Thermal characterization of *Plasmodium falciparum* species specific proteins in Indian geographical area.

Abstract

The paper reveals thermal characteristics of *P.falciparum* species specific proteins. These proteins determine why *P. vivex* species are dominant over *P.falciparum* in Indian geographical area. Outside human host plasmodium parasite survives in poikilothermal mosquitoes. Climatic temperature conditions greatly affect native state of *P.falciparum* species specific proteins in mosquitoes. Study of climatic temperature conditions in Indian geographical area reveals that P_{fsd} protein for *Plasmodium falciparum* can remain in native state between $\geq 7^0$c and $\leq 42.6^0$c temperature conditions. While their best optimum functions (*activity*) was noted between 25.3^0c to 33^0c. P_{fsd} protein were found more heat susceptible for temperature minima and maxima than P_{vsd} proteins of *P. vivex* causing all time high population ratio of *P.falciparum* species.

Body

Introduction

Proteins are building blocks of bio-organism and involved in diverse kind of functions. Loss of functionality of any key protein can result in failure of biological system. Such loss of functionality can be derived from internal factors e.g. misreading of DNA sequence, protein expression etc. or from external factors e.g. pH, temperature, chemicals etc. DNA is solely responsible for unique identity of different species of plasmodium parasite. Malaria, a public health disease is endemic in various countries and ~40% of the world's population is at malaria risk[1]. There is a great need of understanding disease strategy to combat malaria. The malaria parasites are dependent on *Anopheles* mosquitoes for transmission between human hosts. [2] Human malaria infects up to 500 million people and results in almost 3 million deaths per year.[3] Control of the disease is currently limited to antiparasitic drugs and mosquito control.[4] Some proteins constituting plasmodium differ at species level and show differentiating physical properties these can be termed as species differentiating proteins. This difference can also be in terms of heat susceptibility. Heat denaturation of susceptible protein can result failure of biological system of malaria parasite. Protein functions in a specific temperature range (between T_{min} and T_{max}). Thermal stability of species differentiating plasmodium protein (hereafter used as P_{sd}) will vary for specific temperature range (between T_{min} and T_{max}). Unavailability of desired appropriate temperature (below T_{min} and beyond T_{max} situations) will not allow Plasmodium parasite to survive. Such death of malaria parasite species will determine population load of malaria parasite among other species in any ecosystem. Malaria parasite spends their life in eurythermal primary host (human) and poikilothermal secondary host (Anopheles mosquito). Eurythermal human host maintains almost constant body temperature while secondary host

(Anopheles mosquito) provide climate derived body temperature to malaria parasite. Awkward climate (extreme low and high temperature) decline mosquito population simultaneously plasmodium species population also comes down in the ecosystem. In these awkward climatic conditions temperature; a climatic factor deform species differentiating proteins of plasmodium as a result *P. vivex*, *P. falciparum* and other plasmodium species share unequal population in reported malaria cases everywhere. $\boldsymbol{P_{sd}}$ proteins decide the population ratio of a particular species among other plasmodium species in ecosystem. So a study was designed to analyze thermal stability and climatic effect over $\boldsymbol{P_{sd}}$ protein in *P. falciparum* in poikilothermal female *Anopheles* species.

$\boldsymbol{P_{sd}}$ protein in P. falciparum

The proteins constituting plasmodium differ at species level these are proposed $\boldsymbol{P_{sd}}$ proteins. These are found exclusively in a particular species but are absent among other remaining species of plasmodium generally referred as species specific protein. So these are named ***species differentiating plasmodium protein*** ($\boldsymbol{P_{sd}}$). In *Plasmodium falciparum* we termed them $\boldsymbol{P_{fsd}}$ and $\boldsymbol{P_{vsd}}$ in *Plasmodium vivax*. Thus it is a species specific category of proteins which can have one or more of such proteins. These proteins are expressed by unique genomic sequences of particular plasmodium species and may be used as species marker. These proteins are directly responsible for determining population load of a given species among other species in any ecosystem. Table 1.1 intends to provide a good understanding of $\boldsymbol{P_{sdp}}$ protein where protein a, b, c, and d are hypothetical proteins found in plasmodium whose structural or functional activity is a must for the survival of plasmodium.

