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Full Length Research Paper

Seroepidemiological surveillance of antitetanus antibodies in Pakistani childbearing women: A population based survey

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Maternal and Neonatal Tetanus is a severe, often fatal ailment characterized by sweeping augmented strictness and convulsive spasms of skeletal muscles. Vaccination is the most steadfast method of forbearance against this disease and has inculcated to lessen mortalities. The present study was carried out with a view to assess the serum levels of tetanus antitoxins in randomly selected rural and urban women to find out the effect of age, number of pregnancy, parity and number of injections at the time of delivery. The study was cross-sectional in design and hospital-based. The gold standard mouse neutralization assay was employed despite being costly and cumbersome. Antibody titre of each sample was determined by seroneutralization method. 1504 women, randomly selected from various hospitals of age 15 to 40 years, 1064 (0.000) were vaccinated, which are significant while 440 were unvaccinated women and taken as control. The 65% (p = 0.000) women had antibody titre higher than protective level which is significant, 28% equal to protective level and 6.7% below protective level. Average antibody titre of 3rd trimester was higher than 2nd trimester (p = 0.000) which is significant. Immunization awareness and practice was higher in < 30 (78%) than > 30 years in women (63%) of both rural and urban areas but practice was comparatively higher in urban (74%) than rural area (67%). These finding shows that tetanus vaccination in our population is generally effective and cannot be protected by herd immunity, as tetanus is not communicable disease.

Key words: Antitetanus antibodies, seroneutralization, childbearing age, Pakistan.

INTRODUCTION

Tetanus caused by *Clostridium tetani*, is a vaccine-preventable, global infectious disease with significant morbidity and mortality. The global status of serological immunity against tetanus varies between countries as a result of different national vaccination policies and methods, and the criteria used for determination of serum levels of tetanus antitoxin. Protective immunity was noted in 53 to 80% of people aged > 60 years residing in the United States, England, Wales and Sweden, 15.7% of people in Turkey and 23 to 32% of those in Denmark aged > 60 years, while < 75% of Australians aged > 50 years were immune to tetanus (Christenson and Bottiger, 1987; Kjeldsen et al., 1988; Gergen et al., 1995; Maple et al., 2000; Karabay et al., 2005; Gidding et al., 2005).

Maternal and Neonatal tetanus (MNT) is a major health problem in developing countries, and incurs high mortality where compulsory immunization of children is not enforced (UNICEF, WHO, UNFPA, 2000; Vandelaer et

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al., 2003; Griffiths et al., 2004; Pantipa et al., 2007). The World Health Organization's (WHO) implementation of the Neonatal Tetanus Elimination Program in 1989 was a major step toward eliminating this disease (WHO, 1989). However, even though a safe and highly effective vaccine is available, MNT still causes an estimated 309,000 deaths annually worldwide (Vandelaer et al., 2003). Mortality is 135 times higher in the developing countries as compared to developed countries. The incidence is higher in tropical countries and under poor hygienic conditions. In these countries, tetanus in newborns takes a very important route. In most of such cases, use of dirty, rusty scissors to cut the umbilical string of the newborn causes sepsis in navel. An estimated one million infants die due to tetanus in developing countries each year because of poor hygiene. Neonatal tetanus is the second leading causes of death recognized as vaccine preventable disease among children worldwide (WHO, 1998).

The WHO/UNICEF/UNFPA have devised a strategy for achieving elimination of MNT with a provision of at least 2 doses of tetanus toxoid (TT2) to all pregnant women in high risk areas and 3 doses (TT3) to all childbearing age and promotion of clean delivery services to all pregnant women and effective surveillance of MNT. There is a renewed resolve and vigor to achieve MNT elimination in the 57 countries which have not yet done enough for this goal. 90% of the neonatal deaths occur in 27 of the 57 countries (Deming et al., 2002). Pakistan is one of the countries which accounts for 73% of neonatal disease burden and 22,000 neonatal deaths occur every year due to MNT (WHO, UNICEF, UNFPA, 2000). The others include Bangladesh, China, Democratic Republic of Congo, Ethiopia, India, Nigeria and Somalia (UNICEF, WHO, UNFPA, 2000).

Vaccinating pregnant women is one of the major ways of protecting against NT; vaccination rates alone are often considered inaccurate indicators of a population's protection, since they may not reflect the actual biological level of protective immunity (Gergen et al., 1995). Measuring serum anti-tetanus antibody levels yields a more accurate estimate of a population's protection and is a good way to monitor the effectiveness of any vaccination strategy for pregnant women. The proportion of women with tetanus antitoxin titers adequate to provide protection for themselves and for their newborn infants varied from 96% in New Haven, Connecticut that is, industrialized part, to 19% in Santiago, Chile which is under developed country. Women of childbearing age in five of the nine areas (Sao Paulo, Recife, and Pôrto Alegre, Brazil, Ecuador, and Gazankulu, South Africa) had an average of 40% immunity to tetanus and did not differ significantly from one another (Varela et al., 1985).

