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The impact of climatic variables on the population dynamics of the main malaria vector, *Anopheles stephensi* Liston (Diptera: Culicidae), in southern Iran

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ABSTRACT

Objective: To determine the significance of temperature, rainfall and humidity in the seasonal abundance of *Anopheles stephensi* in southern Iran.

Methods: Data on the monthly abundance of *Anopheles stephensi* larvae and adults were gathered from earlier studies conducted between 2002 and 2019 in malaria prone areas of southeastern Iran. Climatic data for the studied counties were obtained from climatology stations. Generalized estimating equations method was used for cluster correlation of data for each study site in different years.

Results: A significant relationship was found between monthly density of adult and larvae of *Anopheles stephensi* and precipitation, max temperature and mean temperature, both with simple and multiple generalized estimating equations analysis (P<0.05). But when analysis was done with one month lag, only relationship between monthly density of adults and larvae of *Anopheles stephensi* and max temperature was significant (P<0.05).

Conclusions: This study provides a basis for developing multivariate time series models, which can be used to develop improved appropriate epidemic prediction systems for these areas. Long-term entomological study in the studied sites by expert teams is recommended to compare the abundance of malaria vectors in the different areas and their association with climatic variables.

KEYWORDS: *Anopheles stephensi*; Climatic variables; Monthly activity; Iran

1. Introduction

According to the 2018 World Health Organization report, about 228 million cases of malaria were recorded worldwide, resulting in 405 000 deaths[1]. Iran is currently at an advanced phase of malaria elimination, but there are still few indigenous cases of the disease reported every year. According to the Iran's Ministry of Health, the most important malaria foci are located in the Sistan & Baluchistan, Hormozgan, and Kerman provinces in the south and southeastern parts of the country[1,2].

Seven *Anopheles* mosquitoes are known to be the main malaria vectors in Iran: *Anopheles (An.) stephensi*, *An. culicifacies* s.l., *An. superpictus* s.l., *An. fluviatilis* s.l., *An. dthali* and *An. maculipennis* complex including *An. sacharovi*[3]. Among these vectors, *An. stephensi* is the main and the most important malaria vector in southern Iran[4]. Various parts of Asia including Iran, Iraq, Afghanistan, Bahrain, Bangladesh, China, India, Kuwait, Myanmar (Burma), Nepal, Oman, Pakistan, Qatar, Saudi Arabia, Thailand, United Arab Emirates, and recently, Ethiopia, Djibouti in Africa, and Sri Lanka in Asia are endemic areas for this species[3,5-7].

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This species has been reported in 10 out of the 31 provinces of Iran, particularly in plain areas, all the southern coastal areas, hills, mountains and in the southern regions up to 900 meters above sea level[3,4]. *Anopheles stephensi* is active in the southern coastal regions of Iran throughout the year, with two peaks of activity in April-May and September-October. In the cold mountainous region of southern Iran, it is active from April to November, with a peak in July[4]. This endophagous and endophilic species is distributed mainly in moderate semi-arid and absolute arid climates, although its presence in high semiarid and slight semi-arid climates has also been reported. Previous studies have indicated that it is the most prevalent anopheline species in the malaria-endemic area of southern Iran[3]. *Anopheles stephensi* has a wide range of breeding places including urban and rural habitats. In urban areas this species has been collected from pools, catch basins, ponds, seepages, and stream margins. Breeding places documented for *An. stephensi* in rural areas include irrigation canals, margins of streams, pools, seepages, streambeds, marshy areas with gentle water flow, palm irrigation canals, and rivers[3].

Mosquito larvae development and the survival and behaviour of adult mosquitoes are dependent on various environmental factors[8,9]. Regions with warm temperatures, humid conditions, and high rainfall are preferred habitats for *Anopheles* mosquitoes[10]. Weather conditions can directly affect malaria incidence through the modification of vector population dynamics, duration of gonotrophic cycles, biting rates, fecundity, survival and development of larvae and adult, as well as the development of the malaria parasites inside the mosquito. Temperature, rainfall and humidity have long been associated with the population dynamics of mosquitoes, which directly affects malaria transmission[8,9].

