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Increasing Trend of Mixed Infections of *Plasmodium falciparum* and *Plasmodium vivax* among Imported Malaria Cases in Kuwait, a Non-Endemic Country

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Abstract

Background: Malaria in humans is caused by five *Plasmodium* species, *i.e. Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale* and *Plasmodium knowlesi*, which are transmitted by female Anopheles mosquitoes. Although a lot of progress has been made in the fight against malaria, an estimated half of the world's population (−3.95 billion people) in 87 countries and territories is still at risk of malaria. Malaria was eradicated from Kuwait in 1963, and no autochthonous cases have been reported afterward. The cases reported after 1963 were imported primarily among expatriates coming to live or work in Kuwait. **Methods:** The epidemiologic and demographic data of all malaria cases from 1992 to 1996 were collected from the Medical Center for Labor Examination and from 2015 to 2019 from the Infectious Diseases Hospital, Kuwait. All the suspected cases of malaria are referred to Infectious Diseases Hospital for diagnosis confirmation and treatment. Blood samples were obtained from 5800 suspected malaria cases. Giemsa-stained thick and thin blood films were performed to identify the malaria cases and the infecting *Plasmodium* species. Selected samples (n = 24) were retested with microscopy and a real-time PCR assay to reconfirm the diagnosis and identify the *Plasmodium* species. **Results:** During the years (2015-2019), 1549 (26.7%) cases (average = 310 cases per year), out of 5800 tested, were identified by microscopy to have malaria. The gender distribution analysis showed that 78.6% of cases were males and 21.4% were females. An overwhelming majority (97.4%) of cases were expatriates coming from malaria-endemic regions, in India. Only 18 cases of malaria were found among Kuwaiti nationals, and all of them had a history of travel to malaria

endemic African countries. The majority (71%) of cases were detected during the months of May and October of each year. The most infecting *Plasmodium* species were *P. falciparum* and *P. vivax* but the majority (69.5%) of cases had mixed infections with these species. The results of microscopy and PCR were 100% concordant with the diagnosis of malaria and the *Plasmodium* species. **Conclusion:** Our data showed that the incidence rate of imported malaria cases is in declining trend over the years. This study confirms that the migrant workers diagnosed with malaria and treated in their home countries before entering Kuwait for the 1st time have been successful in reducing the incidence of imported malaria infections in Kuwait. However, the number of cases infecting from mixed infections (69.5%) with *P. falciparum* and *P. vivax* has significantly increased compared to earlier findings. Mixed infection easily leads to misdiagnosis/misinterpretation of *Plasmodium* species to improper treatment, as the treatments for *P. falciparum* and *P. vivax* species are different. Therefore, in routine clinical laboratories, using an accurate combination of diagnostic procedures to identify suspected patients with mixed infections is crucial for therapeutic decisions, prompt treatment, and effective patient management.

Subject Areas

Infectious Diseases

Keywords

Plasmodium falciparum, *Plasmodium vivax*, Mixed Infection, Expatriates, Imported Malaria, Microscopy

1. Introduction

Malaria is a protozoan infection of global public health concern with the highest burden in sub-Saharan Africa and Southeast Asia where the disease is endemic [1]. Half of the world population (3.95 billion people) in 87 countries and territories is still at risk of malaria. Despite widespread control and elimination efforts, malaria still continues to be the most important parasitic disease worldwide, affecting 241 million people and causing 627,000 deaths in 2020, mostly in tropical and subtropical countries [1]. This represents about 14 million more cases in 2020 compared to 2019, and 69,000 more deaths [1]. Malaria is the third highest infectious disease killer of children in the world. Every two minutes, a child under the age of five dies from malaria [2]. The major burden of malaria infection is in African and Asian countries. Twenty-nine countries accounted for 95% of malaria cases globally [1]. Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%) and Niger (3%) accounted for about 51% of all cases globally [1]. The World Health Organization (WHO) African Region, with an estimated 241 million cases in 2020, accounted for about 94% of cases. About 95% of malaria deaths globally were in 31 countries. Nigeria

(23%), the Democratic Republic of the Congo (11%), the United Republic of Tanzania (5%), Mozambique (4%), Niger (4%) and Burkina Faso (4%) accounted for about 51% of all malaria deaths globally in 2020. India accounted for about 86% of all malaria deaths in the WHO South-East Asia Region [1].

