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Epidemics and diagnostic testing by NS1 and MAC ELISA for identification of dengue suspected cases in district Hoshiarpur, Punjab, India

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Abstract
Mosquitoes are essential creature that plays crucial role in the transmission of communicable diseases (Zika virus, West Nile virus, Chikungunya virus, dengue, and malaria etc.). The present study was conducted to update the status of dengue in Hoshiarpur district of Punjab. A questionnaire was prepared containing ten different questions for data collection. Data was collected from civil hospital of district Hoshiarpur. Two different diagnostic testing methods NS1 and MAC ELISA methods were used to confirm the number of positive cases of dengue. Results of the study revealed that year 2021 has recorded highest outbreaks of dengue in comparison to 2020. The reason behind increased number of cases might be climatic change, late monsoon and mismanagement of waste. Therefore, considering exponential increase in dengue cases in the hoshiarpur there is need to combat the prevalence of this communicable disease using some herbal remedies.

Keywords: dengue, NS1 ELISA, MAC ELISA, Hoshiarpur, climatic conditions

Introduction

Mosquitoes are important insects affecting human beings and other animals. When a mosquito bites any person, it not only causes skin irritation/inflammation in human beings and animals but also capable to transmit various vector borne diseases Mosquitoes are considered as one of the utmost dangerous insect species on the planet due to their potency to transmit various types of dreadful diseases. Cama ra and co-authors (2020) have reported that 700 million individuals get infected due to biting by mosquito-borne diseases annually and 1 million get died. The United State Centre for Disease Control and Prevention (CDC) evidenced that more than one million people dies due to transmission of malaria by mosquitoes. Presently the rate of infection/transmission has showed abrupt increase, and researchers round the world are considering global warming as the root cause for the accelerated increase in mosquito-borne diseases. Mosquitoes transmit various diseases to people such as "Zika virus, West Nile virus, Chikungunya virus, dengue, and malaria".

Dengue is feverish disease caused by flavivirus transmitted through *Aedes aegypti* or *Aedes albopictus* mosquitoes. It is an arthropod borne virus that belongs to genus Flavivirus and family Flaviridae. There are 4 distinct serotypes of dengue (DEN 1, DEN 2, DEN 3 and DEN 4), each of which is able to cause either classic dengue fever or dengue haemorrhagic fever (DHF) (Gubler, 1998)[2]. Dengue fever (DF) is an acute viral infection results in dreadful health issues. Previously (DF) was known as "water poison" related with flying insects in a Chinese medical encyclopedia in 992 from Jin Dynasty (265-420 AD). The word "Dengue" derived from Swahili phrase Ka-dingapepomeaning "cramp-like seizure". In more than 125 countries, Dengue has attained endemic phase in tropical as well as sub-tropical regions. Bhatt et al., (2013) [3] reported that approximately 390 million individuals are infected each year in the entire world and 96 million are diagnosed at clinical level. In the whole world, dengue has become a major health burden even when vaccine has been developed and is available commercially.

Since 18th century, cases of dengue started getting reported in Asia, Africa and North America. However, after World War-II, epidemiology of dengue has been changed due to increase in economic growth and rise in urbanization, especially in South East Asia, where millions of people migrated from rural to urban areas (Murray et al., 2013) [14]. Rapid urbanization, mismanagement of water supply and sewage system favours the breeding, growth and development of *Aedes* mosquitoes (Caprara et al., 2009) [5].

The threat of rising infectious diseases in the entire world has led to the transmission of dengue fever (DF) in India that is caused by four closely associated stereotypically diverse dengue viruses "(DENV-1, DENV-2, DENV-3 and DENV-4)" (Dang et al., 2020). DF has been come out one of the foremost EIDs in the world and has threatened entire world. In 1956 first emergence of dengue was reported in Vellore, Tamil Nadu and then in 1963 major epidemic was reported in Calcutta, West Bengal which mostly includes dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) evidenced in Delhi and Lucknow (1996), Uttar Pradesh and Pune (2006) and Kerala (2008). In 2010, number of DF cases increased because of the change in circulating serotypes (Pandey et al., 2012) [7].

