Seasonal Variation and Identification of Plasmodium Species in Blood Samples of Patients Referred to Labs of Karachi for Suspected Malaria from 2009 to 2011

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Abstract

Objective: To identify the seasonal effect on the frequency of malaria and its species in blood samples of patients referred to private and public Labs of Karachi for suspected Malaria and to observe if there has been any change in the frequency of the malaria species from May 2009 to Feb 2011.
Methods: A cross sectional retrospective review of the laboratory data of the positive blood samples of malarial parasites over a period of 22 months from May 2009 till Feb 2011 via convenient sampling from a private and a public sector laboratory. Blood samples of patients of all ages and gender who were referred from various doctors with suspected diagnosis of malaria were checked for the presence of malarial parasite (MP) using the slide method and/ or Immunochromatographic Technique. The samples found positive for the MP were then analyzed for species identification. The seasonal variation in the frequencies of these species assessed and the number of positive MP results observed from May 2009 to Feb2011 were described.
Results: Plasmodium vivax was found to be the most frequently identified plasmodium species present in 78.6% of the cases followed by Plasmodium falciparum. Adult males predominated over female and children. Frequency of Malaria was highest in the months of September with an overall surge of cases in the months of Aug to Dec. It is also observed that the number of cases in the year 2010 is much higher than in 2009.
Conclusion: The study concludes that P.vivax is the most frequently isolated species causing malaria in Karachi. The frequency of P.vivax causing malaria seems to have increased from 2009 to 2010. Malaria was more frequent in the months of August to December in Karachi affecting adult males more frequently as compared to females and children.

Keywords: Malaria, plasmodium infections, plasmodium falciparum, plasmodium vivax.
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Introduction

Malaria is one of the most common threats faced by the people throughout the world. WHO estimates in 2012 reveals that almost 207 million cases of malaria are found each year globally[1] while 482000 children under five years of age were killed by malaria in 2012. That is 1300 children die every day, or one child almost every minute[1]. It is especially overwhelming in the developing world which reports about 300–500 million cases and >1 million deaths annually[2,3] most of whom are children under the age of 5 years[1,2]. It is endemic in 91 countries predominantly in Africa, Asia and Latin America putting about 40% of the world's population at risk[1]. South East Asia is the second most affected region in the world with India carrying the highest malaria burden of about 24 million cases per year[4]. Globally it is one of the 5 major killers in paediatric population[2,3]. Pakistan with an estimated burden of 290781 cases annually[5] has been categorized in group 3 of the Eastern Mediterranean region along with Afghanistan, Djibouti, Somalia, Sudan and

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Yemen sharing 95% of total regional burden. Malaria is the second most frequently reported disease from public sector health facility after Acute Respiratory Infection (ARI). It is commonly known that Malaria is an infectious blood born disease caused by bite of female Anopheles mosquito containing a parasite, Plasmodium[6]. When most people think of malaria, they usually think of Plasmodium falciparum, the form most prevalent in Africa. However, other types known to infect humans are Plasmodium Vivax, Plasmodium Ovale and Plasmodium Malariae[7]. A fifth one, P. knowlesi, has been recently documented to cause human infections in Southeast Asian countries such as Malaysia, Thailand, Viet Nam, Myanmar and Phillippines[7,8,9,10]. These species carry their own levels of severity, illness, and fatality rates and there are different risks inherent to each form[11]. Malaria also shows considerable spatial heterogeneity on global, regional and local scales and this should make the basis for the type and degree of interventions required to guide malaria control programme in the country[12,13]. Mixed species infections are commonly observed. There is generally great variability in results between studies[12,14]. In Pakistan Plasmodium vivax and falciparum are the only prevalent species. Since the treatment protocols, prognosis and severity of illness caused by the two species are different, we need to update our data regarding the frequency of the malarial parasitic species and their seasonal variations on regular basis. Increase in frequency of a particular species may act as an indicator of inadequate treatment, failure of prevention strategies, failure of malaria Roll back program or the failure of the ministry to educate the doctors especially general practitioners about the standard treatment recommended by our ministry of health. This in turn will guide us to revise the guidelines for treatment and prevention of the disease accordingly. Also the change in frequency of the disease may help us to reset our priorities regarding the management of the major killers in our national child survival programs. We therefore designed this study to identify the seasonal effect on the frequency of malaria and its species in blood samples of patients referred to private and public Labs of Karachi for suspected Malaria and to observe if there has been any change in the frequency of the malaria species in these blood samples from May 2009 to Feb 2011.