It is now well-documented that population movements play an important role in the spread and introduction of malaria in non-endemic areas [2] [3]. Each year, more than 125 million international travelers from malaria non-endemic countries visit malaria endemic countries and fall ill with malaria while visiting malaria endemic countries/territories, and well over 10, 000 people are reported to become ill with malaria after returning home [4]. Similarly, over 35 million people from malaria-endemic countries visit the non-endemic developed world. Furthermore, military conflicts, civil unrests and ecologic changes have also contributed to global resurgence of malaria as large number of unprotected and non-immune refugees have moved into malaria-endemic areas [4].

There have been no reports of local malaria transmission in most of the Middle Eastern countries including Kuwait, except a few reports from Saudi Arabia and Oman [5]-[10]. Malaria was eradicated from Kuwait in 1963 and no autochthonous cases were reported afterward. The cases reported after the year 1963 are imported among expatriates coming to live or work in Kuwait [10]. However, imported malaria was a major public health problem during the 1990s in Kuwait; around 1400 cases were detected annually among migrant workers from malaria-endemic countries [10]. In the years 1993 and 2019, malaria decreased from 1400 cases to less than 200 cases in Kuwait due to active preventive measures. During the year 1985 to 2000, the majority of cases were infected with *P. vivax* (>70%), followed by *Plasmodium falciparum* (>25%) and a small minority (<5%) had mixed infections with *P. falciparum* and *P. vivax* [10]. The aim of this study was to determine the incidence of imported malaria cases during 2015 to 2019, identify the infecting *Plasmodium* species, and evaluate the impact of preventive measures on the incidence of malaria in Kuwait.

2. Materials

Malaria infection is a notifiable disease in Kuwait. All the migrant workers from malaria endemic countries entering Kuwait for the 1st time are required to carry a recent malaria-free certification issued by the designated Diagnostic Laboratories in their home countries. In addition, they are also screened for malaria infection at the Medical Center for Labor Examination, Ministry of Health. Kuwait is a small country consisting of six administrative governorates. The current population of Kuwait is 4.42 million; 1.45 million are Kuwaitis citizens and remaining 2.97 million are foreign nationals from over 100 different countries [11]. All malaria positive cases are referred to the Malaria Reference Laboratory, Infectious Diseases Hospital (MRL, IDH), Kuwait for treatment and then followed by the preventive departments of the area clinics. In addition, any resident in Kuwait suspected of malaria infection is referred to the MLR, IDH for con-

firmation and therapeutic management. For all cases, the information about the civil identification number, age, gender, nationality, referring hospital, date of sample collection, major clinical symptoms, recent travel history, and test results were recorded in the MLR, IDH registry logbooks. The epidemiologic and demographic data of all malaria cases were collected from the registry logbooks of MRL, IDH during the period January 2015 to December 2019.

3. Methods

The following methods were used to diagnose the infection of malaria.

3.1. Diagnosis of Malaria

The diagnosis of malaria infection was performed on the venous blood samples collected in EDTA collection tubes. Thick and thin blood films were prepared from each specimen. The thick film was fixed in acetone and the thin film in methanol and stained with Giemsa stain for 30 minutes to detect the parasite stage/s and identify the species by light microscopy [10] [12].

