JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Oral melatonin improves the detection of parasitemia in malaria

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Abstract

Introduction: Malaria is a growing global threat and a major cause of mortality in the tropics. The gold standard diagnosis is peripheral blood smear examination. It has been demonstrated that melatonin acts as messenger molecule in malaria pathophysiology. This concept was used to evolve a clinical study wherein use of exogenous melatonin could improve the chance of detection of the parasite.

Methodology: In a prospective study, 80 consecutive patients seen in the Department of Medicine at Kasturba Hospital, Manipal, suspected to have malarial fever were enrolled with proper informed consent, and randomly assigned to the groups given oral melatonin 3mg (melatonin group, n = 40) or placebo (control group, n = 40). Blood samples were collected for peripheral smear examination at baseline and then at two, three, four and five hours after drug administration. The primary end point was the parasite detection index.

Results: Baseline characteristics of patients were comparable. In the melatonin group, there was a significant increase of 0.0943 ± 0.22 in the mean parasite index from 0.217 ± 0.42 pre-melatonin to 0.3114 ± 0.5 post-melatonin (p = 0.001), compared to a difference of 0.0025 ± 0.22 in mean parasite index before and after placebo in the control group (p = 0.95). The maximum rise in parasite detection was seen at five hours after melatonin.

Conclusions: In a single centre study, for the first time, it has been shown that a significantly higher proportion of patients was diagnosed with malaria on peripheral smear after oral melatonin administration, maximal at five hours after administration of melatonin.

Key words: malaria; melatonin; fever; sweating.

J Infect Dev Ctries 2020; 14(11):1327-1331. doi:10.3855/jidc.12518

(Received 05 February 2020 – Accepted 11 April 2020)

Introduction

Malaria is one of the most lethal yet curable infections prevalent in the tropics. According to the World Malaria Report 2018 by WHO [1], there were an estimated 219 million cases and around thirty five thousand related deaths in 2017. In spite of continued research and newer preventive strategies, malaria is still a global threat.

One of the most puzzling questions concerning the development of malarial parasites is the search of molecules which might be involved in the signaling process in the parasite which is not exposed to extracellular milieu. Intriguingly, Hotta *et al*. [2] proved in *in-vitro* and *in vivo* studies that melatonin receptors were found both on *Plasmodium falciparum* and *Plasmodium chabaudi* (rodent parasite). These melatonin receptors [3] are membrane receptors and function through calcium modulation pathways via IP3 signal transduction [4,5].

Melatonin [6], otherwise called the dark hormone, is secreted from the pineal gland. Its synthesis and secretion are increased during the dark period of the day and maintained at a lower level during daylight hours. This remarkable diurnal variation in secretion is brought about by norepinephrine secreted from postganglionic sympathetic nerves (*nervii conarii*) that innervate the pineal gland. Nightly rise of melatonin levels in the human body could be detected by these receptors on the intracellular plasmodia and cause the synchronous eruption of numerous RBCs. Subsequently the patient would have spiking fever with chills and rigors due to cytokine release following the RBCs rupture, which attracts the mosquito vector to the patient and thus enhance the transmission.

Based on this knowledge, this study was conducted with the aim of improving the diagnostic accuracy of detection of malaria on peripheral smear examination by exogenous administration of melatonin, to simulate the physiological phenomenon, in order to induce

parasitemia, which could help in better detection of the disease.

Methodology

Patients with fever and chills of less than two weeks duration, suspected to have malaria, seen in the Medicine Department OPD at Kasturba Hospital Manipal, Government District Hospital Udupi and T.M.A Pai Rotary Hospital Udupi associated with Kasturba Medical College, Manipal University, India from January 2007 to June 2008, were consecutively screened and enrolled into the study. Patients with seizure disorders on anti-epileptics, those on treatment for psychiatric disorders, those on steroid therapy, subjects below the age of 16 years, and pregnant and lactating women were excluded from the study contemplating the possible interactions with melatonin [7,8]. Patients with symptoms suggestive of severe malaria [9] such as coma, anuria, jaundice and those refusing to participate in the study were also excluded.

This single center study protocol conformed to the Declaration of Helsinki and was approved by the Institutional Ethics Committee (reference no. KHEC/012/2007). An informed written consent was taken from each patient at the time of enrolment. All authors had access to the study data and have reviewed and approved the final manuscript.

