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SEROEPIDEMIOLOGICAL STUDY OF P. VIVAX MALARIA WITH SUSPECTED LONG INCUBATION IN IRAN*

Gh. H. Edrissian
F. Naimi
A. Afshar

ABSTRACT

P. vivax malaria with suspected long incubation have been frequently observed mostly in the northwest part of Iran in the course of Malaria Control and Malaria Eradication Operations.

A sero-epidemiological study of such cases which present the first symptom in early spring, before anopheles activities season, was carried out in 92 selected villages with a population of 38,726 in two near areas in Hamadan and Bijar, in the west part of Iran. The total number of 3553 blood samples for microscopical and Indirect fluorescent antibody serological technique were collected in 12 weekly total surveillance in the early months of the year 1973 and 1974. Altogether seven cases parasitologically *(P. vivax)* and 98 cases serologically (titers 1/20 to 1/640) positive were observed. From seven parasitological positive cases three were serologically negative. These three cases as they occurred at the beginning of appearance of anopheles

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** Department of Medical Parasitology and Mycology, School of Public Health and Institute of Public Health Research, University of Tehran, P.O. Box 1310, Tehran, Iran.

*** Malaria Eradication and Communicable Disease Control Unit of Hamadan Province, Ministry of Health and Welfare.

could be considered as new transmission.

The results of examination of 1534 total blood samples collected from children up to 15 years old in selected villages of the Hamadan area at the end of the transmission season of 1973 showed only one serological positive case.

This investigation showed that the malaria infection rate in the selected areas, which seemed to be the most appropriate region in Iran, is now too low for such study.

INTRODUCTION AND REVIEW

Two types of *P. vivax* malaria, one with short and the other with long incubation period was demonstrated in the Soviet Union by Nicolaev in 1935. This author (1949) separated *plasmodium vivax* into sub-species *P. vivax* and *P. vivax hibernans.* Coatney et al (1950) on their experimental studies on sporozoite induced malaria, caused by St. Elizabeth strain of *P. vivax,* found that the acute attacks occur in two short (within two months) and long (9–10 months) waves after exposure. The long term latency was not dependent on the sporozoite dosage and also the degree or duration of early patient parasitaemia. Eddleman et al (1951) also reported seven cases of *P. vivax* malaria with long incubation in California among returned people from Korea. Covell in his comments stated that the above reported cases are in harmony with those indigenous *P. vivax* malaria with long incubation which have occurred in Rumania, Italy, Spain, Holland and England, to the so-called Madagascar and St. Elizabeth strains used for malariotherapy in England and the United States. but not to the Chesson strain originating in the South West Pacific in which protracted incubation period and long term latency have never recorded. He stated again in 1954 that protracted incubation and late relapse may be a characteristic of *P. vivax* strains throughout the world excepting only the South West Pacific region. Characteristics of *P. vivax* with long latent period extending to eight months has been described by Winckel (1954) in the Netherlands. In respect to the biology of *A. maculipennis atroparvus* which is a semi-hibernating species and a very domestic Anopheles in the Netherlands, winter transmission should be also considered in this country. In the Soviet Union in addition to the two types of *P. vivax* malaria, one with short (9–21 days) and the other with long (8–14 months) incubation period, another strain has been isolated in Moscow ("MS" strain) from a patient in 1953 that produces both short (9–20 days) and long (216–327 days) incubation. Tiburskaya (1961) demonstrated in the "MS" strains that the length of incubation period does not depend on the number of sporozoites induced. This author (1962) also studied the Korean strain of *P. vivax* and concluded that this strain sometimes produces infection with short incubation period.

Shute and Maryon (1968) claimed that they never observed protracted incubation period in patients who were infected by intravenous sporozoites and they believe that the duration of incubation period has some relationship with the number of injected sporozoites. They mentioned that "the phenomenon of latency of both the primary attack and relapses occur in strains of both tropical and temperate origin".

Moshkovsky (1973) classified the malaria caused by most strains of *P. vivax* (except Chesson type) in respect to the sequence of acute attacks in two types:

I) The short incubation type followed by the so-called remote relapses.

