between 30.34 and 15.76 months. In order to further categorize the individuals, we used their ages to do so. The average duration of symptoms was found to be 5.911.81 days. Patients were then divided into two groups based on the length of time they had been experiencing their symptoms. Malaria parasites in a single milliliter of blood averaged 359.6369.52 parasites per milliliter. 66% of the 93 patients with malaria tested positive for P. falciparum, 22.7 % of those tested positive for P. vivax, and 11.3 % of those tested positive for P. ovale, all of which were proven to be parasites. A thick peripheral blood smear film was used to diagnose Scanty parasitaemia malaria in 52.5% of patients, whereas a thin peripheral blood smear film was used to diagnose malaria in 48% of cases. Scanty parasitemia malaria can be detected using thin peripheral blood smear film because of its high diagnostic specificity, sensitivity, predictive value, and diagnostic accuracy. In these computations, the thick peripheral blood smear film was used as the gold standard. There were 81 patients who tested positively and were given an accurate diagnosis, while there were 80 patients who tested negatively and were given an accurate diagnosis, according to the findings of this study.

Table 1: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Male (n=108)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	47 (95.9)	2 (4.1)	49	0.000*
Negative	6 (10.2)	53 (89.8)	59	
Total	53	55	108	
Sensitivity	Specificity	Ppv	Npv	Accuracy
95.9%	89.8%	88.7%	96.4%	92.6%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

Table 2: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Female (n=69)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	34 (94.4)	2 (5.6)	36	0.000*
Negative	6 (18.2)	27 (81.8)	33	
Total	40	29	69	
Sensitivity	Specificity	Ppv	Npv	Accuracy
94.4%	81.8%	85%	93.1%	88.4%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

There was a 95.3 percent sensitivity, 87.1 percent specificity, 95.2 percent positive predictive value (PPV), and 90.93 percent accuracy level. Additional characteristics were gender, age, symptoms, and the type of malaria that a person was suffering

from. A thin peripheral blood smear film has a high sensitivity, specificity, predictive value, and diagnostic accuracy. There are nine tables in all, starting with Table 1 and ending with Table 9.

Table 3: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Patient With Age≤24 Months (n=69)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	27 (90)	3 (10)	30	0.000*
Negative	2 (5.1)	37 (94.9)	39	
Total	29	40	69	
Sensitivity	Specificity	Ppv	Npv	Accuracy
90%	94.9%	93.1%	92.5%	92.7%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

Table 4: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Patient With Age>24 Months (n=108)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	54 (98.2)	1 (1.8)	55	0.000*
Negative	10 (18.9)	43 (81.1)	53	
Total	64	44	108	
Sensitivity	Specificity	Ppv	Npv	Accuracy
98.2%	81.1%	84.4%	97.7%	89.8%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

Table 5: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Patient With Malaria Symptoms≤3 Days (n=43)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	17 (89.5)	2 (10.5)	19	0.000*
Negative	4 (16.7)	20 (83.3)	24	
Total	21	22	43	
Sensitivity	Specificity	Ppv	Npv	Accuracy
89.5%	83.3%	81%	90.9%	86.04%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

Table 6: Diagnostic Accuracy Of Thin Film In Detection Of Scanty Parasitaemia Malaria Taking Thick Film As Gold Standard For Patient With Malaria Symptoms>3 Days (n=134)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	64 (97)	2 (3)	66	0.000*
Negative	8 (11.8)	60 (88.2)	68	
Total	72	62	134	
Sensitivity	Specificity	Ppv	Npv	Accuracy
97%	88.2%	88.9%	96.8%	92.5%

Chi square test was applied. P-Value ≤0.05 considered as significant. * Significant at 0.05 levels.

Table 7: diagnostic accuracy of thin film in detection of scanty parasitaemia malaria taking thick film as gold standard for patient with p. Falciparum malaria (n=64)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	59 (98.3)	1 (1.7)	60	0.795**
Negative	4 (100)	0 (0)	4	
Total	63	1	64	
Sensitivity	Specificity	Ppv	Npv	Accuracy
98.3%	0%	93.7%	0%	92.18%

Chi square test was applied. P-Value ≤0.05 considered as significant. **Not Significant at 0.05 levels.

Table 8: diagnostic accuracy of thin film in detection of scanty parasitaemia malaria taking thick film as gold standard for patient with p. Vivax malaria (n=22)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	16 (94.1)	1 (1.6)	17	0.579**
Negative	5 (100)	0 (0)	5	
Total	21	1	22	
Sensitivity	Specificity	Ppv	Npv	Accuracy
94.1%	0%	76.2%	0%	72.7%

Chi square test was applied. P-Value ≤0.05 considered as significant. **Not Significant at 0.05 levels.