After informed consent, patients were enrolled into the study. Patients were included in one of the study groups in a 1:1 manner. Patients in the melatonin group received 3mg melatonin and those in control group received a glucose tablet.

According to Brzezinski *et al*. [10], levels of melatonin are supposed to peak in blood one hour after oral administration. After obtaining ethical committee approval, we had done a pilot study initially in 20 patients in whom the peripheral smear was drawn at one hour after oral administration of 3mg melatonin tablet. However they did not show any parasitaemia or any changes on red blood cells in the peripheral smears. Hence after baseline sample collection for peripheral smear examination, the drug or matched placebo (glucose tablet) was given to the patients and repeat blood samples were collected at arbitrary time intervals of two, three, four and five hours after drug administration. This protocol was adopted in our study as no similar studies have been done in patients suspected to be suffering from malaria; the exact time required for the exogenous melatonin to cause the physiological phenomenon of synchronous rupture of numerous RBCs and consequent parasitaemia was not known.

Peripheral smear examination

Peripheral blood smear was selected for our study as it is the gold standard diagnostic test, cost effective and easily available, especially in developing countries [11]. All blood samples were drawn in the daytime between 9 am and 4 pm.

The blood smears were studied under oil immersion microscope after standard Giemsa staining in the Department of Hematology and Pathology, Kasturba Hospital, Manipal. Thick and thin blood film smears were made and stained with Giemsa. The young trophozoites were identified as incomplete rings or spots of blue cytoplasm with a detached chromatin dot. In the late trophozoites of *P. vivax*, the cytoplasm maybe fragmented and Schuffner's dots could also be seen. However the schizonts and gametocytes of these species retain their usual appearance, as do the crescents of *P. falciparum*. Magnification was kept between 500 to 600 X. On thin smears, for the designation of the relative parasite count, a simple code from one to four crosses was used, as follows:

- +: 1-10 parasites per 100 thick film fields
- ++: 11-100 parasites per 100 thick film fields
- +++: 1-10 parasites per one thick film field
- ++++: more than 10 parasites per one thick film field.

The primary outcome was effect on parasitemia defined as a change in parasite index at each time point compared to baseline.

Patients detected to have malaria were treated according to the standard treatment guidelines [12].

Safety of the therapy

Adverse effects of melatonin appeared to be infrequent with a single dose administration. Most clinical trials have involved less than 6 months of daily melatonin administration. The common adverse reactions reported include headache, transient depressive symptoms, dizziness, reduced alertness and abdominal cramps [13], all of which resolve with discontinuation of medication.

Statistical analysis

The primary endpoint was to see the improvement in parasite index with use of melatonin compared to placebo. Since there were no previous studies exploring this concept, we did a time bound study. It was possible to enrol 80 consecutive patients fulfilling the inclusion and exclusion criteria between January 2007 to August 2008 (40 in the melatonin group and 40 in the control group).

Table 1. Baseline Characteristics of patients in the two groups (expressed in mean with standard deviation).

Parameter	Melatonin group (N = 40)	Control group (N = 40)	p value
Male gender, n (%)	33 (82.5%)	31 (77.5%)	0.49
Age (in years)	32 ± 14.9	33.6 ± 13.3	0.67
History of malaria in past	9 (22.5%)	6 (15%)	0.31
Patients with palpable spleen, n (%)	18 (45%)	22 (55%)	0.35

Descriptive statistics were expressed as median (interquartile range) or number (%). Comparison of continuous variables was done by Wilcoxon rank-sum test and categorical variables were compared by Fisher's exact test or Pearson's chi-square test. All statistical tests were performed using SPSS for Windows version 15 (SPSS Inc., Chicago, IL).

Results

A total of 129 patients with fever and chills of less than 14 days duration were screened during the enrolment period between January 2007 to August 2008 (Figure 1). After careful assessment, 80 patients fulfilled the inclusion and exclusion criteria; 40 patients were assigned to the melatonin group and 40 to control group. The two study groups were comparable with respect to demographic characteristics, clinical and laboratory data (Table 1).