3.2. Detection of *Plasmodium* Species by Real-Time Polymerase Chain Reaction (PCR)

Real-time PCR identification of *Plasmodium* species was performed blinded to the microscopy results. A genus-specific primer set corresponding to the 18S rRNA was used to amplify the target sequence. All PCRs were performed using the Light Cycler-Fast Start DNA Master Hybridization Probes kit (Roche Applied Science) as described earlier [13]. Internal control was used to monitor the DNA isolation procedure and to check for possible PCR inhibition. Briefly, each reaction had a final concentration of 4 μM $MgCl_2$, 0.5 μM primer PF1, 1.0 μM primer PF2, 0.2 μM probe PF3, and 0.4 μM probe PF4. The amplification and detection was carried out as described earlier with minor modifications to the thermal profile [13]. Amplification of different *Plasmodium* species yielded products with different melting temperature curves and mixed infections showed more than double curve with species-specific melting temperatures. The limit of detection of the assay was determined at 1 - 5 parasites/μL of blood (≤0.0001% of infected red blood cells).

4. Results

The incidence of imported malaria infection was reported during 1992-1996 and 2015-2019 and evaluated the impact of malaria preventive measures in Kuwait, a non-endemic country and the change of the malaria species shifts among the expatriate brings the malaria infection in Kuwait. The number of imported malaria cases reported in Kuwait during 1985-2019 is given in **Figure 1**. During the period between January 2015 and December 2019, a total of 5800 suspected malaria cases were referred to the Infectious Diseases Hospital for malaria diagnosis. Among them, 1549 (26.7%) cases tested positive using thick and thin blood

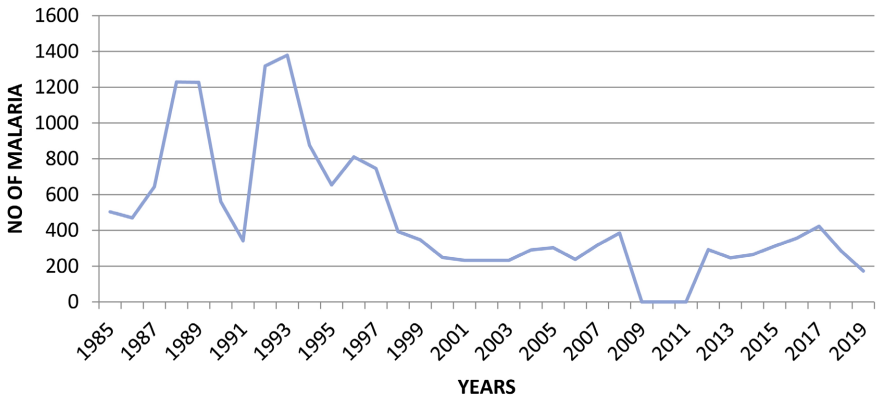

Figure 1. Number of imported malaria cases detected among expatriates in Kuwait between 1985-2019.

films microscopy (**Table 1**). The number of malaria cases was higher among males (n = 1218; 78.6%) as compared to females (n = 331; 21.3%) (**Table 1**). The number of malaria cases detected among the expatriates in different regions during 2015-2019 is given in **Figure 2**. Giemsa stained microscopy of thick and thin blood films was performed to confirm the diagnosis of malaria species. Selected samples were retested with PCR assay to reconfirm the malaria species. During the 5 years (1992-1996) 1112 (0.2%) cases and (2015-2019) 1549 (26.17%) imported cases of malaria were detected, 78.6% were males and 21.3% were females (**Figure 3**). During 1992-1996 the different species of malaria were detected as follows; Pf, 41.3%; Pv, 52.1% and mixed infection of Pf + Pv were 15.1% (**Table 2**; **Figure 4**), whereas in 2015-2019; Pf, 25.7%; Pv, 4.8% and mixed infection of Pf + Pv were 69.5% (**Table 3**) (**Figure 5**). Only 18 cases were reported among Kuwaiti nationals, all with a history of travel to African countries. The majority of malaria cases were detected during May and October.

The age of patients with malaria ranged from 1 to 73 years with a mean of 34.1 years. The mean age of female patients (30.5 years) was significantly lower than that of males (**Figure 3**). Most of the cases had come to Kuwait from several malaria endemic regions in the descending order as follows: Asia (1149; 74.2%), Africa (360; 23.24%), Middle East (29; 1.9%), America (7; 0.5%), and Europe (4; 0.3%) (**Figure 2**). All the patients were cured with ant-malarial treatment, except ten cases of mixed malaria infection (Pf + Pv) that showed the recrudescence/relapse 3 - 4 months after clinical and radical anti-malarial treatment since starting the Coartem and Artesunate five years before.