The information system of Expanded Program on Immunization (EPI) in Pakistan has reported around 80% coverage in children and approximately 38% of the TT2 coverage of pregnant women in the province of Punjab (Annual Report EPI, 2001, 2002). In a pilot study carried out in Pakistan in women of reproductive age about tetanus toxoid vaccine, 81.3% participants had inadequate knowledge; 12.5% had incorrect knowledge, and 6.3% had no knowledge. The same study revealed that 28 out of 54 participants (52%) were not concerned about tetanus toxoid vaccination. This study further revealed that of those who were vaccinated, only 40.6% were properly vaccinated (Zeb et al., 2006). Another study carried out in Peshawar showed 65% vaccination rate among the women of reproductive age, however, the study did not comment on proper or improper vaccination (Afridi et al., 2006).

Two schedules of tetanus vaccination are followed in Pakistan that is, DPT3 in newborn and TT-2 in pregnant women and also protects child. If, at the time of delivery, antibody titre in mother is equal to 0.01 IU/ml, it gives 100% protection to the new born (Hardegeree et al., 1970). The present study was carried out with a view to assess the serum levels of tetanus antitoxins in randomly selected rural and urban women of childbearing age to find out the effect of age, number of pregnancy, parity and number of injections at the time of delivery.

MATERIALS AND METHODS

Study design and setting

The study was cross-sectional in design and hospital-based in Rawalpindi and Islamabad Districts of Pakistan. Women visiting various hospitals of these districts for antenatal care were enrolled in the study during the period of 2008 to 2009. The gold standard mouse neutralization assay was employed despite being costly and cumbersome. Routine use of ELISA and Haemagglutination has proven unreliable for measuring low titers (Simonsen et al., 1984, 1986). Antibody titre of each sample was determined by seroneutralization method (Barile et al., 1970; Gentili et al., 1985). Tetanus toxin, which was already standardized and has 1000 MLD/ml, was used in this test. WHO Standard antitetanus serum was used as positive control. Albino mice 16 to 18 g body weight, were used for seroneutralization. The women having age between 18 to 40 years were selected for this study. The study was approved by the ethical committee of Quaid e Azam University, Islamabad, Pakistan.

Determination of L+ dose of tetanus toxin

Least amount of toxin was combined with minimum unit of ATS (Anti tetanus serum). It was injected in mice and if it causes the death by the end of fourth day, it is called L+ dose. Tetanus toxin levels L+1/10, L+/100, L+/1000, L+/200 were used in this test, which were determined by the neutralization of WHO standard antitoxin for serum.

Seroneutralization test

Equal amount of serum sample and tetanus toxin were mixed and

Table 1. Total number of samples of women of childbearing age.

Age groups	Serum samples	Urban samples	Rural samples	Controls	p-value
15-20	228 (15.1%)	132 (15%)	32 (17.3%)	64 (14.5%)	0.383
21-25	624 (41.4%)	348 (39.5%)	64 (34.7%)	212 (48.2%)	0.566
26-30	496 (32.9%)	320 (36.4%)	68 (36.7%)	108 (24.5%)	0.848
31-35	128 (8.5%)	64 (7.2%)	16 (8.7%)	48 (30%)	0.000
36-40	28 (1.86%)	16 (1.8%)	4 (2.1%)	8 (5%)	0.956
Total serum samples	1504 (100%)	880 (100%)	154 (100%)	440 (100%)	

Table 2. Distribution of study population with respect to pregnancy trimester.

Age group (Yrs)	Trimes (Ist)	Trimes (2nd)	Trimes (3rd)	p-value
15-20	4 (2.4%)	84 (51.2%)	76 (46.34%)	0.000
21-25	0 (0%)	116 (52.4%)	196(48%)	0.412
26-30	8 (2.06%)	148 (38.1%)	232 (59.7%)	0.000
31-35	0 (0%)	40 (50%)	40 (50%)	0.942
36-40	0 (0%)	4 (20%)	16 (80%)	0.000
Total samples (1064)	12 (1.12%)	492 (46.2%)	520 (48.8%)	

Trimes = Trimester.

incubated at room temperature for one hour and 1 ml from each mixture was inoculated subcutaneously into two mice, observation were recorded for 5 days for any sign of tetanus in mice or death and results were recorded. If mice survived without any sign of tetanus in 5 days, it was taken as indication of higher antibody titre than the respective level of toxin. If mice survived in mixture of serum and L+1/10 dose of toxin, antibody titre is higher than 0.1 IU/ml and mice survived in mixture of serum and L+1/100 dose of toxin titre is higher than 0.01 IU/ml. Similarly, if mice survived in mixture of serum and 1/1000 dose of toxin, titre is higher than 0.001 IU/ml, and in mixture of serum and L+1/200 dose of toxin, if mice survived, titre is higher than 0.05 IU/ml.