Primary outcome

Mean of parasite index detected in melatonin group was 0.217 ± 0.42 pre-melatonin and 0.311 ± 0.50 post-melatonin. However, for the placebo group, the results were 0.345 ± 0.63 pre-placebo and 0.347 ± 0.59 post-placebo. The difference in parasite index before and after administration of melatonin was 0.0943 ± 0.22 (p = 0.001) while in the placebo group no significant difference was seen (0.0025 ± 0.22) in the placebo group (p = 0.95). These results are suggestive of a significant rise in the parasite index in the melatonin group.

The % mean of the difference in parasite index before and after administration of melatonin or placebo was calculated also with respect to the time interval after administration. In the melatonin group, a significant rise in parasite index was noted at five hours after administration, when compared with other time intervals. There were no appreciable differences in the parasite index before and after administration of placebo at any of the time intervals. (Table 2 and Figure 2).

Most of patients were found to have *Plasmodium vivax* malaria. In four patients *Plasmodium falciparum* was detected, three (7.5%) in the melatonin group and one (2.5%) in the placebo group.

Figure 1. Study design - Screening and allocation of subjects.

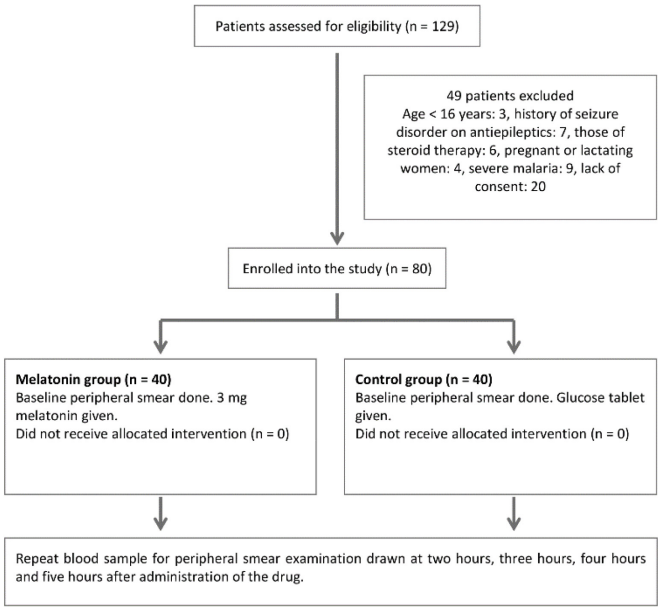


Table 2. Parasite index detection at each time point.

Group	Time point of detection after drug administration	Actual number of patients with malaria	% mean of difference in parasite index	P value
Melatonin group	Two hours	5	6.67	0.02
	Three hours	9	32.8	
	Four hours	12	52.1	
	Five hours	14	165.4	
Control group	Two hours	4	26.6	0.14
	Three hours	5	26.7	
	Four hours	6	18.2	
	Five hours	7	18.7	

Safety of the drug

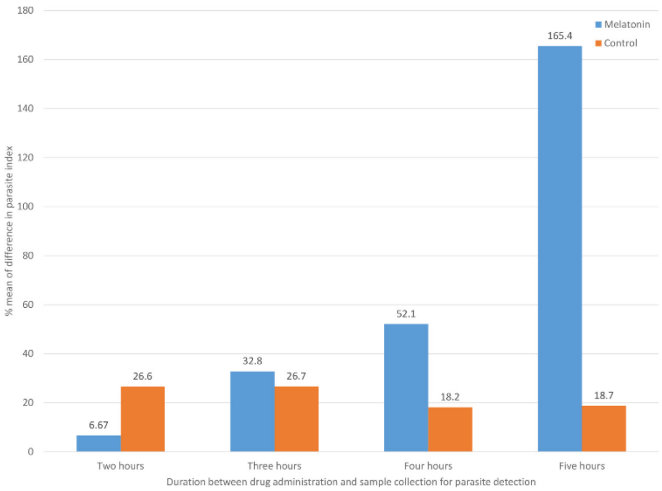
In the melatonin group, two patients had transient dizziness and reduced alertness, one patient reported increased sleepiness, and two patients had a slight increase in body temperature (37.8 °C), which was controlled with paracetamol. None of the patients in the control group reported any side effects.