The global warming is of major public health importance partly because it has provided suitable conditions for the proliferation of vectors of diseases such as mosquitoes and ticks, and has allowed these vectors to adapt to different seasons, migrate and spread to new niche areas that have become warmer[10]. Distribution modelling studies have suggested that climate change can alter the distribution of vector-borne diseases, including malaria, causing rapid spread of these disease into areas that were previously free of the disease[11,12]. Moreover, the quality and quantity of adult mosquito breeding sites may also be significantly affected by meteorological factors. Understanding the relationship between climatic variables and the occurrence of *Anopheles* mosquitoes can provide important information for determining the activity of the parasites and, consequently, the risk of malaria. Detailed information on seasonal mosquito activity in a region is essential for the development of effective vector control programs[9].

Malaria elimination programs may fail due to reintroduction of the disease. To prevent this failure, adequate information about the current and future distributions of malaria vectors is of paramount importance for efficient and evidence-based planning for integrated vector control activities to sustain elimination[13]. The analysis of the correlation between species and environment is always a fundamental issue in ecology, and the quantification of this relationship is the basis of the modeling of geographical prediction[14]. But so far in Iran, the exact relationship between seasonal activity of *An. stephensi* and meteorological data has not been extensively studied. Only the relationship between meteorological factors and the epidemiology of malaria has been investigated in three malaria foci in Iran[15,16]. Therefore, this study was conducted to determine the association between population dynamics of *An. stephensi* and meteorological variables (temperature, rainfall and humidity) in malaria endemic areas of Iran.

2. Materials and methods

2.1. Study area

The three provinces studied in this research (Hormozgan, Sistan & Baluchistan, and Kerman) are located in the south and southeast of Iran. The land area of these three provinces is about 435 675 km^2 (Figure 1).

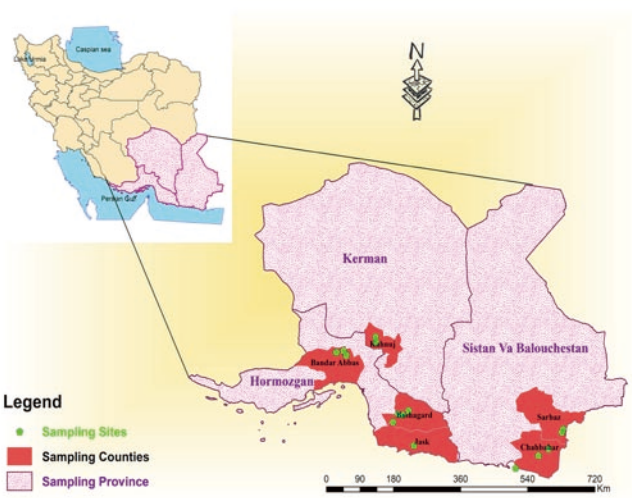

Figure 1. Sampling sites of *Anopheles stephensi* in southern provinces of Iran, 2002-2016.

Hormozgan Province is one of the southern provinces of Iran located in the north of the Strait of Hormuz between the geographical coordinates of 25°24’-28°57’ N and 53°41’-59°15’ E. The climate of the province is tropical, with mostly hot and a short cold season. Warm season with high relative humidity lasts nine months. The cold season lasts about three months which begins in early December and is influenced by the cool wind masses of the West. The temperature rarely reaches zero degree. Basically, the climate of the province is like the climate of the desert areas with extremely small amount of rainfall. In this area, significant rainfall occurs in only one or two occasions throughout the year, and during such occasions, rainfall is often heavy and can cause flooding[17].

Sistan & Baluchistan Province is located between 25°3’-31°28’ N and 58°47’-63°19’ E in the southeast of Iran. It is a desert with dry climate, annual rainfall ranges between 130 mm and 170 mm. The maximum temperature 50 ℃, and the minimum 7 ℃-8 ℃ below zero, with a relative humidity between 50%-95%[17].