Currently, Punjab is facing its worst ever dengue epidemiolo gy. The North Indian state has reported more than 18266 cases of dengue which includes 60 casualties. In 2020 8,435 cases of dengue were reported and 22 deaths occur due to de ngue (https://www.indiatoday.in/india/story/punjab-dengue-outbreak-cases-deaths-mohali-mosquitoes-paracetamol-

1874416-2021-11-08). In year 2017, 2018 and 2019 the cases of dengue were reported 10,289, 14980 and 153908 respectively. Districts which reported higher cases of dengue such as "Mohali, Amritsar, Bhatinda, Hoshiarpur, Pathankot, Muktsar and Ludhiana. Mohali" has emerged as epicentre of the dengue outbreak. Mohali reported 3168 confirmed cases of dengue and 32 deaths, which alone constitutes 17% in Punjab. Punjab government has also formulated a strategy like ramped up testing for dengue in affected districts. Therefore, current study was designed to survey out the status of dengue in Hoshiarpur district of Punjab for two consecutive years i.e. 2020 and 2021 using NS1 ELISA and MAC ELISA testing method.

Methodology

Study Area

Punjab falls in North Western India, having 50362 square kilometres of total area. Punjab is situated at the latitude 30°4' North and longitude 75°5' east. Hoshiarpur is geograp hically situated at the east of Punjab, 296 meters of height above the sea level (Figure 1).

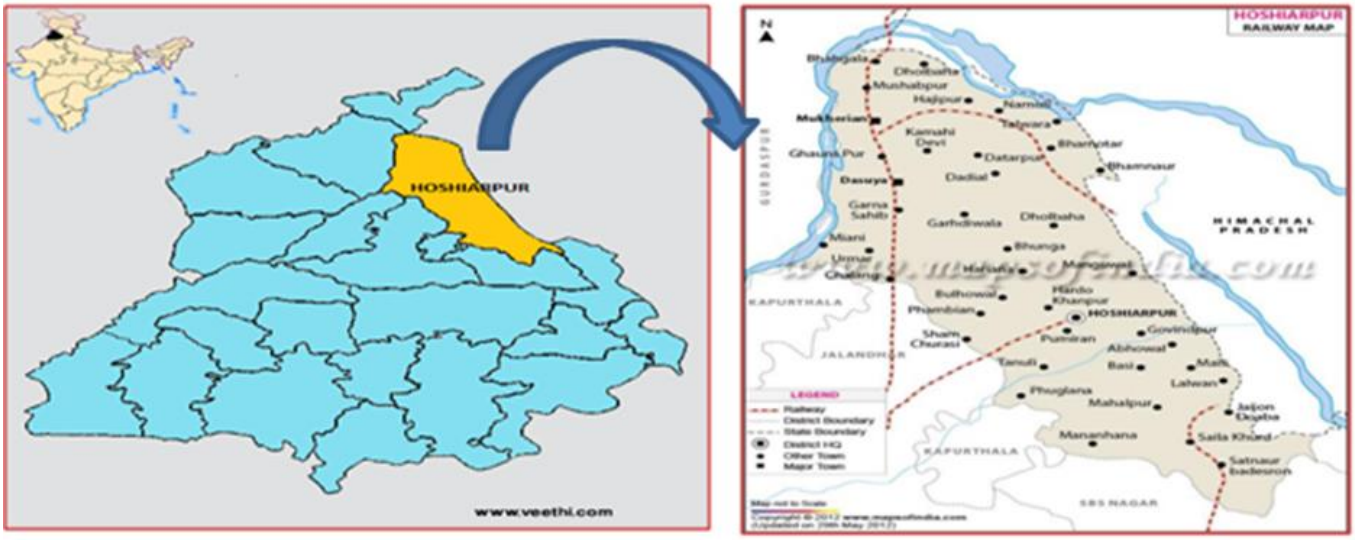

Fig 1: Map of Punjab with district Hoshiarpur.

Questionnaire

1. Total number of patients in 2020 and 2021
2. Total number of samples taken for NS1 ELISA testing in 2020 and 2021
3. Total number of samples taken for Mac ELISA testing in 2020 and 2021
4. number of Positive cases confirmed through NS1 ELISA testing in 2020 and 2021
5. Total number of negative cases confirmed through NS1 ELISA testing in 2020 and 2021
6. Total number of positive cases confirmed through Mac ELISA testing in 2020 and 2021
7. Total number of negative cases confirmed through Mac ELISA testing in 2020 and 2021

Data collection

Data was collected from Civil Hospital of district Hoshiarpur.

Results

Total number of suspected samples

It was found that 1006 and 4057 patients visited the hospital in year 2020 and 2021 respectively (Figure 2). This data indicates significant increase in number of dengue during study period.