Selected (N = 24) samples were blindly tested for both microscopy and real-time PCR to further confirm the microscopy data with respect to malaria positivity and the detection of infecting *Plasmodium* species. The results showed that there was 100% concordance in the microscopy and the real-time PCR results, *i.e.* 20 samples were positive and four samples were negative by both tests (**Table 4**). Further, all the 20 positive samples were found to have mixed infection (Pf + Pv) using both the tests.

Table 1. Yearly and month-wise distribution of microscopy confirmed malaria cases by Gender (2015-2019).

Year	Sex	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	%
2015	M	8	11	11	9	16	13	31	44	48	24	17	12	244	**78**
	F	0	1	4	4	5	4	6	10	10	10	10	5	69	**22**
2016	M	11	10	11	13	34	36	43	38	49	28	17	18	308	**86**
	F	4	1	2	6	8	3	3	9	4	5	2	2	49	**14**
2017	M	13	15	23	18	35	38	46	48	34	35	15	7	327	**79.5**
	F	1	4	9	12	14	3	7	8	8	13	3	2	84	**20.5**
2018	M	18	6	8	17	17	21	33	26	26	21	11	2	206	**70.3**
	F	1	4	4	11	8	8	12	12	12	9	2	4	87	**29.7**
2019	M	8	1	3	6	13	13	24	18	16	14	9	8	133	**76**
	F	1	0	2	2	3	2	4	5	7	4	8	4	42	**24**
Total	**M**	**58**	**43**	**56**	**63**	**115**	**121**	**177**	**174**	**173**	**122**	**69**	**47**	**1218**	**78.6**
	F	**7**	**10**	**21**	**35**	**38**	**20**	**32**	**44**	**41**	**41**	**25**	**17**	**331**	**21.3**
Grand Total														**1549**	

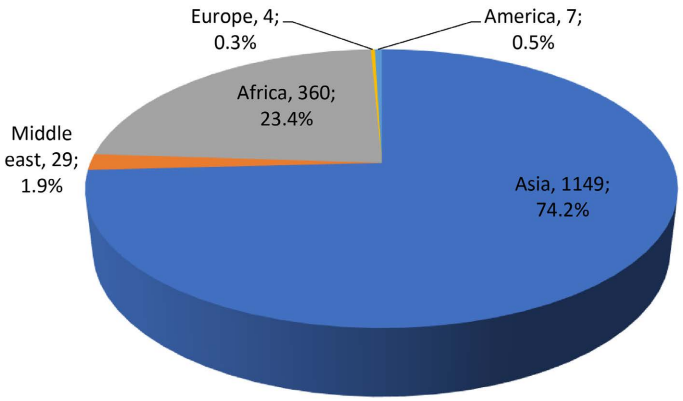

Figure 2. Number of malaria cases detected by microscopy in individuals belonging to different Regions of the world (2015-2019).

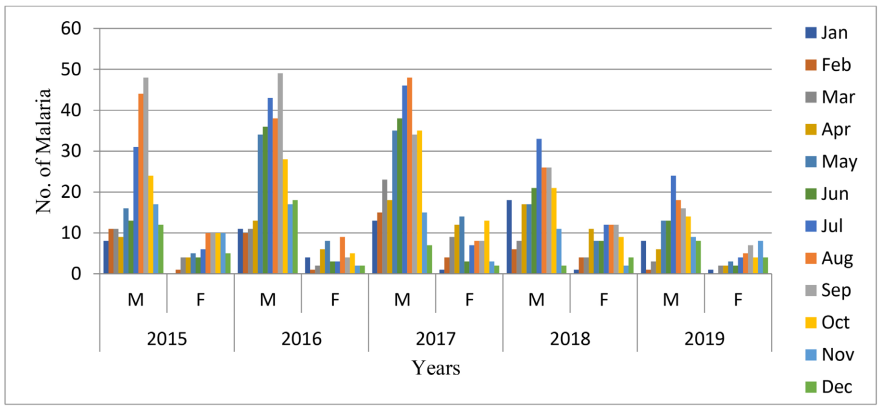

Figure 3. Number of malaria cases detected among males and females between 2015-2019.

