

## Seroprevalence of Hepatitis B and Tetanus among Ezidian (Yazidi) Women Fleeing War

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### Abstract

Women suffer much from wars and forced migrations. In 2014, Ezidians (Yazidis) living in Iraq migrated to Syria and then to Turkey due to civil war. Tetanus is a bacterial infection caused by *Clostridium tetani* spores getting into the body. Hepatitis B is an infection caused by the hepatitis B virus. Both hepatitis B and tetanus are vaccine-preventable diseases. We aimed to evaluate the seroprevalence of tetanus and hepatitis B among Yazidi women who had fled to our country and have been living in a tent city.

This study was conducted in Diyarbakır province, located in Southeastern Turkey, between July 2015 and February 2016. A total of 245 Yazidi women aged between 15 and 49 years, living in a tent city, were included. HBsAg, anti-HBs and anti-HBcIgG were studied by ELISA method with Cobas e 601 (Roche Diagnostics, Switzerland) system autoanalyzer. Antibodies to tetanus toxin were tested by ELISA method with the Virotech ELISA tetanus (Virotech, Germany).

Of the 245 women, 38 (15.5%) had vaccine-induced immunity, while 11 (4.5%) had antibodies against hepatitis B due to previous exposure. Two women (0.8%) had isolated anti-HBcIgG positivity. Among 245 women, 96 (39.2%) had anti-tetanus antibodies.

This population was notably susceptible to both tetanus and hepatitis B. Vaccination of this sensitive population was important for the well-being of the women, their spouses and children to be born.

**Keywords:** Yazidi women, tetanus, hepatitis B, seroprevalence.

### Резюме

Жените страдат много от войни и принудителни миграции. През 2014 г. жителите на Язидис, живеещи в Ирак, мигрират в Сирия и след това в Турция поради гражданска война. Тетанусът е бактериална инфекция, причинена от спорите на *Clostridium tetani*, попадащи в тялото. Хепатит В е инфекция, причинена от вируса на хепатит В. Както хепатит В, така и тетанус са болести, предотвратими от ваксини. Целта ни беше да оценим серологичното разпространение на тетанус и хепатит В сред жените на Язиди, които са избягали в нашата страна и живеят в палатков град.

Това проучване е проведено в провинция Диарбекир, разположена в Югоизточна Турция, между юли 2015 г. и февруари 2016 г. Включени са общо 245 жени от Язидис на възраст между 15 и 49 години. С ELISA метод с автоанализатор на система Cobas 601 (Roche Diagnostics, Switzerland) са изследвани HBsAg, анти-HBs и анти-HBcIgG. Антителата срещу тетанус токсин са проучени чрез ELISA метод с Virotech ELISA тетанус (Virotech, Германия).

От всички 245 жени, 38 (15,5%) са имали индуциран от ваксина имунитет, докато 11 (4,5%) са имали антитела срещу хепатит В поради предишната експозиция. Две жени (0,8%) са диагностицирани с анти-HBcIgG позитивност. Сред 245 жени, 96 (39,2%) са имали антитела срещу тетанус.

Тази популация е особено чувствителна както към тетанус, така и към хепатит В. Ето защо, ваксинирането е важно за благосъстоянието на жените, техните съпрузи и децата, които ще се родят.

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## Introduction

Wars and forced migrations not only cause death, sickness and disability of millions of people but disrupt primary health care. Women of reproductive age and children are the most affected groups from such unusual situations.

Yazidis had lived in the northwestern mountains of Iraq for centuries. Most Yazidis consider themselves ethnically Kurdish and speak Kurdish but are religiously distinct from Iraq's Sunni Kurdish population. Their belief system branded them as heretics among Muslims. By the end of 2014, about 30 000 Yazidi had migrated to Turkey (Asher-Schapiro, 2014). As of May 2015, the total number of Yazidi population in Turkey was estimated as about 19 000. Global forced emigration has increased in recent years. A total of 65.3 million people were displaced by the end of 2015 due to war, general violence, persecution or human rights violations. About 5 million refugees came from the Syrian Arab Republic, and Turkey hosted approximately 2.5 million people worldwide (UNHCR, 2015).