Screening and assessment of dengue through NS1 ELISA test

Detection of the dengue virus through non-structural protein 1 (NS1) antigen is used when a person is having acute dengue infection mainly. Dengue NS1 anti-genemia overlaps with dengue virus viremia and is considered as an acute phase marker for the detection of infection. The dengue testing was conducted through NS1. In case of NS1 testing, total number of cases registered was 674 and 2730 in year 2020 and 2021 respectively (Figure 3). For year 2020 among 674 cases 215 were confirmed as positive through NS1 testing and for year 2021 among 2730 cases 986 were confirmed positive for dengue tested through NS1 ELISA (Figure 4). However, 459 out of 674 cases were found negative for dengue for year 2020. Whereas in year 2021, among 2730 suspected cases 1744 were confirmed negative for dengue (Figure 5).

Screening and assessment through MAC ELISA test

In MAC ELISA testing, total number of cases registered were 332 and 1327 in year 2020 and 2021 respectively (Figure 6). For year 2020, out of 270,62 cases were confirmed positive and for year 2021 among 1327 cases 352 were confirmed positive for dengue when tested through MAC-ELISA (Figure 7). However, number of negative cases was 270 during 2020 and 1075 for the year 2021 (Figure 8).

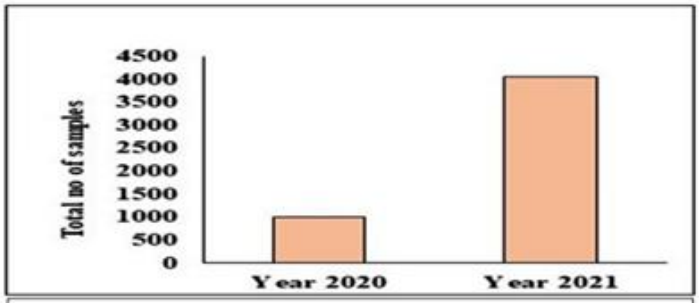

Figure 2: Histogram represent the total number of dengue suspected patients visits the hospital in year 2020 and 2021.

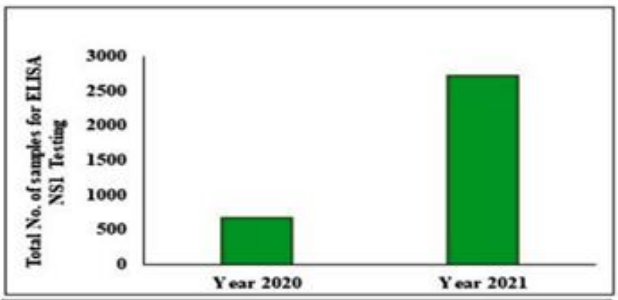

Figure 3: Histogram represent the total number of dengue suspected patients tested through NS1 ELISA test in year 2020 and 2021.

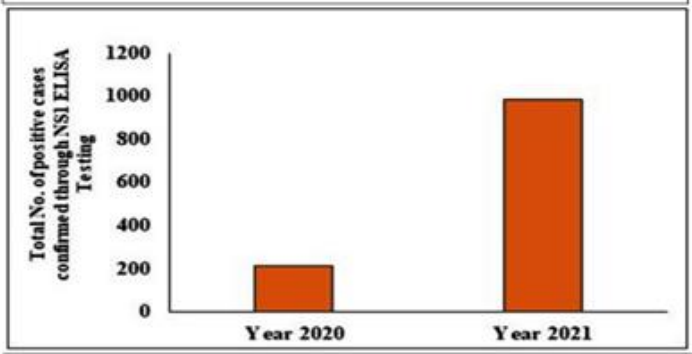

Figure 4: Histogram represent the total number of dengue suspected patients visits the hospital in year 2020 and 2021.

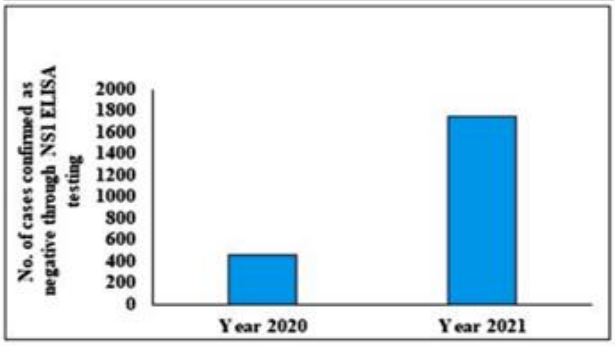

Figure 5: Histogram represent the total number of dengue suspected patients visits the hospital in year 2020 and 2021.

Fig 2, 3, 4, 5

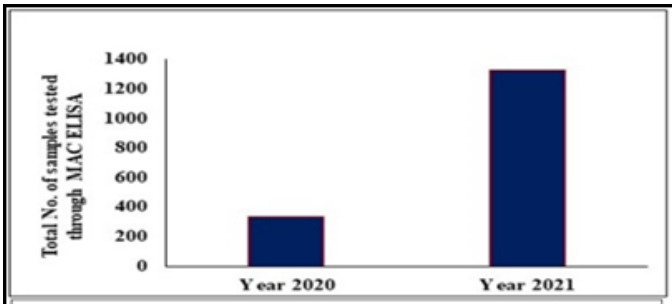

Figure 6: Histogram represent the total number of dengue suspected patients tested through MAC ELISA test in year 2020 and 2021.