Discussion

Malaria detection by quality assured microscopic examination of Giemsa-stained blood smears is the preferred technique because it is cost effective, easily available across the world and most importantly, still the gold standard diagnostic method [11]. In the 1990s, new methods to detect malaria were introduced, including molecular methods that can detect infections at very low parasite levels [14]. Tests for *P. falciparum* that target the histidine rich protein II antigen have the highest detection scores at low parasite density. A smaller number of tests detecting *P. vivax*, targeting plasmodium lactate dehydrogenase or aldolase antigens also perform well [15]. Advanced testing techniques to detect malaria include the quantitative buffy coat method, Falcivax testing kit with a sensitivity of more than 85% and specificity of 95% [14,15]. However, in developing and underdeveloped countries, especially in rural settings, these rapid diagnostic tests may not be available, hence the continued reliance on peripheral smear examination for malaria detection.

The classic symptomology of malaria [12] is fever is marked by paroxysms, which correspond to the development of the parasites in red blood cells. The peaks of fever coincide with the release of successive broods of merozoites into the blood stream. The total duration of a typical attack is 8-12 hours. Most paroxysms start in the night or at the latest in early afternoon [14]. The classical periodicity of febrile paroxysms develops only if the patient is untreated, until the infection becomes synchronized so that sufficient numbers of erythrocytes containing mature schizonts rupture at the same time. In the life history of malaria [14], the female anopheles mosquito transmits the malarial parasite. The flight activity of these mosquitoes is at its peak during the night, when she sucks blood from the host after mating peaks in the evening, to enable her to raise her progeny. During the febrile paroxysms which are predominantly at night, the blood is highly attractive to mosquitoes, raising the probability that the host will get bitten. Obviously from the perspective of a plasmodium, timing is crucial. Nightly release of melatonin is probably responsible for this timing.

Figure 2. Bar diagram showing the percentage of mean of difference in parasite index between the two groups at different time points.



Our study is based on this novel concept. We attempted to detect parasitemia after exogenous administration of melatonin to simulate this endogenous pathway of malaria transmission. For the first time, it was clearly demonstrated that in patients administered melatonin, there was a significant rise in parasite index compared to those given a placebo. We studied arbitrary time intervals of two, three, four and five hours as the time required for the exogenous melatonin to cause the physiological phenomenon of synchronous rupture of numerous RBCs and the consequent parasitaemia was not known. We demonstrated that five hours after melatonin administration, there was significantly increased parasitaemia, while no difference was seen with placebo. Throughout the course of the study, none of the patients in the melatonin group showed any clinical signs of overt parasitemia or any complications due to malaria. To reduce the confounding effects of evening/nocturnal circadian rhythm rise of serum melatonin on the study, all the patients were tested in the day time between 9 am and 4 pm.

There were a few limitations in our study. The blood melatonin levels were not measured during the study to determine the amount of melatonin required to cause the parasitemia. Confounding factors such as the light intensity in the hospital wards and the sleep patterns of patients were not recorded, although they could have a possible effect on the physiological melatonin levels. However, the studies were done in daytime when melatonin should be low. The efficacy of the use of melatonin in the detection of malaria was tested only using peripheral blood smears, not by other methods such as QBC or falcivax kits. Other possible

modes of exogenous melatonin administration, such as transdermal delivery systems, or sublingual or nasal sprays, were not studied. Lastly, it was single center study with a relatively small number of patients enrolled.

Conclusions

For the first time, it was clearly demonstrated that oral melatonin could improve the detection of malaria parasites on peripheral smear examination, maximally at five hours after melatonin administration. More data needs to be collected regarding the timing and dosage of the melatonin to be administered because of the half-life clearance of the drug through the kidney.

Further studies are required to further understand and evaluate the role of melatonin administration in the increased probability of microscopic parasite detection and to explore this fascinating chapter of malariology.

Acknowledgements

We thank departments of Haematology and Pathology, Kasturba Hospital Manipal for their prompt help in the study of the peripheral smears.

Authors' Contributions

CKK and BLS were responsible for the study concept and design; acquisition of data was done by CKK; statistical analysis was done by SV; CKK drafted the manuscript; critical revision of the manuscript for important intellectual content was contributed by SV; administrative and technical support came from SV. All authors have read and approved the final manuscript.

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Conflict of interests: No conflict of interests is declared.