Hepatitis B is an infectious disease, which affects all age groups and can be prevented by vaccination. It is estimated that around 2 billion people in the world have been exposed to hepatitis B virus (HBV), about 400 million have chronic HBV infections, and about 600 thousand people die of HBV-related diseases every year (Dienstag, 2010). Hepatitis B surface antigen (HBsAg) is the first serological marker, detected in the presence of hepatitis B infection. Antibody to HBsAg (anti-HBs) appears after antigen clearance from the blood. Antibodies to the core part of the virus (anti-HBc) can be detected in the period when surface antigens had been cleared and surface antibodies had not appeared yet. HBs Ag positivity indicates the HBV infection but cannot distinguish whether it is acute or chronic (Winn *et al.*, 2006a). Since the only major reservoir is human, the carriers and persons with chronic infection are the main sources of transmission. Screening of pregnant mother for HBsAg and postnatal immunoprophylaxis of infants prevent perinatal infection. Both hepatitis B immunoglobulin (HBIG) and vaccine are recommended for carrier mothers' babies immediately after birth (CDC, 1999; Mast *et al.*, 2006). In the absence of HBIG, vaccination alone is reported to provide a high level of protection, especially in stages other than viral replication (Wasmuth, 2009).

Tetanus is a vaccine-preventable infectious disease caused by toxins of *Clostridium tetani*. In

most cases, *C. tetani* spores enter the body through open wounds, grow in the anaerobic state and elaborate neurotoxins causing spastic paralysis. In developing countries, 20-30% of tetanus cases are seen in infants, of which 70% die (Winn, 2006b). Susceptible newborns are born to mothers with inadequate maternal tetanus antitoxin levels. Advisory Committee on Immunization Practices (ACIP) recommendation for the unknown status of tetanus vaccine is 3 doses in case of injury, followed by booster doses every 10 years (Kretsinger *et al.*, 2006). Measurement of serum antibody levels against tetanus toxoid (TT) helps to determine the protection against the disease.

The aim of this study is to determine the immune status and vaccination needs against hepatitis B and tetanus among Yazidi women who had fled to our country. With this work we hope to guide the immunization efforts for such populations.

## Materials and Methods

This cross-sectional study was conducted in Diyarbakır province, located in Southeastern Turkey between July 2015 and February 2016. Necessary permits were obtained from Diyarbakır Governorship and Diyarbakır Metropolitan Municipality. The tent city had housed 2667 people when the study was conducted. About 650 of them were women aged 15-49 years and 580 of them were married. A total of 248 married women aged 15-49 years participated in the study and were questioned about demographic information and vaccination status. Venous blood samples were obtained after the questionnaire. Since three of the samples were not enough to study, a total of 245 samples were included in the seroprevalence study. Blood samples were stored at room temperature for 15-30 minutes, then centrifuged at 1200 rpm for 10 minutes to separate their sera. Serum samples had been kept at -20°C till the test time.

HBsAg, anti-HBs and anti-HBcIgG were studied by ELISA method with Cobas e 601 (Roche Diagnostics, Switzerland) system autoanalyzer. HBsAg value above 1 cut off index (COI) was considered positive and Anti HBs value above 10 IU/L was considered positive. Since anti-HBcIgG was measured qualitatively by competitive ELISA method, values below 1 COI value were considered positive.

Anti-HBs positivity is seen in those who have had the disease or those who have been vaccinated. Anti-HBcIgG is an immunoglobulin G (IgG) class

antibody against viral core antigen showing current or prior infection. AntiHBs > 10 and positivity of Anti HBcIgG were counted as immunity based on the previous infection while AntiHBs > 10 and negativity of anti-HBcIgG were counted as immunity by vaccination. HBsAg is detected during replication of the virus (Murray *et al.*, 2013).

The serum levels of immunoglobulin G (IgG) antibodies against TT were studied by Virotech ELISA test kit (Genzyme Virotech GmbH, Germany) according to the manufacturer's instructions. Antibodies to TT concentrations were measured in International Units (IU/mL) according to the World Health Organisation (WHO) Standards. Anti TT IgG-antibody concentration of >0,1 IU/mL was indicated as immune protection (Stark *et al.*, 1999).

## Results

The majority of the women participating in the survey comprised 25-29 (23.4%) and 35-39 age (21%) groups (Table 1). The mean marriage age of the women was  $17.8 \pm 3.4$  years while the mean age at first birth was  $19.4 \pm 3.3$  years. The mean number of births was  $4.6 \pm 3.2$ . The majority of the women (91.4%) had been living in rural areas before coming to Turkey. Most of the women participating in the study (95.2%, 236 persons) had arrived in Turkey in August 2014.

