Introduction

Malaria is one of the most important public health problems in Indian subcontinent. 100 million malaria cases and 1 million deaths due to malaria had been reported in 1935 in Indian subcontinent. Most of the subcontinent falls within the temperature transmission window of malaria, which is between 16° and 34°C (Mordecai et al., 2013). India is the largest country of Indian subcontinent. The topologically and climatically diverse condition of India is due to its unique geographical position (Singh et al., 2009). The country is situated between 8˚4’ and 37˚6’ north latitude and 68˚7’ and 97˚25’ east latitude. India is seventh largest country in the world with total area of 3,287,263 km. India is one of the greatest contributor of global malaria. Unique geography of India makes it hot spot for malaria transmission. India is one of the worst malaria affected country in Indian subcontinent (Alam et al., 2008). India alone contributes more than 70% of total malaria cases in Southeast Asia (Kondrachine, 1992). India is 2nd largest country by population. About 95% of India’s population is residing in malaria endemic areas (Gupta and Chowdhury, 2014). Out of this 4.2%, 32.5% and 43.8% of population is living in high, moderate and low risk area of malaria transmission (Dash et al., 2008). Eighty percent cases reported in country are confined to individuals living in tribal, hilly and inaccessible areas. Malaria is reported to the areas it was not found previously due to changing environmental conditions. Various studies have examined the isolated relationships between malaria epidemic in India and temperature (Jhajharia et al., 2013), rainfall (Bouma and Van der kaay, 1996) or irrigation practice (Baeza et al., 2011; Joshi et al., 2006; Sharma, 1996; Tyagi et al., 1995). Malaria in India is exhibiting changing trends from rural to urban malaria, from forest to plain malaria and from industrial to travel malaria (Sharma et al., 2006). Malaria is prevalent in every part of country. Orissa, Uttar Pradesh, Gujarat, West Bengal, Maharashtra, Madhya Pradesh, Rajasthan, Karnataka, Andhra Pradesh and Northeast are most affected states.

Two main threats for controlling malaria in India are insecticide resistance and antimalarial drug resistance (Das et al., 2012). Insecticides were considered as most effective tools for malaria vector (Trigg and Kondrachine, 1998; Shiff, 2002; Mabaso et al., 2004). Indoor house spraying and insecticide treated bed nets are among the
cheapest, most effective and best proven methods of controlling malaria globally (Lengeler, 2004; Wakabi, 2007). Unfortunately, mosquitoes can rapidly evolve resistance to all presently used insecticides. Insecticide resistant mosquitoes are one of the main hurdles faced by global malaria eradication plan in last century (Davidson and Zahar, 1973; Harrison, 1978; Hemingway et al., 2002; Nauen, 2007; Kelly-Hope et al., 2008). Growing insecticide resistance in predominant malaria vectors such as An. culicifacies and An. stephensi is a major concern (Singh et al., 2009). Drug resistance in Plasmodium parasite is 2nd major reason of global burden of malaria. Resistance to chloroquinone was first reported in 1973. Resistance to drug is thought to be originated in 1973 in Thailand, Burma, Bangladesh (Shah et al., 2011). All of them are neighboring countries of India. Resistance then spread across India between 1978 and 2007.

Diversity in Malaria epidemiology in India is due to presence of diverse malaria vector species (Das et al., 2012). India is home of about 60 morphologically distinct Anopheles species. Most of these Anopheles species differ in vectorial capacity. Out of these nine species of Anopheles genus belonging to Cellia subgenus have been designated as vectors of human malaria parasites. Among these, six species (An. minimus, An. fluviiatilis, An. culicifacies, An. dirus, An. stephensi, An. sundaicus) are considered as the primary vectors, while three (An. annularis, An. nivipes/An. philippinensis and An. varuna) are designated as secondary vectors (Dash et al., 2007). Except An. stephensi all primary vectors exists as species complex (Singh et al., 2009).

In natural conditions, An. stephensi is known to exist as three ecological variants, i.e. ‘mysorensis’, ‘type’ and ‘intermediate’ which can be morphologically identified by the number of ridges on their egg floats (Rao et al., 1938). The ‘type’ form has 16–22 ridges, ‘intermediate’ 12–17 and ‘mysorensis’ has 9–15 ridges. As no genomic differences between these forms are known till now, therefore, the three forms of An. stephensi cannot be considered as species complex. Of these three variants, ‘type’ form is an efficient vector causing malaria cases in urban areas, whereas ‘mysorensis’ is confined to rural area only (Subbarao et al., 1987). Despite of its high zoophilic nature and poor vectorial capacity, the ‘mysorensis’ form shares a substantial percentage of the total malaria. ‘mysorensis’ form has considerable importance as a malaria vector in Iran (Subbarao, 1998). The ‘intermediate’ form is found mainly in
rural and semi-urban area and very less information is available about its status as a malaria vector. *An. stephensi* is major urban malaria vector in India. It is responsible for about 12% of malaria cases (Adak *et al*., 2005).