Material and Methods

A retrospective cross-sectional study was conducted for 22 months from May 2009 till Feb 2011 to include a maximum available adequate sample size incorporating seasonal changes to observe its effects along with the changes in frequency of malaria from May 2009 to Feb 2011. The minimum required sample size was 1040 using the frequency 17% with e=3% and confidence level =99% using the software "openepi by the formula of "proportion" [15].

Sampling technique was convenient sampling. Inclusion criteria were positive blood samples for malaria from patients of all ages and gender, while negative blood samples were excluded. Data collected were positive samples of malaria parasite (MP) from two sources, from a private lab and other from Tertiary care public health center, Civil Hospital Karachi (CHK).

The blood samples of the patients referred to the laboratories with the suspected diagnosis of malaria were checked for the presence of MP using the slide method and/ or Immunochromatographic Technique for malaria parasite (ICTMP). The samples found positive for the MP were then recruited in the study and analyzed for species identification.

Two methods were used for the detection and identification of the malaria parasite. Slide Method which detects MP by making thick and thin peripheral blood smear. Mostly thin peripheral blood smear is used which is prepared by using Ethylene Di Amine Tetra Acetic Acid (EDTA) sample by the method which includes a blood smear made by push technique and stained by Leishman's staining with 1:3 dilutions. The slide is then washed with tap water and dried in air. Oil is applied to the slide which is then observed under an appropriately se-

lected field of microscope to identify different stages of malaria parasite.

The second method is the ICTMP which is a rapid, in vitro diagnostic test for the detection of plasmodium falciparum antigen and an antigen which is common to all four species of malaria. The test uses two antibodies that have been immobilized across the test strip. One antibody is specific for the histidine-rich protein II antigen of plasmodium falciparum (HRPII).The other antibody is specific for an antigen that is common to P. falciparum, P.ovale, P. malariae. A total of 5µl of whole blood was applied to the test strip and Reagent A was added which lyses the blood sample and allows migration past the purple pad which contains colloidal gold conjugated antibodies that are directed against the malarial antigens. When positive sample is applied, the malarial antigens bind with gold conjugated antibodies and continue migration along the strip where they are captured by immobilized antibodies. When capture occurs, a pink-purple Control line (C) will form and either one or two pink-purple Test lines (T) will form in the window. When a negative sample is applied, only the control line will appear. The test is invalid if the Control line does not appear whether or not a Test line is present.

Data was then analyzed for the frequencies of the identified plasmodia species. It was also assessed for the seasonal variation in the frequencies of these species in addition to the demographic data consisting of ages and gender of the affected subjects. The study was approved by the IRB of Dow University of Health sciences.

Results

Data of a total of 2576 samples positive for MP were analyzed. Plasmodium vivax was found to be the most frequently identified plasmodium species present in 2025(78.6%) of the cases followed by Plasmodium falciparum 551(21.4%). No case of P.malariae or P.ovale was seen.

As per gender variation analysis, males predominated over females and children with a frequency of 65.5% as compared to 24.4% females. Children below the age of twelve years constituted 10.1% of the patients.

In this study Malaria was found to hit the highest point in the month of September with an overall surge in cases in the months of August to December Fig. 1. It is also observed that the number of cases in the year 2010 is much higher than