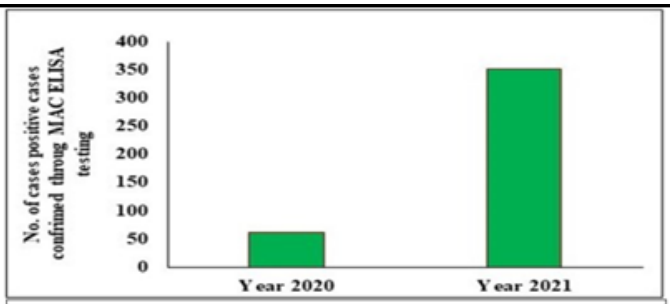

Figure 7: Histogram represent the total number of positive cases of dengue tested via MAC for year 2020 and 2021.

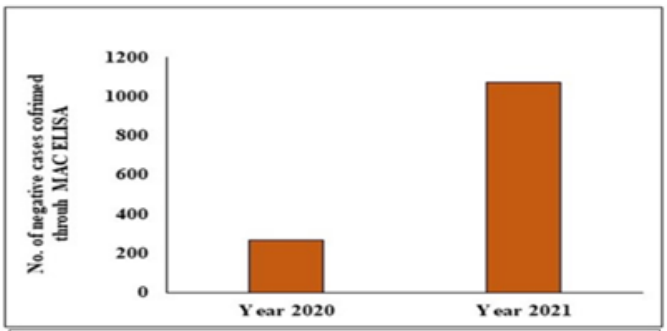

Figure 8: Histogram represent the total number of negative cases of dengue tested through MAC for year 2020 and 2021.

Fig 6, 7, 8

Discussion

The results of the present study revealed that in year 2021, Hoshiarpur has witnessed the unprecedented rise in dengue cases, reason being delay and prolonged monsoon season. (i.e. from September to October). In monsoon season, people in the study area become very careful and uses different strategies to protect themselves from transmission of dengue, however, due to delayed monsoon people ignore to take precautionary measure (Marsa, 2013)[9]. This delay in monsoon lead to accumulation of rain water on road sides remains stagnant in different ditches, which provide favourable medium to multiply mosquito larvae. Larvae used to grow in plastic glasses, coconut shells, cisterns, discarded containers among others. The prevalence of

dengue has elevated from last decades in the entire world and India as well. In India, Punjab is also having considerable prevalence of dengue and has created health burden (Ralapanawa et al., 2018; Bowman et al., 2016) [10, 11].

In the present study, highest number of suspected people visited the Civil Hospital during 2021 in comparison to year 2020. Similarly, in year 2021 highest number of cases were observed that were diagnosed through ELISA NS1 and MAC ELISA method. This revealed that year 2021 showed eruption of dengue in district Hoshiarpur. World health Organization (WHO, 2022) reported that COVID-19 has created considerable pressure on populations and makes people more susceptible to communicable diseases. Both COVID-19 and Dengue has induced greater pressure on health management system in the entire world. Moreover, this disease is season dependent, which means change in climatic condition acts as driving force in the prevalence of dengue (Descloux et al., 2012). Salam (2018) [13] studied that fast increase in dengue is associated with change in temperature, humidity. Due to heavy rainfall breeding ability of mosquitoes increases due to rise in the level of stagnant water at different place and also leachate formation in waste that was thrown on small water bodies, digs and road sides. Delayed monsoon results in the propagation of larvae in different places and items plastic glasses, coconut shells, cisterns, discarded containers among others (Murray et al., 2013) [14]. Dengue has created alarming situation due to its adverse effect on human health and global economy.

C**onclusion**

Dengue has become a major vector-borne disease in the second half of 20th century in tropical and sub-tropical regions of world with wide range of adverse health issues such as haemorrhagic fever to dengue shock syndrome. Impact of climatic conditions is considered as major causes of out-break and re-outbreak of infectious diseases having multiple adverse effects on humans and other animals. In India, during the boreal summer, south-western winds bring moisture from the Indian oceans to the land, which in turn leads to the massive rainfall during the southwest monsoon period, followed by the northeast monsoon. Therefore, it may be suggested that temperature is an essential factor for the development of pathogens in various climatic areas and might be beneficial to understand spatio-temporal variations in dengue risk.

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