Table 9: diagnostic accuracy of thin film in detection of scanty parasitaemia malaria taking thick film as gold standard for patient with p. Ovale malaria (n=11)

	Thick film			P-value
Thin film	Positive	Negative	Total	
Positive	6 (75)	2 (25)	8	0.338**
Negative	3 (100)	0 (0)	3	
Total	9	2	11	
Sensitivity	Specificity	Ppv	Npv	Accuracy
75%	0%	66.7%	0%	54.5%

Chi square test was applied. P-Value ≤0.05 considered as significant. **Not Significant at 0.05 levels.

DISCUSSIONS

In clinical trials, innovative diagnostic tests, epidemiological studies, and clinical treatment, light microscopy is regarded the "gold standard" for detecting malaria. The flaws of microscopy are well-known, even among scientists. 15-17 The amount of education, experience, and motivation of the person conducting the microscopy, as well as the laboratory's equipment, all play a role in the accuracy of the results. Despite the availability of modern technologies such as malaria rapid diagnostic tests (RDTs) and PCR, no clinical trial has shown that either one is useful. When the blood contains a large number of parasites, RDTs are more likely to give false negative results. Plasmodium falciparum can only be detected using RDTs based on HRPII, which are more reliable and cost less money. Due to new techniques' limitations, microscopy will continue to play an important role in clinical trials for many years to come. Additional to this, it will continue to be used as an evaluation tool for new diagnostic devices. [18]

False-positive malaria smears led to serious problems in two distinct Phase III clinical trials that took place on different continents (unpublished observations). False positive smear tests may be caused by interpreting thick films of malaria, according to current research. [19–22]: People are working hard to identify diagnostic approaches that can be combined to reduce or eliminate this issue.. Thin-film examination of positive smears could be an option if they need to be viewed rapidly before the research subject is treated. This is especially true if the results need to be re-checked. [23]

Depending on where you are, there are many different ways to apply the malaria thin film. Once malaria has been found on the thick film, people are usually instructed to use the thin film for correct species identification and for counting high density parasitaemia. After the thick film has been studied, this is done. Thin films, on the other hand, are rarely investigated and, in many cases, are not even manufactured in poor nations. Thin films aren't used by microscopists because they take too long and aren't sensitive enough to detect low parasite densities. [23]

Laboratory workers in the West, on the other hand, rarely utilize thick film. Instead, they base their findings purely on a study of the thin film. The thin film was chosen in this case because it is thought to be easier to both make and understand.. A three-minute reading of a thick smear is similar to a ten-minute reading of a thin

film, according to Dowling and Shute's well-known study. According to Dowling and Shute, Plasmodium ovale can be found more easily with the thin film. As a further discovery, they found that the thick smear caused between 60 and 90 percent of the parasites to die. [15]

When the results of the thick film were positive, it was necessary to calculate the minimum parasite density required to prove the existence of parasites and species in thin film. As a result, the accuracy of malaria prevention trials can be significantly affected by false-positive thick smears [19–22] and very low rates of false positive malaria smears. Two malaria preventive trials that used routine weekly screening and microscopy at the time of malaria symptoms to confirm parasitaemia obtained histories of parasite density. The presence of parasitaemia was confirmed using one of these procedures. Plasmodium falciparum infections were found in 95% of non-immune study subjects [23-25], while Plasmodium vivax infections were found in 83%, 54%, and 30% of non-immune study subjects. Some 62%, 54%, and 30% of an adult Kenyan population who were only partially immune to malaria had more than one hundred parasites per milliliter in their bloodstream, respectively. Malaria prevention trials routinely collect hundreds of samples, yet only a small percentage of those samples are positive. Using the thin film as a teaching tool, microscopists might be educated to always look at the thin film if the density is expected to be positive. These calculations provide a positive result if there are 5 or 8 parasites per 200 white blood cells (WBCs) (200 or 500 parasites per l). [23]

The microscopist should proceed in this manner when they are doubtful of the conclusion (e.g., 100 percent typical appearance). The sole apparent advantage of the thin smear over the thick film when it comes to species identification is the ability to identify P. ovale when it is in the hands of an expert. 79 Due to the fact that when P. ovale does not have a "red zone" on the thick film, it is frequently mistaken for P. malariae. There were 36 cases in this study where P. ovale was found to be a contributing to the diagnosis. Due to a lack of the other species' presence in adequate numbers, mixed infections could not be established most of the time. PCR may be used in future studies to determine whether or not this claim is true. [23]

Some laboratory employees are still unable to use thick malaria smears with acceptable sensitivity and specificity, even after two weeks of training, according to one study. According to the study, this is the case.

[21] Thin smears were commonly used in the West since it was thought that they were easier to form and understand. There has to be an investigation into whether or not the thin-film smear is useful in health care settings in disadvantaged countries. [23]

Study Limitations: Due to the fact that this study does not use randomization and instead relies on observations, there is a problem with patient selection bias. A small sample size and confinement to a single institution limited the scope of this investigation. Consequently, it is possible that the findings do not apply to bigger populations.

CONCLUSION

The results of the study showed that thin blood smear films are accurate diagnostic tools 90.9% of the time. It could be used as a second way to confirm a diagnosis of malaria.

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