ESTIMATION OF ANTI-GLYCOPROTEIN ANTIBODIES AGAINST RABIES VIRUS INFECTION IN IRAQI POST EXPOSURE AND HIGH RISK GROUPS VACCINATED WITH HUMAN DIPLOID CELL VACCINE

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ABSTRACT

Aim: Since little information is available in Iraq concerning antibody level in vaccinated individuals both pre and post exposure subjects and the need for monitoring the immune status and efficiency of human diploid cell vaccine (HDCV) used in Iraq, the present study was done to determine the level of anti-glycoprotein antibodies.

Materials and methods: Antibody level estimation have been done using sera of 55 vaccinated subjects with human diploid cell vaccine (HDCV) pre and post exposed to rabies using enzyme-linked immunosorbent assay (ELISA) technique.

Results: Results indicate significance decline in antibody level of old age subject when compared with younger age. It was found that antibody titer was decline to less than 0.5 IU/ml in 8.57% of vaccinated subjects lasting up to 2-17 wks of post-exposure fifth dose. In general the level of antibody in Iraqi subjects was low as compared with other studies. Results also indicate that 25% of high risk subjects need booster dose after about 2 years of last vaccinated dose according to WHO recommendation.

Conclusion: Serology after last vaccinated dose can be considered necessary, since some vaccinated subjects had low antibody levels (<0.5 IU/ml).

INTRODUCTION

Rabies is a zoonotic disease caused by RNA virus in the family Rhabdoviridae genus lyssavirus (Lyles and Rupprecht, 2007). Human’s contract rabies most commonly from bites by animals infected with rabies virus (Knobel et al., 2005). After entry to the central nervous system the virus causes acute progressive fatal encephalomyelitis. Incubation period usually ranges from 1-3 month (Shankar, 2009). Prompt wound care and administration of rabies immunoglobulin (RIG) and vaccine are highly effective in preventing human rabies following exposure (CDC, 2010). In many Asian and African countries where canine rabies has not been adequately controlled, dogs account for 90% or more of animal rabies cases (Fishbein and Robinson, 1993). Antibodies play a central role in prophylaxis against many infectious agents (Moore and Hanlon, 2010). Glycoprotein in rabies virus envelope induces neutralizing antibodies essential for protection against rabies. Measurement of these antibodies reflects a level of humoral immunity both during post exposure therapy and during prophylactic vaccination (Welch et al., 2009). Individual at high risk of exposure (Such as veterinarians, lab worker and others) should undergo pre-exposure prophylaxis with rabies vaccine because this disease is highly dangerous and causes death after exposure to rabid animal (Wilde et al., 2003; Krause et al., 2005). Since little information is available in Iraq concerning antibody level in vaccinated individuals both pre and post exposure subjects and the need for monitoring the immune status and efficiency of human diploid cell vaccine (HDCV) used in Iraq, the present study was done to determine the level of anti-glycoprotein antibodies using quantitative indirect enzyme-linked immunosorbent assay (ELISA) and also to estimate the relation between age range and the change in antibody level after time period up to 17 wks.

MATERIALS AND METHODS

Sample collection:

The blood samples were collected in serum separation tubes from 55 Iraqi subjects during December 2008 to March 2009; they included 47 post-exposure subjects ranging from 5-66 years old vaccinated with five doses HDCV (Pasteur-Mériieux-Lyon, France), and 8 highly risk subjects vaccinated with three doses HDCV which are compromised of 2 veterinarians and 6 staff members of Pasteur institute for communication diseases in Baghdad Province. Also, blood sample was collected from fifteen follow up subjects after fourth and fifth post-exposure doses vaccination. Blood sample was allowed to clot for 30 minutes before centrifugation for 15 minute at 1000 g. Serum was removed and stored at -20c until testing commenced.
Detection of rabies anti-glycoprotein antibodies:

A quantitative indirect ELISA using human rabies virus antibody (IgG) kit (Cusabio biotech co., LTD), was used to detect rabies virus anti-glycoprotein antibodies. This kit included a microplate that was pre-coated with purified rabies virus antigen. The optical density (OD) values for the test samples were compared with the OD values of positive controls and antibody titers expressed as micro international units per ml (mIU equivalent to 1000 IU) were obtained from a standard OD antibody titer curve. Because the serum samples were diluted, the concentration read from the standard curve should be multiplied by the dilution factor. All steps were conducted in accordance with the instructions of the manufacture (www.cusabio.com) and results were read using an ELISA reader at wave length of 450±2 nm. Subjects were considered to be immune against rabies virus infection if they produced ELISA titers of ≥ 0.5 IU/ml (Stantic-Pavlinic et al., 2006).