Kerman Province is situated between 53°26’-59°29’ E and 25°55’-32° N. The weather in this province is quite variable. Changes in temperature are very sharp between cold and hot seasons, and even between nighttime and daytime, the temperature may show a sharp change. The average annual rainfall is about 185 mm. The runoff of precipitation begins in November and continues until May of the following year, with the highest rate from January to May[17].

2.2. Species data collection

We carried out an extensive electronic literature search to gather all the relevant data on the monthly abundance of *An. stephensi* larvae and adult from earlier studies conducted from 2002 to 2019. Articles and theses published in English and Persian languages were included in our review. We limited our inclusion criteria to studies that explicitly cover the monthly adult and larval abundance of *An. stephensi* in south and southeast of Iran.

The search was run in electronic databases including: Scopus, Web of Science, PubMed, Google Scholar, Digital Library of Tehran University of Medical Sciences (TUMS) (for MSc/PhD thesis), Scientific Information Database (SID), and Irandoc. Searching time range was from 2000-2019, but after 2016 there was no published ecological study on this species in Iran. Different keyword combinations used to capture the fullness of the topic included *Anopheles*, malaria, monthly activity, Iran, Sistan & Baluchistan, Hormozgan, and Kerman. Out of 463 papers and theses that were conducted over the specified time period retrieved from our literature search, seven studies met our inclusion criteria *i.e.* conducted in the south and southeastern part of Iran on the monthly activity of *An. stephensi* over a year period in consistent with the purpose of this study (Figure 2). Total catch and dipping methods were used in these seven studies[15,18-23], respectively for adult and larvae sampling. These studies were carried out in more than 20 villages in six counties of the three provinces: Chabahar and Sarbaz counties in Sistan & Baluchistan Province; Bandar Abbas, Jask, and Bashagard counties in Hormozgan Province; and Kahnooj County in Kerman Province (Figure 1). Monthly activity of larvae and adult *An. stephensi* over a year for the studied counties are shown in Figures 3 and 4.

2.3. Climatic data

Data from all the synoptic stations in each city are provided to the National Meteorological Organization on a daily basis. These data include temperature, humidity, pressure, wind direction, precipitation, velocity, radiation, *etc.* Data on the average maximum and minimum monthly temperature, total monthly precipitation and average monthly relative humidity were included and analyzed in the present study. These data for the studied areas were obtained from the National Meteorological Organization[24].

