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## Prevalence of West Nile virus in Mashhad, Iran: A population-based study

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### ABSTRACT

**Objective:** To evaluate the prevalence of West Nile virus seropositivity in the general population of Mashhad, Northeast of Iran. **Methods:** One hundred and eighty two individuals living in the city of Mashhad were studied using cluster sampling method. Both IgM and IgG antibodies against WNV were detected by ELISA method. **Results:** In this study, the overall IgG seroprevalence of positive West Nile virus was 11%; however, IgM antibody was not found in the participants. **Conclusions:** Our study suggested that the prevalence rate of West Nile virus is considerable in Mashhad city. It seems necessary for clinicians and health care workers to be aware of WNV infection in the Northeast Iran.

## 1. Introduction

West Nile virus (WNV) is a member of Flaviviridae family, genus Flavivirus that includes small and enveloped viruses. WNV is found in Africa, Eurasia, Australia, and North America. The genome of the virus is a single-stranded, positive sense RNA by approximately 11 000 bp in length[1,2]. WNV is a mosquito-borne virus that infects various mammal species specially horses and humans[3]; however, the most commonly WNV infected animals are birds that serves as the reservoir host[2,4].

The main mode of WNV transmission is by the bite of an infected mosquito. Other transmission routes in humans include blood

transfusion ,organ transplantation, breastfeeding and even during pregnancy from mother to baby that are less common[5]. The majority of infected people with the virus (approximately 80%), have no symptoms and up to 20 percent of them have mild flu-like symptoms, such as muscle aches and a fever. Serious symptoms are mostly included encephalitis and meningitis that occur in about one in 150 infected people with WNV[6]. Till now, several studies in Iran have been discussed about the seroprevalence of WNV[7]; however, the prevalence of the virus in recent years has not been studied in Mashhad city. The aim of this study was to determine the WNV prevalence in Mashhad, Northeast of Iran.

## 2. Materials and methods

### 2.1. Sampling

In this study, 182 individuals were employed from March 2011 to March 2012. Multistage Cluster Sampling method was applied

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to determine the prevalence of WN among selected population[8]. Samples were collected in different seasons. Number of collected samples in spring, summer, autumn and winter were 50, 40, 45 and 47, respectively. Following registration of 182, informed consent was obtained and a questionnaire was filled for each participant. Five milliliter of venous blood was obtained and the sera were collected. The prevalence of anti-WNV, both IgM and IgG, was determined by ELISA method.

## 2.2. IgG detection

For IgG ELISA, wells were coated overnight at 4 °C with mouse hyperimmuneascitic fluid diluted 1:1 000 in 0.05% Tween-20 PBS containing 5% skim milk as a saturating reagent. Antigen and sera were diluted by this solution. Native antigen at 1:500 dilution was incubated for 1 h at 37 °C and after washing with 0.05% Tween-20 PBS, human samples diluted 1:100 was added and incubated for 1 h at 37 °C. Peroxidase labeled anti-human immunoglobulin was added at 1:1 000 for 1 h at 37 °C. After 10 min incubation with 3,3',5,5'-tetramethylbenzidine (TMB) substrate (KPL, USA), the OD was read at 450 and 620 nm.

## 2.3. IgM detection

For IgM ELISA, the ELISA plates were coated with goat IgG fraction to human IgM (anti-M chain) diluted in PBS r1 and incubated overnight at 4 °C. After addition of diluted native antigen, diluted immunoascite was then added. After a definite incubation, peroxidase-labelled anti-mouse immunoglobulin was added and incubated. The plates were then washed three times with PBST containing 0.5% Tween. Finally, hydrogen peroxide and TMB were added and after a short incubation, the enzymatic reaction was stopped by the addition of 4 N sulphuric acid. The plates were then read by an ELISA reader at 450 nm[5].

## 3. Results

Of 182 participants, 136 (74.7%) were female and 46 (25.3%) were male. The age range was from 15 to 65 years and the mean of age was 36.8 years. None of them were found to be positive for WNV IgM antibody by ELISA. The overall prevalence of positive WNV IgG antibody was 11.0% (20 out of 182 participants) using ELISA method, which included 13 female (7.1%) and 7 male (3.8%). The association between WNV IgG antibody positivity and gender was not statistically significant ( $P = 0.289$ ). The prevalence of antibodies against WNV was not similar in different age groups and it was not statistically significant ( $P = 0.527$ ). The highest prevalence rate was 14.5% in the age group 31-45 years (Figure 1).