Table 2. Yearly distribution with respect to number and percentage of malaria cases during 1992-1996 by the infecting *Plasmodium* species.

years	Number of samples checked	Total positive			
		No. of positive	Different species		
			Pf	Pv	Pf + Pv
1992	99,672	214	80	188	25
1993	153,000	400	157	168	74
1994	97,862	178	82	79	30
1995	96,321	117	41	63	13
1996	106,456	203	99	81	22
	553,311	**1112** **0.2%**	**459** **41.3%**	**579** **52.1%**	**168** **15.1%**

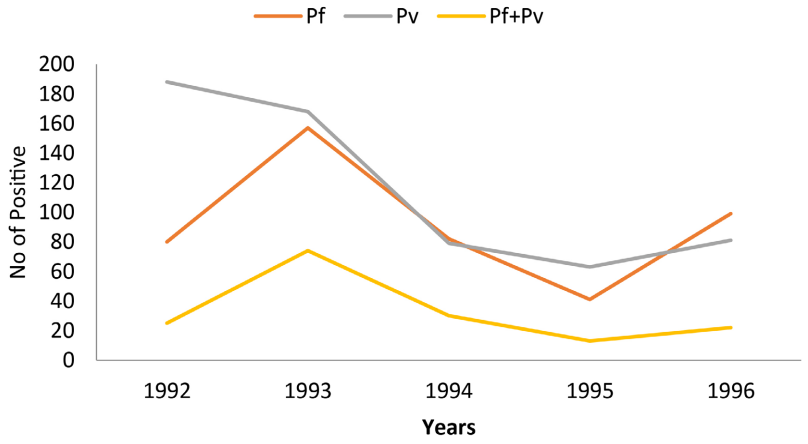

Figure 4. Yearly distribution (1992-1996) of malaria cases by the infecting *Plasmodium* species, Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*.

Table 3. Yearly distribution with respect to number and percentage of malaria cases during 2015-2019 by the infecting *Plasmodium* species.

years	Number of samples checked	Total positive			
		No. of positive	Different species		
			Pf	Pv	Pf + Pv
2015	1180	312	89	20	203
2016	1159	356	54	24	278
2017	1547	423	101	18	304
2018	1019	285	81	12	192
2019	895	173	73	1	99
Total	**5800**	**1549**	**398**	**75**	**1076**
%		**26.7%**	**25.7%**	**4.8%**	**69.5%**

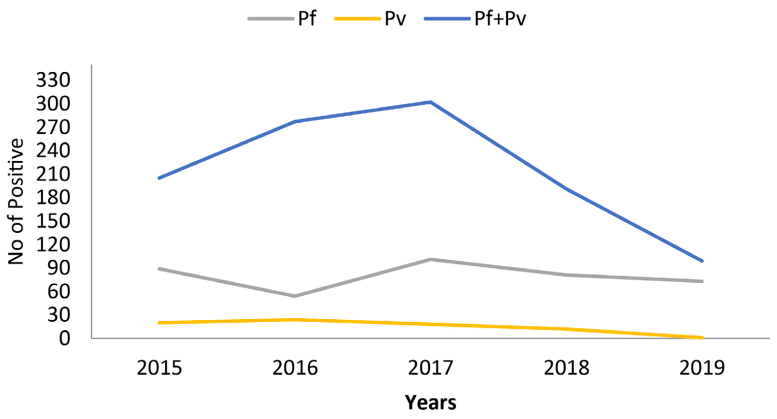

Figure 5. Yearly distribution (2015-2019) of malaria cases by the infecting *Plasmodium* species, Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*.

Table 4. Comparison of the blood samples for malaria diagnosis by PCR and microscopy.