Twenty five women had delivered a baby after fleeing to Turkey; 72% of these laboring women received prenatal, while 40% received postnatal health care services, and 8 of them were vaccinated after birth (Table 2).

Women participating in the study had a total of 1125 pregnancies, of which 1033 (91.8%) ended with birth and 92 (8.2%) were aborted. A total of 633 (61.6%) deliveries occurred at home and 396 (38.3) in hospital. They were asked some questions about injury in order to determine the status of tetanus vaccination. Twenty one (8.6%) of the women had been injured during the previous 5 years, 11 (4.5%) were injured during the journey to Turkey, and 57.1% of them (12 persons) were vaccinated after sustaining an injury. A total of 96 women (39.2%) had anti-tetanus antibodies of above 0.1 IU/mL.

Of the women participating in the study, 4.5% (11 persons) were immunized due to past hepatitis B infection, 15.5% (38 persons) were vaccinated, and 78.8% (193 persons) were susceptible to hepatitis B (Table 3). Isolated anti-HBc IgG was detected in two women and one woman was positive for both HBsAg and anti-HBc IgG.

**Table 1.** The age ranges of the women applying the questionnaire

| Age ranges | Number n (%) |
|------------|--------------|
| 15-19      | 13 (5,2)     |
| 20-24      | 39 (15,7)    |
| 25-29      | 58 (23,4)    |
| 30-34      | 52 (21,0)    |
| 35-39      | 44 (17,7)    |
| 45-49      | 28 (11,3)    |
| Total      | 248 (100)    |

**Table 2.** Delivery and postnatal care status of Yazidi women after migrating to Turkey

|                                   | n (%)    |
|-----------------------------------|----------|
| Have you received antenatal care? |          |
| Yes                               | 18 (72)  |
| No                                | 7 (28)   |
| Total                             | 25 (100) |
| Have you received postnatal care? |          |
| Yes                               | 10 (40)  |
| No                                | 15 (60)  |
| Total                             | 25 (100) |
| Have you been vaccinated?         |          |
| Yes                               | 8 (32)   |
| No                                | 17 (68)  |
| Total                             | 25 (100) |
| Has your baby been vaccinated?    |          |
| Yes                               | 14 (56)  |
| No                                | 11 (44)  |
| Total                             | 25 (100) |

## Discussion

Causes such as war and forced migration severely hamper prenatal care and negatively affect maternal health. Especially the women 15-49 years of age need very special health care during pregnancy and childbirth. The situation of female refugees was reported as lower than that of the society in which they migrated in terms of meeting their health services and needs (Carballo and Nerurkar, 2001). The average marriage age for women participating in the study was  $17.8 \pm 3.4$ , and the average age at first delivery was  $19.4 \pm 3.3$ . The rate of adolescent married women participating in the study was 65.2%. In a study among the Syri-

**Table 3.** Hepatitis B immune levels of the women participating in the study

| Hepatitis B immune level             | Number n (%) |
|--------------------------------------|--------------|
| Sensitive to hepatitis B             | 193 (78,8)   |
| Vaccine-related immunity*            | 38 (15,5)    |
| Immunity due to previous infection** | 11 (4,5)     |
| Others***                            | 3(1,2)       |
| Total                                | 245 (100)    |

\* AntiHBs>10 COI and AntiHBcIgG negative

\*\* AntiHBs>10 IU/L and AntiHBcIgG positive

\*\*\* 2 women had isolated AntiHBcIgG antibodies and 1 had both HBsAg and AntiHBcIgG

an refugee women, it was reported that 59.6% of the women were married at the age of 18 years and earlier (Demir, 2016). According to the Turkish Demographic and Health Survey (TDHS) 2013 data, adolescent marriage rate in Turkey was 7% (Hacettepe University Institute of Population Studies, 2014). Being a minority and living in rural areas are factors that lead girls to marry at an earlier age. In Yazidi women, early marriage and the birth of a child in the first years of marriage give the woman value in society, therefore marriage and first delivery ages are lower. The average birth number of women participating in the study was  $4.1 \pm 3.2$ . In Turkey, the average number of births was reported as 1.9 in Western Anatolia, and 3.4 in Eastern Anatolia (Hacettepe Üniversitesi Nüfus Etütleri Enstitüsü, 2014). The average number of births in our study group was higher than the average in Turkey. In a survey conducted with Syrian refugee women, the total number of pregnancies was  $3.6 \pm 2.6$  (0-14) and the total number of live births was  $2.7 \pm 2.2$  (0-10) (Bahadır *et al.*, 2015). The rate of births attended by health personnel among the women participating in the study was 70.2% (62.5% in hospital, 7.7% at home). According to the WHO World Health Report, births attended by skilled health personnel in Iraq between 2006 and 2012 was 91%, while this rate was 72% in the world (WHO, 2014). According to the TDHS 2013 data, 97.4% of women in Turkey gave birth with the help of medical staff (Hacettepe University Institute of Population Studies, 2014).