2.4. Statistical analysis and mapping

Regression models are popular in modeling association between a

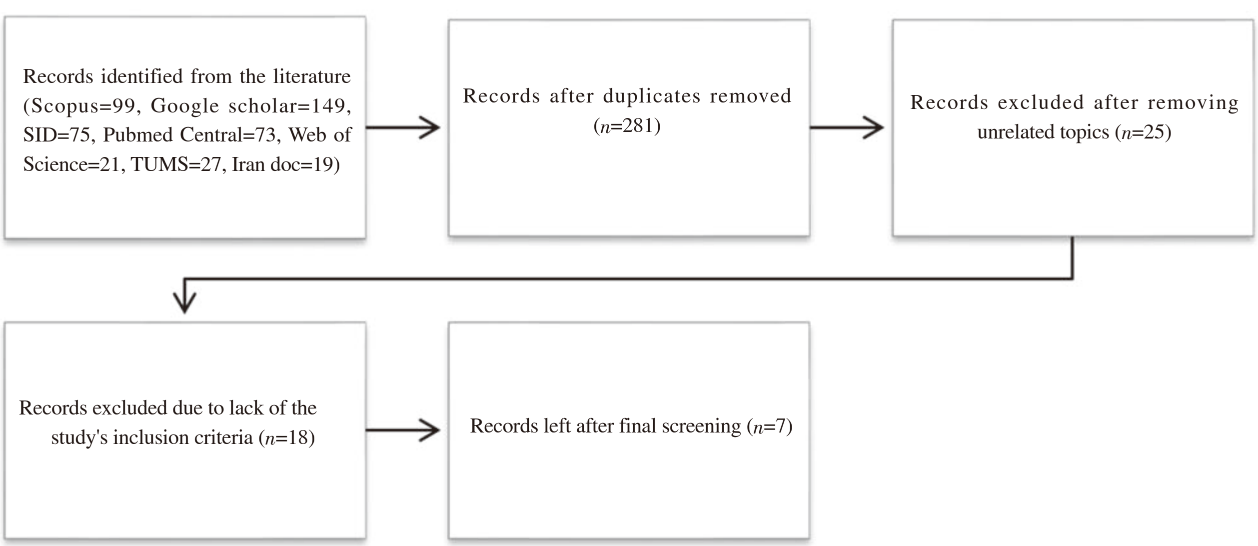

Figure 2. A flow chart showing the literature selection process.

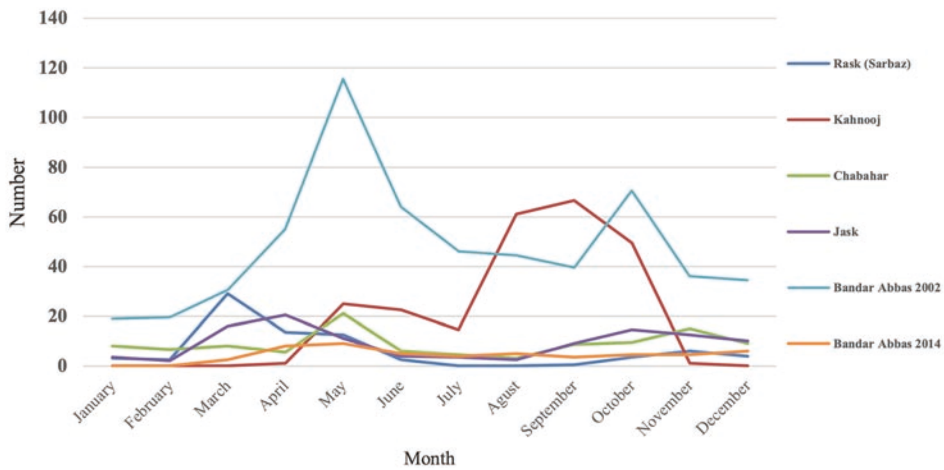

Figure 3. Monthly activity of adult *Anopheles stephensi* according to counties in south of Iran, 2002-2016. In Bashagard study, they just collected larvae, so there was no data about adults. In Bandar Abbas, we found two studies conducted in 2002 and 2014. According to the results of the studies used, the highest adult density of *Anopheles stephensi* in two studies conducted in Bandar Abbas (2002) and Kahnooj. In other studies, the density was less than 20 during the year. There were two peaks of activity in most studies.