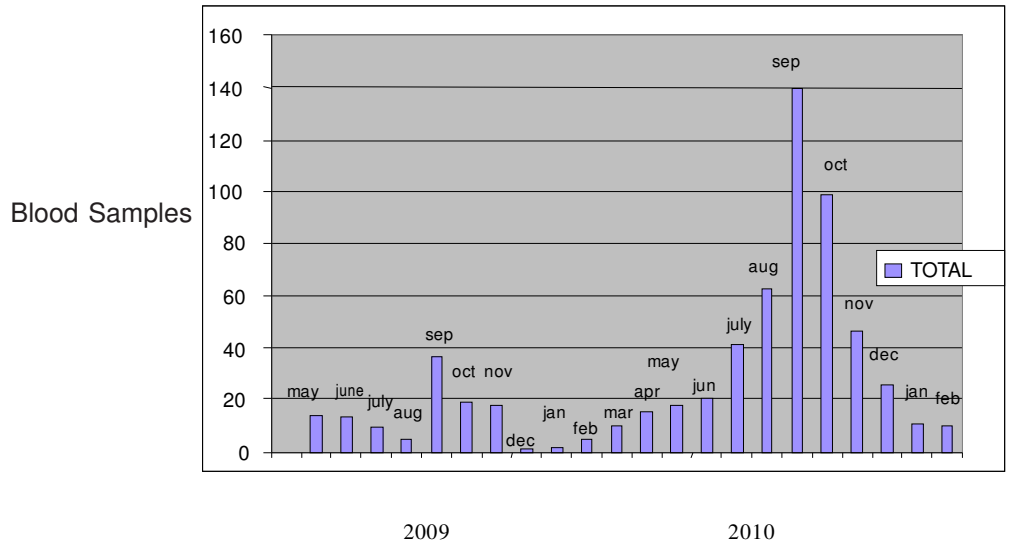

Fig. 1: Seasonal variation of the frequency of Malaria in blood samples of patients referred to private and public Labs of Karachi for suspected Malaria from May 2009 to Feb 2011.

in 2009 although the general pattern of increase in cases in the post monsoon season stays the saFig. 1. Seasonal variation of the frequency of Malaria in blood samples of patients referred to private and public Labs of Karachi for suspected Malaria from May 2009 to Feb 2011.

Discussion

Our data emphasizes the observation that plasmodium vivax is the most common species among the inhabitants of Karachi. The result is comparable to other reports in literature from around the world showing P.vivax to be the most geographically wide spread of human malarias. The frequency is especially consistent with the reported figures of 70-90% occurring in most of Asia, Central and South America and the Middle East. In South East Asia, French Guiana and western pacific it is responsible for more than 50% of the malaria cases[10,16]. Variable frequencies have been noted in different parts of neighbouring India where some areas reported predominance of P.falciparum responsible for 30-90% of malaria burden while in most areas it is P.vivax accounting for nearly 50% of the total malaria burden. Contrary to the results of Asia and America the majority of infections in Africa amounting to about 85% are caused by Plasmodium falciparum, estimating an average prevalence of 63% in western Africa and 39% in eastern and southern Africa,[5,10,16]. As in India, Pakistan also reports variable frequencies of the plasmodium species from various areas. While the aggregate frequency reported by WHO has always shown P.vivax to take the lead in Pakistan ranging from 70% in 2008 to 61% in 1998-2001[5] to 60% in 2004-2005, there are other studies reporting higher frequencies of P.falciparum in various parts of Sindh ranging from 58% as studied by malaria control programme to 65% at Baqai Medical University Karachi and at Jhangara to 88.5% at CHK and Anklesaria Hospital Karachi (Aug 2003-Dec2005) and in Larkana district of Sindh (2005)[17]. While Data of Provincial Malaria Control Programme of Sindh reports an average of P.falciparum ratio in years 2004-2005 as 33% and 37.2% respectively[18]. Similarly Balochistan also reported 57.1% of falciparum against 42.8% of P.vivax[19] but the heterogeneity could be witnessed here as well as Sibbi reported more of P.vivax as compared to Duki, Harnai and Khuzdar district where p.falciparum was more prevalent. Higher frequencies of P.vivax (58%) were also reported from Kohlu[20] and Ziarat[21] and from Quetta in earlier years of 1994-1998[22]. P.falciparum has also been reported as the major problem for Afghan refugees in Khyber Pakhtoonkhwa (KPK)[23]. On the other hand results from Punjab are consistent with our results reporting predominance of P. vivax (60.5% and 39%) in Multan[24] and south Punjab[25]. the highest frequency of P.vivax reported so far of 90.4% was observed in Kashmiri refugees settled in Muzaffarabad[26]. These variations not only in different areas of the country but within the city of Karachi as well corroborates the already established fact in literature which says that malaria prevalence can vary significantly between sites, even at local scale and shows a high rate of heterogeneity[14] as has been reported by Malawi and Cambodia also[12,13]. The predominance of malaria in male gender is also consistent with the results of other studies[13, 18].