RESULTS

Finding summarized in table 1 showed no significance statistical change (P>0.05) has been achieved in antibody titer for the age range of 5-19 years old when comparing the values of 1-12 wks with those after 12-17 wks, while this change was statistically significant for 20-40 years old and 45-66 years old. There is no statically significant difference (P<0.05) between antibody levels within high risk vaccinated subjects. Results of the present study (Fig 1) showed that antibody titer of 8.57% of post-exposure subjects was less than 0.5 IU/ml after 2-17 wks of the last immunizing dose. Also the results showed that 25% pre-exposed subjects have a titer of less than 0.5 IU/ml after about 2.5 years and taking in consideration that all of these pre-exposed subjects had no administration of rabies immunoglobulin (RIG). Results showed the antibody titer for two (13.33%) of 15 subjects in post-exposure were 0.36, 0.39 IU/ml after fourth dose (Fig. 2), a levels that were below the WHO recommended minimal acceptable level of 0.5 IU/ml. After fifth dose, these subjects titer were raised to 1.48, 0.55 IU/ml, respectively. All other subjects in this group had antibody titers more than 0.5 IU/ml of both doses.

DISCUSSION

The induction of rabies virus-specific antibody response is one important immunologic component of response to vaccination (Plotkin et al., 2008). We found the antibody titer of post exposure and pre exposure Iraqi subjects reached lower levels ranging from 0.4366-3.1764 IU/ml in post exposure and 0.3916-4.1475 IU/ml in pre exposure cases, these values were low compared to results of other study including 0.7-1605 IU/ml (Ghaffari et al., 2001). An important use of rabies pre-exposure prophylaxis is to prime the immune response to enable a rapid anamnestic response to post-exposure booster vaccination and simplify the post-exposure prophylaxis requirements for previously vaccinated persons (CDC, 2008). All healthy persons tested in accordance with Advisory Committee on Immunization Practices (ACIP) guidelines after completion of at least a 4-dose regimen of rabies post-exposure prophylaxis should demonstrate adequate antibody response against rabies virus (CDC, 2010). Meanwhile we observed the antibody titer after 4-doses (28 days after initiation of vaccine) may be inadequate to protection against rabies virus infection. Fluctuation of antibody titer arising between fourth and fifth dose was noticed in subjects group of post-exposure due to humoral immune response to rabies antigens. This process of which is controlled by many factors include the amount of antigen, route of delivery, the expression and involvement of major histocompatibility complex genes, and the health status of the individual, among others (Moore and Hanlon, 2010). Most studies have shown that serum neutralizing antibodies are detectable in 20%-40% of young healthy vaccine recipients by day 7 after vaccination and that close to 100% of healthy vaccine recipients have rabies antibodies by 14 days after receiving 2 doses of the HDCV (Rupprecht et al., 2009). Results of
present study indicate significant decline in antibody titer level in older than younger age groups. Declining immune function with age substantially contributes underlying complex changes in the immune system are collectively termed immunosenesence and they affect all cell types of both the innate and the adaptive immune system (Weinberger et al., 2008). On the other hand a study on vaccination with rabies vaccine found that 85% of young 2-3 years are seroprotected if a booster dose is given at 12 months, seroprotection may last for more than 10 years (Leder et al., 2008). In a study by Brow et al. in which 23.6% of participants were failed to demonstrate titers of rabies antibodies greater than 0.5 IU/ml within a period of 4-10 years after last immunization, so booster immunization has been suggested (Brown et al., 2011). Since antibody titer of greater than or equal to 0.5 IU/ml was considered indicative of seroconversion providing an adequate titer in the line of world health organization WHO recommendation, the booster dose was considered necessary for antibody levels rising in risky group.

**CONCLUSION**

Our conclusion is that boosting is necessary for 8.57% of post exposure cases and this fact disagree with the recommendation which suggests 2 doses of vaccine for those who have been received complete vaccination regiem and being exposed to rabies again (CDC, 2008). Antibody level 25% of high risk group was (0.3916-0.4327) which suggests boosting after 2 years of last immunizing dose according to the report of WHO which states that all persons who work with live rabies virus in a diagnostic research or vaccine production laboratory should have a serum sample tested for rabies virus neutralization antibodies and a booster administered when the titer falls below 0.5 IU/ml (Moore and Hanlon, 2010). Future studies are needed for the completion of information concerning Iraqi population.

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**References**