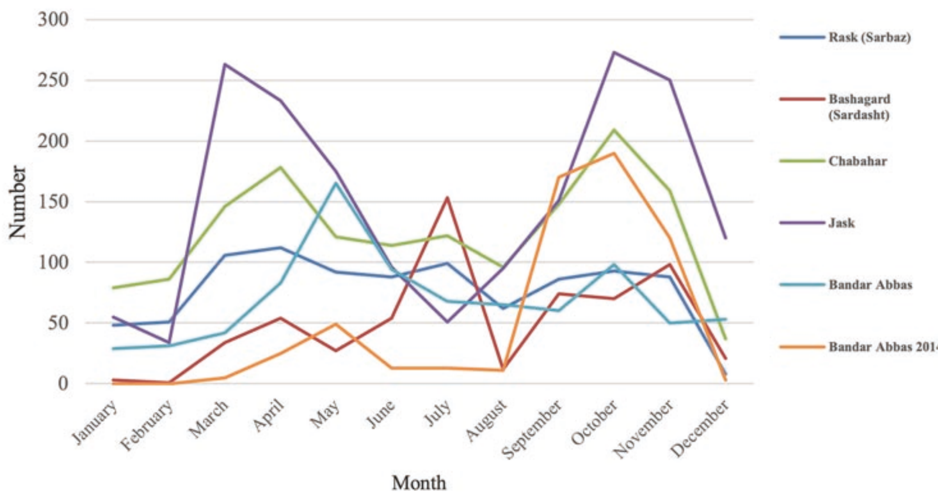

Figure 4. Monthly activity of *Anopheles stephensi* larvae according to counties in south of Iran, 2002-2016. In Kahnooj study, they just collected adults, so there was no data about larvae. In Bandar Abbas, we found two studies conducted in 2002 and 2014. The highest density of larvae was found to be in Jask County. In the case of monthly activity, the density of larvae of *Anopheles stephensi* in Sarbaz County was almost constant during the year. In the study of Bashagard County, the highest density was in July and the trend of increase and decrease has been seen during the year. In other studies, there were two peaks during the year, one in October and another in the spring between March and May.

response variable and a set of explanatory variables. Independence of observations is an assumption underlying regression models. If this assumption is violated, as in clustered or longitudinal data, common regression models are not applicable. Generalized estimating equations (GEE) is among options to deal with correlated observations that assesses marginal associations between mean response and explanatory variables. In general, if μ represents the mean of response variable y and **X** is a vector of explanatory variables, then the relation between μ and X can be written as:

$$g(\mu_{ij}) = \eta_{ij} = \mathbf{X}_{ij}\beta$$

Where i=1,2, ..., I represents the i-th cluster and j=1,2, ..., n_i is the j-th observation within cluster i that includes η_i related obsevations. β is the vector of regression coefficients. Here g (.) is called 'link function' relating μ to **X** and is chosen according the nature of response variable y. Here, our response is a count with Poisson distribution and the natural choice would be the log link as:

$$\log(\mu_{ij}) = \mathbf{X}_{ij}\beta$$

For each explanatory variable, exp (β) is interpreted as the expected change in incidence rate ratio (IRR) by 1-unit increase in the corresponding covariate. It is needed to specify how the response variables are correlated by defining a working correlation matrix from possible correlation structures. In this study we used GEE methodology to account for cluster correlation of data for each city in different years. Selection among different correlation structures were based on their fit to the current data according to quasi-likelihood information criterion (qic) proposed for GEE models[25]. The data were analyzed using Stata version 12. Statistical significance level was set at 0.05. Shape files of administrative boundaries of Iran were provided by National Cartographic Center and used for plottnig the original map of the study area in ArcMap10.5.

3. Results

The results of simple and multiple GEE analysis between climatic variables and monthly density of adult and larvae of *An. stephensi* are given in Table 1.

3.1. Relationship between climatic variables and monthly density of adult An. stephensi

In simple GEE analysis, relationship between monthly density of adult *An. stephensi* and precipitation, max temperature and mean temperature was significant (P<0.05). However, the analysis with one month lag, showed a significant relationship between monthly density of adult mosquitoes and max temperature (P<0.05). Similar to simple GEE analysis, in multiple GEE analysis there was a significant relationship between monthly density of adult *An. stephensi* and precipitation, max temperature and mean temperature (P<0.05). However, there was significant relationship between monthly density of adult *An. stephensi* and max temperature (P<0.05) when analysis was done with one month lag.

3.2. Relationship between climatic variables and monthly density of An. stephensi larvae

In simple GEE analysis, there was a significant relationship between monthly density of larvae of *An. stephensi* and precipitation and mean temperature (P<0.05). When the analysis was conducted with one month lag, significant relationships were found between larval density and relative humidity, precipitation and min temperature (P<0.05). In multiple GEE analysis, the relationship between monthly density of *An. stephensi* larvae and precipitation, and min temperature and was significant (P<0.05). Running analysis with one month lag, it showed there was a significant relationship between monthly density of larvae, and relative humidity and precipitation (P<0.05).

4. Discussion

Anopheles mosquitoes are sensitive to temperature during their life cycle, and development, so that their longevity and mortality rates all dependent on temperature[26]. The *Anopheles* mosquito life cycle

Table 1. Results of generalized estimating equations between climatic variables and density of *Anopheles stephensi*, Southern Iran, 2002-2016.