Several studies have shown in tropical settings the strongly favorable effects of temperature, humidity, and rainfalls on transmission of malaria infection[12] with substantial regional and year-to-year variations[13] as has been the case in our study as well where a surge in the cases of malaria was noted in the post monsoon months of August to December. Similar experience was reported from Sibi, Baluchistan. Variable seasonal effects were noted in Duki, Baluchistan with highest infection of P.falciparum in April and lowest in December while In Harnai area highest infection of P.falciparum was reported in December and lowest in January[18]. Although the predominance of P.vivax in our study is consistent with the results of older studies from the late 90's to mid 2000 including those from WHO and Provincial Malaria Control Programme, the frequency has gone up to more than 78%. Currently 97 percent of the available funds in the world are focused on the research and prevention of

P.falciparum while other types of malaria especially P.vivax have been kept on the medical back burner which is said to be a big mistake[11,27]. With this increasing frequency of P.vivax we need to re-channelize our malaria control programmes giving more importance to P.vivax as well. Limitation of the study is that since this study consists of data taken from two laboratories only, the results cannot be generalized to the whole community.

We strongly recommend a large scale, community based study to determine the malaria load of the community and to identify the most prevalent Plasmodium species causing malaria in our country. Predicting and mapping malaria risk with species identification all over Pakistan is also suggested. Updating these malaria maps should then be carried out on a regular basis as new data become accessible. More attention should be given to prevention, control and treatment of P.vivax also in addition to P.falciparum in our National and Provincial Malaria Control Programmes. The Health ministry and the personnel involved with National and Provincial Malaria Control Programmes should identify and rectify the causes for the surge in frequency of P.vivax.

Conclusion

The study shows that P.vivax is the most frequently isolated species causing malaria in blood samples of patients referred to private and public Labs of Karachi from May 2009 to Feb 2011 responsible for over 78% cases of malaria. The frequency of P.vivax causing malaria has increased from 2009 to 2010. No case of P.ovale or P.malariae was isolated. Malaria was seen more frequently in the months of August to December in Karachi. It was more frequently seen in males as compared to females and children.

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References

1. World Health Organization. Factsheet on the World Malaria Report [Internet]. Geneva: World Health Organization; 2014. Available from: http://www.who.int/entity/campaigns/malaria-day/en/.
2. Krause PJ. Malaria. In: Kliegman RM, Behrman RE, Jenson HB, Stanton BF, editors. Nelson Textbook of Pediatrics. 18th ed. Philadelphia: Saunders Elsevier; 2007. p. 1477-85.
3. Sultan MA. Malaria in Children. Pak Ped J 1999;23:69-74.
4. World Health Organization. Factsheet on the World Malaria Report [Internet]. Geneva: World Health Organization; 2012. Available from http://www.who.int/malaria/media/world_malaria_report_2012_facts/en/. Accessed on: September 19, 2014.
5. World Health Organization. Global Health Observatory Data Repository [Internet]. Pakistan statistics summary. Geneva: World Health Organization; 2014. Available from: http://apps.who.int/gho/data/node.country.country-PAK. Accessed on September 19, 2014.
6. Singh B1, Kim Sung L, Matusop A, Radhakrishnan A, Shamsul SS, Cox-Singh J, et al. A large focus of naturally acquired Plasmodium knowlesi infections in human beings. Lancet. 2004;363:1017-24.
7. World Health Organization. International travel and health. Malaria [Internet]. Geneva: World Health Organization. Available from: http://www.who.int/ith/diseases/malaria/en/. Accessed on September 19, 2014.
8. White NJ. Plasmodium knowlesi: The Fifth Human Malaria Parasite. Clin Infect Dis 2008;46:172-3.
9. Kakkilaya BS. The Malaria Parasites. Dr. B.S. Kakkilaya's Malaria Web Site [Internet]. 2011-2013. Availabale from: http://www.malariasite.com/malaria/MalarialParasite.htm.
10. Tordrup D1, Virenfeldt J, Andersen FF, Petersen E. Variant Plasmodium ovale isolated from a patient infected in Ghana. Malar J 2011;10:15.
11. Foley DH, Klein TA, Kim HC, Wilkerson RC, Rueda LM. Malaria risk assessment for the Republic of Korea based on models of mosquito distribution. US Army Med Dep J 2008;5:46-53.
12. Kazembe LN, Kleinschmidt I, Holtz TH, Sharp BL. Spatial analysis and mapping of malaria risk in Malawi using point-referenced prevalence of infection data. Int J Health Geogr 2006;5:41.