A number of efforts have been made to eradicate this vector of malaria from India but burden of disease caused by this vector is still increasing. Conventional vector control measures such as indoor residual spraying and insecticide-treated nets are effective but operationally difficult, logistically demanding and relatively less effective against exophilic and exophagic vectors (Greenwood, 2009). Insecticide resistance is causing operational problems for control programmes. Resistance to DDT, dieldrin and malethion is reported in *An. stephensi* in Iran, Iraq, Saudi Arabia and Indian subcontinent (Busvine and Pal, 1969; Zahar, 1974; Abai *et al*., 2008). In India insecticide resistance has been reported in *An. stephensi* from Delhi, Goa, Haryana, Rajasthan, Gujarat and Karnataka (Ganesh *et al*., 2003; Tiwari *et al*., 2010; Shetty *et al*., 2013; Quiñones *et al*., 2015). Resistance to DDT and HCN-dieldrin in *An. stephensi* has been already reported and spread of the insecticide resistance is posing a big obstacle in malaria control in India (Singh *et al*., 2009). Due to such problems, novel vector control strategies are required.

Multi-disciplinary approaches using complimentary strategies appear to be the most promising for the control of this disease. The plan outlines the research agenda to develop tools, including new drugs, vaccines, and mosquito control strategies. The two very potential techniques tested in the past for genetic manipulation of vector species include genetic engineering and sterile insect technology. The technique of genetic manipulation had provided certain successes in managing insect populations (Alphey *et al*., 2002). It has been suggested that the manipulation of vectors through the introduction of genes conferring refractoriness to the parasite (Collins and Besansky, 1994) could be one such approach, but this will require knowledge of their population genetics and the extent of gene flow between populations. Population genetics of mosquitoes give information of gene flow between populations. Such information is useful in inferring dispersion pattern of vector and understanding role of vector in disease epidemiology. Determining genetic structure can help to understand heterogeneities in disease transmission due to genetically distinct vector populations and to predict the spread of genes of interest, such as those involved in insecticide
resistance or refractoriness (Ndo et al., 2010). Information on the population genetics of malaria vectors is important in allowing more rational management of insecticide resistance and in explaining the role of vectors in the geographical variation in malaria transmission. The success of a genetic modification programme requires the precise identification of appropriate gene for introduction into natural populations, and a clear understanding of the population genetic structure of vector.

The population genetic study can enable not only the identification of parasite origins but also routes of population movement, and therefore the likelihood of successful elimination within a given region with respect to parasite diversity, population movement and the burden of imported cases (Moonen et al., 2010). Methods used to identify genetic variation in natural population include analysis of chromosomal variations, isozymes and DNA markers (Coluzzi and Sabatinni, 1967; Mahon et al., 1976). Most commonly used methods that have been applied in studying genetic variations in Anopheles mosquito population are analysis of inversion polymorphism in polytene chromosomes and most recently there has been focus on use of DNA markers. DNA markers provide an easy approach and have been applied widely in population genetic studies. Among various DNA markers Microsatellite DNA markers has been widely studied. Microsatellite DNA has become a popular tool for genetic studies of Anopheles mosquitoes. Up to 150 polymorphic loci have been characterized for seven species in An. gambaie complex (Zheng et al., 1996).

The field of population genetics has been revolutionized by discovery of hyper-variable microsatellite DNA sequences (Zhang and Hewitt, 2003). Microsatellites are DNA fragments consisting of relatively short regions of tandemly repeated sequences such as dinucleotides, trinucleotides or tetranucleotides. In most of eukaryotic organisms, microsatellite sequences are abundant, widely dispersed throughout the genome and are highly polymorphic in length due to variation in the number of repeats within a particular locus as a result of uneven cross over or slippage of the DNA polymerase during replication (Jeffreys et al., 1985; Tautz, 1989). Microsatellites loci have been described as powerful markers for mapping qualitative and quantitative traits of mosquitoes and in the field of population genetics for measuring intraspecies differentiation because of their high polymorphism, co-dominance, abundance
throughout genome (Zheng et al., 1996). All these properties make microsatellite loci highly versatile marker.

There seems to be no information available about the population genetic structure and gene flow of *An. stephensi* in India, except in Rajasthan (Vipin et al., 2010a) where gene flow in *An. stephensi* was studied across the Aravalli mountain range, Haryana (Vipin et al., 2010b) where genetic differentiation between three ecological variants i.e. ‘type’, ‘intermediate’ and ‘mysorensis’ and Madhya Pradesh (Sharma et al., 2015) was studied using microsatellite markers. No such study appears to have been carried out covering whole India. The availability of accurate genetic information on *An. stephensi* paves the way for future development and testing of novel vector control approaches in this region. Therefore, the present investigation was undertaken with a view to analyze the genetic structuring in *An. stephensi* populations in India.