S. No.	Position of tubes	Sample No.	Malaria Results		
			Results	PCR	Microscopy
1	A1	N1	Positive	Pf + Pv	Pf + Pv
2	B1	460	Positive	Pf + Pv	Pf + Pv
3	C1	560	Positive	Pf + Pv	Pf + Pv
4	D1	556	Positive	Pf + Pv	Pf + Pv
5	E1	538	Positive	Pf + Pv	Pf + Pv
6	F1	539	Positive	Pf + Pv	Pf + Pv
7	G1	518	Positive	Pf + Pv	Pf + Pv
8	H1	588	Positive	Pf + Pv	Pf + Pv
9	A2	466	Positive	Pf + Pv	Pf + Pv
10	B2	597	Positive	Pf + Pv	Pf + Pv
11	C2	598	Positive	Pf + Pv	Pf + Pv
12	D2	567	Positive	Pf + Pv	Pf + Pv
13	E2	594	Positive	Pf + Pv	Pf + Pv
14	F2	463	Positive	Pf + Pv	Pf + Pv
15	G2	584	Positive	Pf + Pv	Pf + Pv
16	H2	581	Positive	Pf + Pv	Pf + Pv
17	A3	T1	Negative	Negative	Negative
18	B3	T2	Positive	Pf + Pv	Pf + Pv
19	C3	T3	Positive	Pf + Pv	Pf + Pv
20	D3	T4	Positive	Pf + Pv	Pf + Pv
21	E3	T5	Positive	Pf + Pv	Pf + Pv
22	F3	17035553	Negative	Negative	Negative
23	G3	17035554	Negative	Negative	Negative
24	H3	17035550	Negative	Negative	Negative

Pf = *Plasmodium falciparum*, Pv = *Plasmodium vivax*.

5. Discussion

Malaria is a common and life threatening disease of the poor in tropical and subtropical countries of the world and a leading cause of sickness and death. In areas with high transmission, the most vulnerable groups are young children, who have not developed the immunity to malaria yet, and pregnant women, whose immunity has been decreased by pregnancy. The costs of malaria to individuals, families, communities and nations are enormous [1].

The relative risk of malaria infection in travelers visiting different countries can be roughly estimated by using the surveillance data and the disease characteristics in different areas. Approximately, 2.5 million of 4.207 million inhabitants in 2019 in Kuwait were expatriate workers from several malaria-endemic countries. The survey of imported malaria cases in Kuwait between January 2015 and December 2019 was conducted and the data obtained are compared with a previous survey of 1992-1996.

Our data of this study showed that 1549 malaria cases were detected among 5800 suspected cases in Kuwait with yearly low of 173 cases in 2019 to a high of 423 cases in 2017. This rate is considerably lower than the data reported in an earlier study conducted during 1993-1994 showing an infection rate of >1400 cases/year of imported malaria cases (**Figure 1**) [10]. Since the total population of Kuwait has changed drastically in recent years due to influx of large number of expatriates mainly from malaria-endemic countries, it was of interest to determine the incidence rate of malaria during this study period. The incidence of total malaria cases are in declining trend in the number of imported malaria cases over the years, especially after 1994 (**Figure 1**). This is likely due to the pre-screening of all migrant workers from malaria-endemic countries in their home countries before their travel to Kuwait. Further, screening of all expatriates for malaria is performed after entry into Kuwait. The number of malaria cases reported among returning resident expatriates increased after visiting their home country due to reinfection. Similar observations have also been made in other adjoining Gulf countries including Bahrain [14], Qatar [15] [16], United Arab Emirates [17] and Saudi Arabia [18] [19] [20] [21] [22].

The clinical and epidemiological features recorded in our study showed that an overwhelming majority of malaria cases (1149; 74.2%), were detected among expatriates coming from malaria-endemic countries, such like India, Pakistan and Afghanistan. Only 18 cases of malaria were detected in Kuwaiti nationals, all with a history of travel to malaria-endemic African countries. The majority of malaria cases were detected among Indians who also form the majority single ethnic group among expatriate residents in Kuwait [23]. Similar data have also been reported from other Arabian Gulf countries, such as Bahrain, Qatar, United Arab Emirates and Saudi Arabia which also have large expatriate population, particularly from the Indian sub-continent [14]-[22].