Table 1.1 Occurrence of proteins in two different species of Plasmodium

Protein	*Plasmodium vivex*	*Plasmodium falciparum*
a	Yes	Yes
b	Yes	No
c	No	Yes
d	Yes	No

On the basis of table 1.1 it is clear that protein b and d are $\boldsymbol{P_{vsd}}$ protein for *Plasmodium vivex* while protein c is $\boldsymbol{P_{fsd}}$ protein for *Plasmodium falciparum*. Protein c can also serve as molecular marker for *P. falciparum*. Any climatic/ physical/ chemical or biological factor resulting loss of structural and functional activity of protein c will affect the population share of *P. falciparum* among others species of plasmodium in ecosystem.

Material and methods

Area of study

Bharatpur district was chosen as study area which lies in Aravalli foothills of Indian subcontinent. Bharatpur is situated between 26° 22' to 27° 83' North Latitude and 76° 53' to 78° 17' East Longitude. It is situated 100 meters above the sea level. It is 184 km. away from Delhi in South-East.

Data collection

Human malaria cases from Bharatpur were collected for a year (Jun 2008-May 2009). We collected total 2025 malaria cases from Bharatpur city and all 10 towns under its territory with grand population of 2494247 in year 2008 and 2550368 in year 2009. On the basis of malaria cases months with disease burden are identified to frame a malaria calendar. On the basis of climatic conditions of corresponding month annual malaria rhythm was prepared. Population of Bharatpur District in table 1.2 and malaria cases in table 1.3 were obtained from Office, CM & HO, Bharatpur, Rajasthan, India. Whether report in table 1.3 was collected from India Meteorology Department, Rajasthan.

Monthly *P. falciparum* malaria cases reflect degree of functional *activity* of $\boldsymbol{P_{fsd}}$ protein of *P. falciparum*. Low malaria cases in any month reflect low *activity* while high cases reflect high *activity* of $\boldsymbol{P_{fsd}}$ protein of *P. falciparum.* A comparison of monthly malaria cases between *P. vivex* and *P. falciparum* gives comparative functional efficiency of $\boldsymbol{P_{fsd}}$ protein of *P. falciparum* and $\boldsymbol{P_{vsd}}$ protein of *P. vivex* in the climate of concerned geographical area. Climatic conditions of the given study area are determining factor for functional efficiency of $\boldsymbol{P_{sd}}$ protein of both species; as a result malaria cases of both species are differed.

Table 1.2 Population of Bharatpur District

Population in the year 2008	Population in the year 2009
2494247	2550368

Table 1.3 Malaria status in Bharatpur district correlated with whether report

PLACE	Jun 08	Jul 08	Aug 08	Sept 08	Oct 08	Nov 08	Dec 08	Jan 09	Feb 09	Mar 09	Apr 09	May 09
P.vivex cases	54	105	604	761	139	33	6	7	4	14	74	145

P. falciparum cases	1	10	25	20	8	6	5	0	1	1	2	0
Total cases of Malaria	**55**	**115**	**629**	**781**	**147**	**39**	**11**	**7**	**5**	**15**	**76**	**145**
Rain fall (mm)	341.5	173.7	177.9	189.1	1.5	0.0	0.0	0.0	1.8	2.4	5.9	38.1
$\mathbf{T_{max}}$	34.9	33.5	33.0	33.7	35.0	29.2	24.6	21.7	27.1	33.1	38.9	42.6
$\mathbf{T_{min}}$	26.0	26.3	25.3	23.9	19.5	11.5	8.8	7.0	8.2	13.5	19.8	25.3
Relative Humidity	85	90	92	88	80	85	95	96	90	78	43	53

Results and Discussion

Table 1.2 presents population of study area in study time. Malaria cases coming from a large population produces unbiased study results and minimizes the chance of error while putting any conclusion.