Variable		No lag			Lag=1 month		
		IRR	95% *CI*	*P*-value	IRR	95% *CI*	*P*-value
Larvae							
Simple	RH	1.008	0.998-1.018	0.104	1.019	1.001-1.038	0.034
	Precipitation	0.994	0.989-0.998	0.016	0.993	0.988-0.998	0.012
	Min temperature	1.034	1.018-1.050	<0.001	1.041	1.016-1.066	0.001
	Max temperature	1.009	0.969-1.050	0.650	1.007	0.969-1.047	0.680
	Mean temperature	1.027	0.997-1.059	0.077	1.030	0.997-1.063	0.070
Multiple	RH	1.004	0.994-1.012	0.400	1.013	0.997-1.029	0.100
	Precipitation	0.996	0.991-1.000	0.090	0.995	0.994-0.997	<0.001
	Min temperature	1.025	1.004-1.046	0.018	1.022	0.991-1.053	0.150
	RH	1.008	0.998-1.018	0.110	1.018	1.000-1.036	0.047
	Precipitation	0.994	0.988-0.999	0.042	0.995	0.992-0.998	0.001
	Max temperature	1.001	0.957-1.047	0.940	1.016	0.986-1.048	0.280
	RH	1.007	0.996-1.018	0.190	1.016	0.999-1.033	0.060
	Precipitation	0.995	0.990-1.000	0.070	0.995	0.993-0.997	<0.001
	Mean temperature	1.016	0.978-1.055	0.390	1.020	0.989-1.052	0.200
Adult							
Simple	RH	0.994	0.972-1.016	0.620	0.995	0.988-1.003	0.257
	Precipitation	0.988	0.977-0.999	0.033	0.987	0.972-1.002	0.090
	Min temperature	1.013	0.964-1.065	0.580	1.011	0.949-1.078	0.720
	Max temperature	1.097	1.075-1.120	<0.001	1.085	1.052-1.120	<0.001
	Mean temperature	1.076	1.024-1.131	0.004	1.066	0.990-1.149	0.087
Multiple	RH	0.994	0.967-1.022	0.70	0.997	0.967-1.028	0.880
	Precipitation	0.982	0.964-1.000	0.06	0.987	0.969-1.005	0.180
	Min temperature	1.005	0.929-1.088	0.88	1.004	0.909-1.108	0.930
	RH	1.010	0.996-1.024	0.160	1.012	0.999-1.024	0.056
	Precipitation	0.995	0.993-0 .997	<0.001	0.998	0.992-1.004	0.550
	Max temperature	1.107	1.079-1.136	<0.001	1.101	1.045-1.159	<0.001
	RH	0.995	0.978-1.013	0.640	0.998	0.981-1.016	0.870
	Precipitation	0.989	0.981-0.998	0.017	0.993	0.982-1.004	0.250
	Mean temperature	1.061	1.006-1.117	0.023	1.057	0.975-1.146	0.170

IRR= incidence rate ratio; RH= relative humidity; *CI*= Confidence interval.

consists of three aquatic juvenile stages (egg, larva and pupa) and adult. Also their blood feeding behavior depends on temperature[27]. The results of this study show the significant effect of climatic variables on the abundance of *An. stephensi* in the south and southeastern parts of Iran. There was a positive correlation (P<0.01) between *An. stephensi* abundance and average maximum and mean temperature. On the other hand, 1 ℃ increasing in maximum and mean temperature will increase the density of adults as 0.097 and 0.076, respectively. However, the increase total precipitation was negatively correlated (P<0.05) with the abundance of *An. stephensi* in the studied areas. Significant positive relationship was observed between mean temperature and larval abundance (P<0.001), while it was negative between total precipitation and the density of larvae (P<0.05).

Similar to our results, temperature showed a strong positive correlation with the total number of mosquitoes in a Nigerian state[28], but a negative correlation with mosquito larval abundance was reported in Saudi Arabia[29]. Moreover, the abundance of *An. gambiae* s.l. and *An. funestus* showed a strong negative association with mean temperature in Nigeria[30]. In another study conducted in Bangladesh, there was no significant association between temperature and the abundance of *An. willmori*, *An. baimaii*, *An. annulari*, and *An. jeyporiensis*. The only species with a reported negative association between abundance and temperature is *An. karwari*[9]. Because the studied counties are in tropical and subtropical area, mean and maximum temperrature is important in establishing *An. stephensi*, the main vector of malaria in these areas. Several studies on other *Anopheles* species in different regions of the world have shown the same or different results[9,28,30,31].