The majority of malaria cases were detected among males from May to October, which coincides with the rainy season/peak malaria infection period in the home

countries of expatriate workers who either came or returned to Kuwait after summer/winter holidays [24]. Males also comprised >90% of all imported malaria cases in Qatar [15], UAE [17] and Saudi Arabia [21]. Furthermore, similar seasonal trend was also reported from other Gulf countries with most imported cases detected during the months of August and September [15] [17] [22]. The malaria cases among returning expatriates could be the result of declined immunity due to their prolonged stay in non-endemic Kuwait which probably resulted in increased susceptibility to malaria infection during their subsequent visits to home countries. Presently, there is no sensitive biomarker available to measure the anti-parasite immunity in the residents or travelers [25].

The interesting finding of our study was the change of the trend of malaria species infection from (1992-1996) to (2015-2019). During 1992-1996 the distribution of malaria species was as follows; *P. falciparum* 41.3%, *P. vivax* 52.1% and mixed infection of (Pf + Pv) 15.1%, whereas, in 2015-2019 the distribution of malaria species was found different like, *P. falciparum* 25.7%, *P. vivax* 4.8% and mixed infection of (Pf + Pv) 69.5%, respectively. However, the mixed infection (Pf + Pv) in 2015-2019 was the dominant species than the previous studies. Most of the cases of mixed infection (97%) were found among Indian nationals. The possibility of misdiagnosis of malaria species was ruled out by retesting several selected samples by a molecular (PCR) assay, *i.e.* real line PCR (**Table 4**). In an earlier survey carried out during 1992-2000 in Kuwait, *P. vivax* was the dominant *Plasmodium* spp. detected among the migrant workers, including expatriates from India [10]. It is important to mention here that *P. falciparum* which had dominated malaria species among Indians earlier, is now showing a decreasing trend over the last few years, 41.3% in 1992-1996 to 25.7% in 2015-2019 while majority of malaria cases are now caused by the mixed infection of Pf + Pv. Several recent studies from India which had employed molecular (PCR) methods for the diagnosis of malaria had also reported the mixed Pf + Pv infections in 11% to 45% of patient samples [26] [27]. Several studies have also concluded that since field diagnosis of malaria at the primary health care level in India is mostly performed by microscopy therefore, cases of mixed species infection are usually misdiagnosed due to inexperienced microscopists [28] [29] [30] [31]. The microscopy technique is very sensitive and accurate but it requires very experienced and trained microscopists. This is also evident from the fact that 17% of mixed infections were initially identified as monoinfections due to *P. falciparum* in one study from India [28]. Recently a field-applicable ultrasensitive CRISPER-based diagnostic assay is developed for the detection of DNA sequences from all malaria species in symptomatic and asymptomatic malaria patients [32].

The detection of mixed *P. falciparum* and *P. vivax* infections in 69.5% of malaria cases in Kuwait is the highest reported so far from among the GCC countries. The majority of imported malaria cases in Qatar and United Arab Emirates are caused by *P. vivax* followed by *P. falciparum* [16] [17], and mixed *P. falciparum* and *P. vivax* infections are less frequent, occurring only in 2% of the cases

[16] [33]. On the contrary, *P. falciparum* malaria is more common in locally acquired malaria cases while an increasing proportion of imported cases were caused by *P. vivax* in Saudi Arabia [5] [18] [22]. Mixed *P. falciparum* and *P. vivax* infections were reported in only 7% of malaria cases in one study [6], while in another study, nearly 2% of PCR-confirmed malaria cases (n = 369) were due to mixed infections that were missed by microscopy alone [34]. Misdiagnosis is the main reason for this malaria species variation though the expatriate population is the same in all the Gulf countries, therefore, the microscopists must be well trained/experienced to differentiate malaria species correctly. In Kuwait, all the malaria negative and positive slides are confirmed by a very experienced consultant, whereas in other Gulf countries the technical staff is the sole responsible for the diagnosis and they misdiagnose the mixed infection due to lack of training/experience.