II) The long incubation type in which the primary attack occurs after a long period between the acquisition of the infection and the onset of remote relapses.

Some strains of *P. vivax* may cause cases of only one of these types and in other strains both types are encountered in varying proportions. He explained these differences by a hypothesis that *P. vivax* may produce two types of sporozoites. Type 1 causes *P. vivax* malaria with short and type 2 with long incubation period. Some strains produce sporozoites of only one type and some sporozoites of both types.

Bray (1975) believes that Moshkovsky's theory is not tenable.

Garnham et al (1975) stated "it is probable that certain sporozoites in *P. vivax* fail to develop in the normal time and that they are reactivated by an unknown factor a year or more after innoculation."

Shute et al (1974) showed that dosage of sporozoites is essential factor and 10—1000 sporozoites caused delayed incubation between 257 and 628 days.

P. vivax malaria with long incubation period has been reported by Gokburk (1968) in Turkey. In Iran in the course of Malaria Control and Malaria Eradication Operations cases of *P. vivax* with suspected long incubation have been observed in the North-Western part of the country which presented, apparently, the first symptom of infection in early spring when it is prior to the active season of the local malaria vectors.

In regard to the findings that the pre-erythrocytic form of plasmodia do not produce malaria antibodies detectable with indirect fluorescent antibody (IFA) test and this test becomes positive about four to seven days after patient parasitaemia (WHO/MAL 69.703) by using the IFA technique we may be able to separate *P. vivax* with long incubation from relapsing cases of vivax malaria.

Sero-epidemiological study of such *p. vivax* malaria cases (using IFA test) with suspected long incubation period was carried out with recommendation and the support of the World Health Organization

in the western part of Iran where cases of this type of vivax malaria are more likely to occur.

MATERIALS AND METHODS

The investigation was carried out in 92 villages, with a population of 38,726, located in two neighbouring areas in Hamadan and Bijar, in the west part of Iran. The selection of villages was according to the number of *P. vivax* cases recorded during the early months of the five years, 1968 to 1973, in the local Malaria Eradication Units files.

The weather in the area is rather cold and temperatures vary from −33°C in February to +38°C in July and relative humidity is high in February, maximum 99% and minimum 62%, and low in September, maximum 65% and minimum 8% (Hamadan Meteorological Station report in 1973).

The potential malaria vectors in the area are: *A. (M.) superpictus, A. (A.) sacharovi* and *A. (A.) maculipennis typicus* (School of Public Health 1970). At the present time the most important one seems to be *A. (M.) superpictus.*

From human plasmodia, *P. vivax, P. falciparum* and *P. malariae* which had been found before the malaria eradication programme (School of Public Health 1970) the most prevalent species was *P. vivax* and now this parasite is almost the only species which is transmitted by anopheles. The Malaria Eradication Programme has been started in these areas from 1957. The area had been under irregular spraying till 1970 and afterwards the focal spraying has been done whenever it was felt necessary for elimination of new malaria foci which occasionally appear in these areas.

The study was started in 1973. During one month (from 21st June to 21st July) in four weekly total surveillance carried out in selected villages, 1002 blood samples were collected from all fever cases and those who were suspected to have malaria infection.

In order to estimate the malaria incidence during 1973, at the end of the transmission season (November 1973) 1534 total blood samples were collected from children up to 15 years in 31 villages with a population of 18,351 in the Hamadan area.

This study was repeated in 1974 as early as it was possible and during two months from 6th May to 6th July, in the same selected villages of 1973, 2551 blood samples were collected in eight weekly total surveillances. Usually thick and thin smears were prepared on slide for parasitological examination which was carried out in the local Malaria Eradication laboratories in Hamadan and Bijar.

The simple weekly entomological survey was carried out in selected villages of Hamadan in 1974 in order to estimate the beginning of anophele activity in the area.