Table 1.3 shows annual malaria rhythm. During monsoon season (July, August, and September) malaria cases are increasing it is *First log phase* for malaria. An increase can be noticed in both *P. vivex* and *P. falciparum* cases. Other than these two species of Plasmodium no other species was reported from study area.

In terms of $\boldsymbol{P_{sd}}$ protein for P. falciparum climatic conditions during monsoon seasons are favorable for $\boldsymbol{P_{fsd}}$ protein expression. But $\boldsymbol{P_{vsd}}$ proteins for P. vivex are expressed much better resulting higher population share of P. vivex than P. falciparum in the ecosystem. Outside human host these parasites are residing in climate affected poikilothermal mosquitoes. Maximum cases of P. falciparum are obtained in the month of August. Maximum temperature in the month of August is 33^{o} centigrade and minimum temperature is 25.3^{0} centigrade indicating optimum temperature range for $\boldsymbol{P_{fsd}}$ proteins in terms of (structural/ expression/ functional property; hereafter called *activity*) however, even within this temperature range (25.3^{0} to 33^{o} c) expression/ *activity* of $\boldsymbol{P_{fsd}}$ proteins can not overtake $\boldsymbol{P_{vsd}}$ proteins. So *P. vivex* population ratio exceeds than *P. falciparum* species in the ecosystem of study area.

Maximum temperature in the month of September is 33.7^{0} centigrade and minimum temperature is 23.9^{0} centigrade. Increased maximum temperature ($\mathbf{T_{max}}$) and/ or decreased minimum temperature ($\mathbf{T_{min}}$) in the month of September must be responsible factor for declined *activity* of $\boldsymbol{P_{fsd}}$ proteins so in the month of September *P. vivex* cases are exceeding while *P. falciparum* cases faced a decline.

After monsoon season winters arises in the month of October, November, December, January, and ends in February in Indian subcontinent as reflected through climatic conditions of study area in table 1.3. Total cases of malaria (including *P. vivex* and *P. falciparum* cases each) are decreasing in this season. It is the *first decline phase* of malaria. Minimum temperature during winters touched 7.0^0 centigrade during study time. Due to low temperature of winters (7.0^0 centigrade) in study area *activity* of $\boldsymbol{P_{vsd}}$ proteins and $\boldsymbol{P_{fsd}}$ proteins is reduced significantly resulting a decline in *P. vivex* and *P. falciparum* population. Nil cases of P. falciparum in the month of January reflect that $\boldsymbol{P_{fsd}}$ proteins lose their *activity* below 7.0^0 centigrade temperature. Although *activity* of $\boldsymbol{P_{vsd}}$ proteins is also declined in winters due to low temperature but they serve comparatively better than $\boldsymbol{P_{vsd}}$ proteins

After winters spring season arise in Indian subcontinent it increases $\mathbf{T_{min}}$ in March, April, and May as evidenced with table 1.3. Increase in $\mathbf{T_{max}}$ can reduce the length of spring season and results summers.

$\boldsymbol{P_{fsd}}$ proteins get their *activity* on due to increased $\mathbf{T_{min}}$ in March, April, and May (spring) as a result they find their optimum temperature range to function. *P. falciparum* malaria cases are increasing due to this reason in spring. In the month of May maximum temperature ($\mathbf{T_{max}}$) is 42^0 centigrade which denatures $\boldsymbol{P_{fsd}}$ proteins as a result *P. falciparum* no longer survives. This can be evidenced with almost zero cases of *P. falciparum* malaria in the month of May; table 1.3. Despite of 42^0 centigrade temperature $\boldsymbol{P_{vsd}}$ proteins could not be denature, evidenced with 145 cases of *P. vivex* malaria during May in table 1.3. Such high temperature reduces mosquito population significantly resulting low malaria cases in June. Due to next rainfall season mosquito population increases once again from June onwards (341.5 mm rain fall in June, 2008). As a result of increased mosquito population *P. falciparum* malaria cases touches its peak ($\mathbf{T_{max}}$ 33^0c and $\mathbf{T_{min}}$ 25.3^0c).