All the mixed uncomplicated infections with Pf + Pv were treated with Coartem (artemether 20 and lumefantrine 120) as follows: (4 tablets stat, 4 tablets 8 hrs later, 4 tablets 24 hrs after the first dose, 4 tablets 36 hrs. after the first dose, 4 tablets 48 hrs after the first dose, final 4 tablets 60 hrs after the first dose (total 24 tablets) in Infectious Diseases Hospital. The complicated cases of malaria are treated with Artesunate (The recommended dosage of Artesunate for Injection is 2.4 mg/kg administered intravenously at 0 hours, 12 hours, and 24 hours, and thereafter, administered once daily until the patient is able to tolerate oral antimalarial therapy). The clinical treatment was followed by radical treatment in the preventive department of the area health clinic with the standard dose of primaquine (0.5 mg/kg/day) and chloroquine (5.0 mg/kg/day) weekly for 8 weeks to clear out the hypnozoite stage of the *P. vivax* and *P. ovale* and asexual stages of residual blood stage malaria parasites not killed during clinical treatment. However, the WHO has recommended the dosage of primaquine (0.25 mg/kg/day) for 14 days. However, at lower dose of primaquine the high percentage of relapse has been observed after 3 - 4 months with no travel history to the malaria endemic countries [35]. We are using the lower dose of primaquine by increasing the time period to minimize the side effect of hemolysis especially in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. All the malaria infected patients were checked for glucose-6-phosphate dehydrogenase (G6PD) before the radical treatment to avoid the hemolysis. A number of studies have reported in mixed malaria, an increase in relapse rates by *P. vivax* following treatment of *P. falciparum* [36]. Though, the most common cause of *P. vivax* relapse is poor adherence to the duration of treatment or inappropriate dose. In Kuwait the radical treatment is given to the patient by the health inspectors in the preventive department and the patient has to swallow the medicine in their presence, therefore, the dose, duration and schedule were strictly followed. However, the failure in treatment may be only due to impairment in CYP2D6 enzyme and should be investigated [37].

Autochthonous malaria cases have been reported only from two GCC countries (Oman and Saudi Arabia) [7] [19], whereas, autochthonous malaria cases

have not been detected in Kuwait so far since 1963. However, the Kuwait is very vulnerable to reintroduction of malaria infection due to certain risk factors, like: i) change in the ecological conditions due to an enthusiastic drive for making Kuwait green by plantation throughout the country, which may increase the breeding of the mosquitoes by increasing the humidity and lowering the temperature. There are three species of Anopheles are found in Kuwait and these are; *A. stephensi*, *A. pulcherrimus* and *A. pharoensis*. The breeding of the mosquitoes are below the threshold for transmission due to arid conditions and the breeding places are far from the residential areas, ii) due to development in farming the extensive use of water for agricultural irrigation and domestic purposes.

6. Conclusion

The interesting finding of our study is the change in the trend of malaria species distribution/infection now from about a decade before. During a decade before *P. vivax* was the dominant species followed by *P. falciparum*, and mixed infection of (*P. falciparum* + *P. vivax*), whereas, after a decade the mixed infection of (*P. falciparum* + *P. vivax*) is dominant species followed by *P. falciparum* and *P. vivax*. The most striking finding of this study is a very high incidence of mixed infection with *P. falciparum* and *P. vivax* among workers from India (97.4%). The increase in the mixed infection with *P. falciparum and P. vivax* has to be evaluated carefully to know the reasons for the trend change. Only ten cases of mixed malaria infection showed recrudescence/relapse 3 - 4 months after clinical and radical anti-malarial treatment since starting the Coartem and Artesunate five years before. A significant change in the drug sensitivity pattern of *P. falciparum* isolates from these countries and the finding of imported mixed *Plasmodium* infections from India will have an important impact on the future therapeutic, as well as prophylactic policies for *P. falciparum* infections in Kuwait.

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Conflicts of Interest

The authors declare no conflicts of interest.

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