In the present study, total monthly precipitation showed significant relation with abundance of adult *An. stephensi* but not in one-month delay. Humidity did not show any significant relation. In some previous studies[9,28], these two climatic factors showed positive Pearson's correlation with the abundance of the adults *An. willmori* and *An. baimaii* in Bangladesh, as well as mosquito larval abundance in Nigeria. However, in some other studies, rainfall and humidity negatively correlated with the abundance of other species such as *An. karwari, An. annulari, An. jeyporiensis* in Bangladesh. The abundance of *An. gambiae* s.l., the main malaria vector in Africa, had a positive correlation with monthly rainfall in Nigeria (r=0.735, P<0.05) and Uganda (r=0.119; P=0.713). The abundance of *An. funestus*, the second main vector in Africa, had similar correlation in Nigeria, but it is negatively correlated with rainfall in Uganda (r= 0.099; P=0.761)[30,31]. *Anopheles* species use standing water as breeding sites. It seems that the southeastern provinces of the country have tropical climate, where humidity sometimes exceeds 70%, and rainfall in these areas often leads to flooding. Flooding has negative effect to mosquito abundance. There could be multiple reasons such as: i) killing a significant proportion of larvae and washing larvae out of habitat[32], ii) diluting food, iii) introduction of pollutants, iv) a lot of rainfall might decrease mosquito questing behaviour, *etc.*, v) change in water storage practices.

In a study conducted in Kahnooj, south of Kerman Province, it was reported that temperature is the most important climatic factor affecting the incidence of malaria, whereas rainfall and humidity did not have any significant effect. Based on the results of the regression model in this study, it is expected that a one degree increase in maximum temperature will increase malaria incidence by 15% in the same month and by 19% in the following month[16].

Climate warming has been linked with mosquito metabolic rate and physiology[33]. It has been suggested that the metabolic rate of *Anopheles* mosquitoes in highland areas will experience a larger shift due to rapidly increasing global temperature. The development rate of mosquito larvae is highly dependent on temperature. Developmental delays and high mortality of mosquitoes are associated with low temperature conditions[34]. Increase in ambient temperature shortens the duration of larval stage maturation[35], and in the adult stage, this leads to an increase in the rate of blood meal digestion and consequently, increased human biting frequency and parasite sporogonic development, all of which increase fecundity, reproductive fitness and malaria transmission efficiency[36,37].

Based on meteorological forecasting HadCM2 model, by the years 2020, 2050, 2075 and 2100, it is expected that surface temperature in Iran will increase by 1, 1.7, 2.4 and 3 degrees, respectively. Also, a decrease in rainfall especially in southern area is predicted[38]. It seems that these changes may alter breeding places of this species in the south of the country in the future, which may favor an increase in abundance of this species. By decreasing rainfall and increasing temperature in the future years, the micro-environment of the larval breeding places may be altered, but due to the maintenance of water in artificial sources, the abundance of larvae may increase. In some parts of the country, climate change will provide areas with high potential for growth of this species, and it may change the distribution of this species, and consequently, malaria transmission.

To conclude, relationship between different meteorological factors and vector density was found in this study. More detailed entomological data and appropriate mathematical models need to be developed to fully understand the importance of different factors in the genesis of malaria epidemics in these areas. Long-term entomological studies in selected sites by expert teams are recommended to compare the abundance of malaria vectors in the endemic different areas and their association with climatic variables.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Authors' contributions

MA contributed to the literature search and data acquisition. ARF and TJK contributed to the statistical analysis. KP contributed to the data acquisition data acquisition. AAHB designed and supervised the project. HV revised the manuscript. All authors contributed to the drafting the reviewing the manuscript.

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