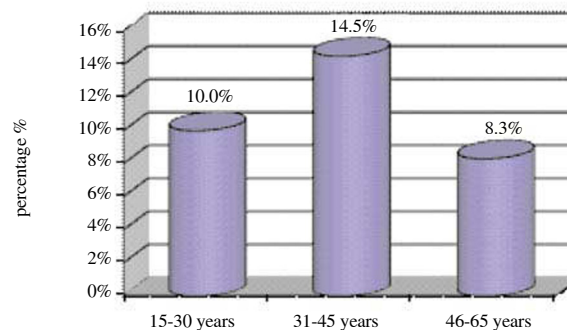


Figure 1. Frequency of WNV IgG antibody according to the age groups.

The highest prevalence of WNV IgG antibody was observed in collected samples during spring (Figure 2); however, there was no statistically significant association between WNV IgG antibody positivity and the season of sample collection ( $P = 0.831$ ).

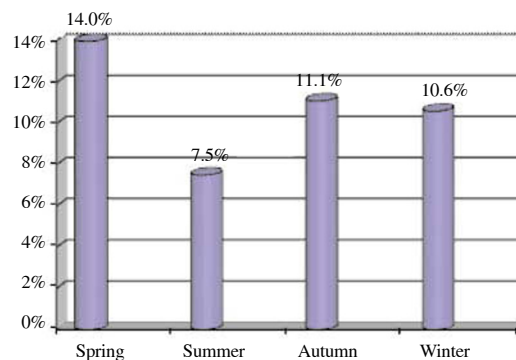


Figure 2. Frequency of WNV IgG antibody according to the season of sample collection.

## 4. Discussion

WNV is an arbovirus that is found in temperate and tropical regions of the world. The most WNV infections are asymptomatic. The incubation period is 3-15 days and the symptoms of the WNV disease are fever, malaise, myalgia, fatigue, vomiting, diarrhea, skin rash and lymphadenopathy[3]. In some patients, the virus can cause a potentially serious illness with severe symptoms including West Nile meningitis, West Nile encephalitis, and acute flaccid paralysis[9, 10].

The virus is widely distributed in different parts of world including Africa, Asia, Middle East and Europe[11, 12]. Previous seroepidemiology studies in Iran demonstrated that the presence of WNV antibody in different regions of Iran[7, 13–15]. Since, there is currently no specific antiviral therapy for the treatment of WNV infection and no effective vaccine for prevention, on-going monitoring and surveillance studies regarding WNV prevalence is an important broadcasting tool for health care authorities to have

a prospective concerning this life-threatening arbovirus in order to choose the best preventive strategies.

In this study, 182 participants who were residents of Mashhad city, WNV IgM and IgG seroprevalence were determined by ELISA. Accordingly, the prevalence of WNV IgG antibody was 11.0 %.

In a previous study in 2010, the prevalence of WNV among blood donors in Tehran city, which was determined by ELISA method for anti -WNV IgG, was 5%, while all samples were negative for WNV-specific IgM antibody at the time of the study[15]. In another study, 1976, the prevalence of antibody against WNV was reported to range between 0.0% among people living in Tabriz (urban area of East Azerbaijan province) to 95.8% among people living in Deiqi (rural area of Khozestan province) of Iran[7]. In this study, the prevalence of WNV in Mashhad was reported 2% by virus neutralization test[7] that is lower than our study. The differences may be due to the different times of sampling. Also, climate change due to global warming in recent years may increase mosquito activity period that consequently can lead to escalating virus prevalence[16].

To sum up, because of climate differences in Iran, the distribution and abundance of mosquito varies in different provinces of Iran. Accordingly, those provinces with warmer climate and longer period for mosquito activity may show higher prevalence of WNV infection[17, 18]. Seroepidemiological studies are essential to determine the prevalence of WNV in other parts of Iran especially in endemic area for better decision making in the future.

To sum up, this study demonstrates that the prevalence of WNV is considerable in Mashhad city, Iran. It can be claimed that continuous surveillance is essential to monitor the prevalence of WNV infections and also in hospitalized patients with encephalitis and meningitis. In addition, community-based mosquito control programs play a critical role in the elimination of WNV infection[19].

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## Conflict of interest statement

We declare that we have no conflict of interest.

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