Among the 245 women tested, 20% were found to be protected (women with HBsAb presence and HBsAg absence) against HBV. Of these protected women, 11 (4.5%) had immunity due to previous exposure while 38 (15.5%) had vaccine-induced immunity. The majority of women participating in the study (193 persons, 78.8%)

were found to be susceptible to hepatitis B. Isolated anti-HBc IgG positivity was found in 2 (0.8%) women, and HBsAg was found to be positive in one woman (0.4%) in our study.

According to WHO data, worldwide HBsAg positivity was 3.5% in women and 3.9% in men (WHO, 2012). HBsAg positivity rates varied between 6-9% in Turkey, while it rose to 13% in Diyarbakır (Çakaloğlu *et al.*, 1990; Yılmaz *et al.*, 2004). Turkish Red Crescent Association reported HBsAg positivity as 0.63% in blood donors in 2012 (Tosun *et al.*, 2013). In a study conducted with 5234 persons in the southeast of Turkey (Diyarbakır, Batman, Şanlıurfa, Mardin provinces) HBsAg positivity was found to be 8.2% in rural areas and 6.2% in urban areas (Dursun *et al.*, 2005). According to the results of the Turkish Department of Viral Hepatitis War reports, HBsAg positivity rates in Southeastern Anatolia, Aegean and Central Anatolia regions were 4.1%, 1.4%, and 3.4%, respectively (Tosun *et al.*, 2013). A systematic review study about age-and region-specific hepatitis B prevalence revealed that Eastern and Southeastern regions had higher prevalence (6.72%) than other regions of Turkey (Toy *et al.*, 2011). The vaccination program conducted by the Turkish Ministry of Health since 1998 has significantly reduced the risk of HBV infection especially in children of 0-14 age. But HBV infection risk of adult age groups still remains high (Özekinci *et al.*, 2014).

Immunity status against tetanus differs between countries and regions. The protective levels of antitoxins are particularly important when injuries occur and the baby is born. In a study conducted in a teaching hospital of Iraq, 69.7% of perinatal deaths were associated with not having tetanus toxoid vaccine (Abdulhameed and Aljammal, 2016). Immunity rates to tetanus varies worldwide; it was reported as 72% in the USA

(McQuillan *et al.*, 2002). In a study conducted in the Central Anatolia region of Turkey, 25.3% of adults above 40 years had protective anti-tetanus antibody levels (Öztürk *et al.*, 2003). A multi-centered study involving Samsun, Antalya and Diyarbakır in 2000-2001 revealed that immunity against tetanus was lower in Diyarbakır than Samsun and Antalya. Protective antibody level rates were reported as 59.9%, 73.5% and 75% in Diyarbakır, Antalya and Samsun, respectively (Kurtoglu *et al.*, 2004). In a community-based study conducted in the northwest region of Turkey, 98.6% of the participants had immunity against tetanus (Tansel *et al.*, 2009). Another study conducted in the north-western part of Turkey reported that there was about 80% immunity against tetanus and a decrease in immunity with increasing age (Dundar *et al.*, 2005). Many years of wars have seriously affected Iraq's health system. About 65% of children had completed the vaccine series of diphtheria-pertussis-tetanus (Al Hilfi *et al.*, 2013). In the present study, 39.2% of the Yazidi women had protective levels of anti-tetanus antibodies. Tetanus immunity among these women was found to be lower than the average immunity in Turkey.

## Conclusion

The individuals fleeing to other countries should be considered to be high priority as they are in the risk group and these individuals should be paid special attention for protection and improvement of health. Particularly, immigrant women taking refuge in other countries should be offered unpaid healthcare services. Since this population is remarkably susceptible to hepatitis B virus and tetanus (about 80% of the women were susceptible to hepatitis and about 60% of them to tetanus), vaccination is of utmost importance for the protection and well-being of themselves, their spouses and babies to be born.

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