Table 1.4

Serial no.	**Conclusion**
1	Optimum temperature for *Plasmodium falciparum* protein ($\boldsymbol{P_{fsd}}$) ranges between 25.3^0c to 33^0c.
2	Loss of *activity* for $\boldsymbol{P_{fsd}}$ protein of *P. falciparum*; $\mathbf{T_{max}}$ $\geq 42.6^0$c
3	Loss of *activity* for $\boldsymbol{P_{fsd}}$ protein of *P. falciparum;* $\mathbf{T_{min}}$ $\leq 7^0$c
4	*Plasmodium falciparum* parasite and $\boldsymbol{P_{fsd}}$ proteins can survive between $\geq 7^0$c and $\leq 42.6^0$c temperature conditions.

5	*Plasmodium vivax* parasite and **P_{vsd}** proteins can tolerate comparatively higher and lower temperatures than *P.falciparum*. So *P. vivax* is better adapted in Indian climate.
6	Low temperature (7^0c) is quite more effective in halting life cycle of *Plasmodium vivax* than high temperature (42.6^0c) in geographical climate of study area.
7	**T_{min}** of optimum temperature for plasmodium falciparum is somewhere between □25.3^0c and □23.9^0c

Discussion

After discussing the results we can point out few important conclusions about **P_{fsd}** proteins. First, these proteins lose their *activity* beyond 42.6^0c and below 7^0c. While at 42.6^0c temperature **P_{vsd}** proteins still perform better than **P_{fsd}** proteins so 42.6^0c temperature lies in optimum temperature range of **P_{vsd}** proteins. Second, **P_{fsd}** proteins are less adapted in Indian geographical area than **P_{vsd}** proteins as a result all time inferior population of *P. falciparum* than *P. vivex* is seen in study area including a large area of India. The places in India (e.g. Andhra Pradesh) where **P_{fsd}** proteins find optimum temperature range in climate *P. falciparum* malaria cases exceed than *P. vivex* cases.[5] Third, **P_{vsd}** proteins are able to maintain their native state at comparatively higher temperature than **P_{fsd}** proteins. Fourth, optimum temperature range for **P_{fsd}** proteins in study area is found within 25.3^0c - 33.0^0c. University Corporation for Atmospheric Research (UCAR) reports that at temperatures below 20°C (68°F) the parasite *Plasmodium falciparum* can not complete its growth cycle in the mosquitoes so it can not be transmitted. [6] But our results demonstrate a decline in *activity* of **P_{fsd}** proteins at 23.9^0c strongly indicating that this temperature does not lie within optimum temperature range of **P_{fsd}** proteins and $\leq 7^0$c temperature is needed to halt *Plasmodium falciparum* growth cycle in the mosquitoes (Table 1.3) instead of 20°C. At 33.7^0c *activity* of **P_{fsd}** proteins is found to be decreased while **P_{vsd}** proteins still serve better (Table 1.3). Fifth, minimum temperature at which **P_{fsd}** proteins and **P_{vsd}** proteins lose their *activity* has a little difference compared with maximum temperature at which they lose their *activity*.

Future perspective of P_{fsd} proteins

It is further matter of research to isolate, purify, clone and characterize **P_{fsd}** proteins. Their exact function in terms of structural / enzymatic/ or regulating is needed to be known. The pathway that is blocked due to their absence at $\leq 7^0$c and $\leq 42.6^0$c is further matter of research. Climate change / drug induced factors resulting mutations and gene adaptations provide new area of research in **P_{fsd}** proteins.[7, 8] A comparative study will reveal how **P_{vsd}** proteins are better adapted in Indian climate than $\boldsymbol{P_{fsd}}$ proteins. How climate change in a geographical area affects $\boldsymbol{P_{fsd}}$ and $\boldsymbol{P_{vsd}}$ proteins altering population of *P. vivex* and *P. falciparum*.

Author Contributions

Bhardwaj M. and Bharadwaj L. wrote the manuscript and developed and implemented the factors behind population ratio. Bhardwaj M. designed and implemented the databases. Bharadwaj L. processed the databases required for the protein stability. We declare that we have no conflicts of interest.

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