13. Incardona S, Vong S, Chiv L, Lim P, Nhem S, Sem R et al. Large-scale malaria survey in Cambodia: Novel insights on species distribution and risk factors. Malar J 2007;6:37.

14. Mueller I, Widmer, Michel D, Maraga S, McNamara DT, Kiniboro B, et al. High sensitivity detection of Plasmodium species reveals positive correlations between infections of different species, shifts in age distribution and reduced local variation in Papua New Guinea. Malar J 2009;8:41.8:41

15. Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health, Version. www.OpenEpi.com, updated 2014/09/22, accessed 2014/10/20. Available fromhttp://www.openepi.com/Menu/OE_Menu.htm

16. Barnadas C, Musset L, Legrand E, Tichit M, Briolant S, Fusai T, et al. High Prevalence and fixation of Plasmodium vivax dhfr/dhps mutations related to sulfadoxine/pyrimethamine resistance in French Guiana. Am J Trop Med Hyg 2009;81:19-22.

17. Mahmood K, Jiramani KL, Abbasi B, Mahar S, Samo H, Talib A, et al. Falciparum malaria: various presentations. Pak J Med Sci 2006;22:234-7.

18. Nizamani MA, Kalar NA, Khushk IA. Burden of Malaria in Sindh Pakistan: a two years surveillance report. J Liaqat Univ Med Hlth Sci 2006;5:76-83.

19. Yasinzai MI, Kakarsulemankhel JK. Frequency of various human malaria infections in hottest areas of central balochistan, Pakistan: duki, harnai,andsibi. Pakistan Armed Forces Medical Journal 2008.

20. Yasinzai MI, Kakarsulemankhel JK. Incidence of human malaria infection in bordering areas adjoining with Punjab: Barkhan and Kohlu. Pak J Med Sci 2008;24:306-10.

21. Yasinzai MI, Kakarsulemankhel JK. Prevalence of human Malaria infection in district Ziarat and Sanjavi, Pakistan. Pakistan J Zool 2009;41:475-82.

22. Sheikh AS, Sheikh AA, Sheikh NA, Paracha SM. Endemicity of malaria in Quetta. Pak J Med Res 2005;44:41-45.

23. Howard N, Durrani N, Sanda S, Beshir K, Hallet R, Rowland R. Clinical trial of extended dose of chloroquin for treatment of resistant falciparum malaria among Afghan refugees in Pakistan. Malar J 2011;10:171.10:171.

24. Yar HM, Masood K, Maqbool A, Malik GQ. Prevalence of malaria parasite species in Multan district. The Professional 1998;5:183-7.

25. Shehzadi S, Akhtar T, Hanif AH, SaharS, Niaz S. Molecular surveillance of Malaria in south Punjab with higher proportions of mixed infections. 31st Pakistan Cong. Zool. (Int.), Univ. AJK, Muzaffarabad, 2011; Abstract: 101.

26. Jan AH, Kiani TA. Haematozoan Parasites in Kashmiri refugees. Pak J Med Res 2001;40:10-12.

27. Shepard W. Different Types of Malaria, Geographic Dispersal, Risk Assessment, and How Travelers Can Prepare [Internet]. Published on December 6, 2011. Available from: http://www.vagabondjourney.com/different-types-of-malaria-geographic-dispersal-risk-assessment-and-how-travelers-can-prepare/.