A certain amount of blood (about 50 µl) was also spread on Whatman filter paper by heparinized microhaematocrit capillary tubes or approximately 0.1 ml of blood from a finger prick flow directly into the circle printed on special thick filter paper, Ropaco No. 1023. 0.038 received from Dr. Henry Mathews, Parasitology Division, C. D.C., U.S.A., through WHO grants. The dried blood samples on filter paper were tested by IFA technique in Protozoology Laboratory, School of Public Health and Institute of Public Health Research, Teheran.

P. vivax human malaria antigen, prepared from malaria patients at the Field Medical Research Station in Bandar Abbas, in the southern part of Iran, according to Sulzer et al. (1971) in 1973 and Aotus *P. vivax* malaria antigen, received from Dr. A. Voller, N.I.C.M. the Zoological Society of London, in 1974 were used in IFA test.

Wellcome Fluorescent Anti-Human Immuno-globulin was used as conjugate in dilution 1/20 with 0.1 per cent Evansblue.

Performance of IFA test was according to Voller and O'Neil, (1971) and Leitz Ortholux Fluorescence Microscope equipped with UG_5 and BG_{12} primary filters and 470 m. occular filter.

RESULTS AND CONCLUSION

From 1002 blood samples prepared in four total weekly surveillances from 21st June to 21st July 1973 six cases parasitologically *(P. vivax)* and 29 cases serologically (titers 1/20 to 1/640) were positive. From parasitological positive cases two were serologically negative by IFA technique (two boys, 4 and 9 years old in Hamadan and Bijar areas found on 1st and 17th July respectively).

In examination of 1534 total blood samples prepared from children up to 15 years in Hamadan area in November 1973 only one serologically positive case in titer 1/80 was detected.

From 2551 blood samples prepared in eight total weekly surveillances from 6th May to 6th July 1974, one parasitological *P. vivax* case was found in a three year old boy in Hamadan area on 2nd July. This case was serologically negative. Among these 2551 blood samples 69 cases were serologically positive in titers 1/20 to 1/320 (Table 1).

In a simple entomological survey carried out in Hamadan area in 1974, the first adult anopheles was caught on 16th June.

Altogether in the selected villages, seven cases of *P. vivax* were detected in the early months of the years 1973 and 1974. Three cases which were serologically negative were found in three boys of the ages three to nine years without previous malaria history. These cases, as they occurred at the beginning of appearance of anopheles activity season in the area, could be considered as new transmission.

The serological rate in all fever cases and those who were

suspected to have malaria in 12 weekly surveillances of selected villages during the early months of 1973 and 1974 was 2.7%. The total malaria parasitological positive cases discovered in the selected villages in the usual malaria eradication surveillance campaign during all the year 1973 was 25 *P. vivax* classified as four indigenous, 18 relapsing and three imported cases (annual parasite incidence 0.6/1000).

There was no record of any malaria case in the area in the early months of the year 1974 before the start of this study in this year.

The above obtained data show that the infection rate is too low to disclose the occurrence of *P. vivax* with long incubation period in the area.

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Table 1

Sero-Epidemiological Study of *P. vivax* Malaria with Suspected Long Incubation in Iran (1973–1974)
Serological and Parasitological Positive Cases

	Age Group (years)	Number Examined	Parasitological + ve Cases (P. vivax)	Serological + ve Cases (IFA Titres)						
				1/20	1/40	1/80	1/160	1/320	1/640	Total
1973	<2	39	0	0	0	0	0	0	0	0
	2–4	106	1	1	0	0	0	0	0	1
	5–9	109	2	1	2	1	1	0	0	5
	10–16	99	0	1	1	0	1	0	1	4
	17–30	219	2	1	1	2	2	0	0	6
	Over 30	430	1	8	2	2	0	0	1	13
		1002	6	12	6	5	4	0	2	29
1974	<2	86	0	0	0	0	0	0	0	0
	2–4	221	1	0	2	0	1	0	0	3
	5–9	243	0	2	1	0	2	0	0	5
	10–16	316	0	1	4	0	1	0	0	6
	17–30	587	0	2	8	5	1	0	0	16
	Over 30	1098	0	5	15	11	5	3	0	39
		2551	1	10	30	16	10	3	0	69
Total		3553	7	22	36	21	14	3	2	98

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