Statistical analysis

Statistical analyses were done by SPSS 15.0 for WINDOWS, and other analyses were done by using T-test and examination of variance (ANOVA).

RESULTS

A total of 1504 samples were collected randomly from women visiting Gynecology Departments of the various hospitals of Rawalpindi and Islamabad Districts of Pakistan for antenatal care. Among these, 440 women were unvaccinated and taken as control group. The age of women ranges from 15 to 40 years and were divided into five groups. The first group comprised 228 women of 15 to 20 years, 2nd group 624 women of 21 to 25 years, 3rd group 496 women of 26 to 30years, 4th group 128 women of 31 to 35 years and fifth group 28 women of 36 to 40 years of age. The distribution of women both from rural and urban area are also shown in Table 1, which are non significant except in the age group 31 to 35 in rural and urban women ($p = 0.000$). The distribution of study population with respect to their pregnancy trimester shows that only 12 women were in first trimester, 492 (46.2%) women were in 2nd trimester and 520 (48.8%) women were in 3rd trimester ($p = 0.000$) (Table 2). The pregnancy in first trimester that is, 28 (87.5%) are higher in urban women than rural. The number of pregnancy with respect to rural and urban areas is elaborated in Table 3. The women in 35 to 40 years group had 100% multiple pregnancies. Conception and parity of study population are explained in Table 4. Mean conception of first group was 1.51 and parity was 1.31. Mean conception of second group was 3 and parity was 2. Third group had mean conception 4 and parity 2.37. In 4th group, mean conception was 4.45 and parity was 3.28. Fifth group had mean conception 5.2 and parity 3.4. With increase in age, number of conception was also increased in turn parity increased. Trend for increase in conception is same for rural and urban in different age groups.

Vaccination status and serosurveillance

Vaccination status and serosurveillance of study population is described in Table 5. In first group, percentage of tetanus vaccinations was 71.9% and mean

Table 3. Vaccination percentage of women by age, residence and No. of pregnancies in study population.

Age groups (Yrs)	Urban			Rural			p-value
	Pregnancy Ist, 2nd, 3rd			Pregnancy Ist, 2nd, 3rd			
15-20	128 (71.1%)	40(22.3%)	12 (6.6%)	28 (87.5%)	4 (12.5%)	NIL	0.000
21-25	88 (25.2%)	112 (32.3%)	148 (42.5%)	8 (11.7%)	24 (35.2%)	32 (47%)	0.000
26-30	20 (6.25%)	72 (22.5%)	228 (71.25%)	16 (23.5%)	12 (17.6%)	40 (58.9%)	0.000
31-35	4 (5%)	12 (15%)	40 (80%)	4 (25%)	NIL	12 (75%)	0.000
35-40	NIL	NIL	16 (100%)	NIL	NIL	4 (100%)	

Table 4. Mean conception and mean parity of study population.

Age groups (Yrs)	Mean conception	SD	Mean parity	SD
15-20	1.51	±0.87	1.31	±0.60
21-25	3.00	±1.52	2.00	±1.12
26-30	4.00	±1.66	2.37	±1.15
31-35	4.45	±2.24	3.28	±1.28
36-40	5.20	±0.84	3.40	±0.55

PREG = pregnancy, SD = standard deviation .

antibody titre of this group was 0.033 IU/ml (SD ± 0.027). The minimum protective antibody titre of tetanus is 0.01 IU/ml. Vaccination percentage of second group was 66% and mean antibody titre 0.039 IU/ml (SD ± 0.022). In 3rd group, vaccination percentage was 78.2% and mean antibody titre 0.042 IU/ml (SD ± 0.052). Fourth group had vaccination percentage of 62.5% and mean antibody titre was 0.04 IU/ml (SD ± 0.023). In the fifth group, vaccination percentage was 71.4% and mean antibody titre was 0.043 IU/ml (SD ± 0.038). The vaccination percentage was higher in < 30 years groups and lower in > 30 years groups, but antibody titre was found higher in > 30 years than < 30 years groups in both rural and urban area.

Trimester wise distribution of antibody titre

Trimester wise distribution of antibody titre in study population is explained in Table 6. In the first group, only four samples fall in first trimester of pregnancy and had antibody titre 0.01 IU/ml. Second trimester had 84 women and the mean antibody titre was 0.023 IU/ml (SD ± 0.02), 76 women of this group were in third trimester, and had mean antibody titre 0.05 IU/ml. In 2nd group, none of the women were in first trimester and 216 women in second trimester had mean antibody titre of 0.04 IU/ml (SD ± 0.03) and 196 women in third trimester had mean antibody titre of 0.042 IU/ml (SD ± 0.02). Only 8 women of 3rd group were in first trimester and had mean antibody titre of 0.005 IU/ml, in second trimester, 148 women had mean antibody titre 0.04 IU/ml (SD ± 0.02) and 232 women were in third trimester, and had mean antibody titre of 0.05 IU/ml (SD ± 0.06). In fourth group, none of the women in first trimester, 40 in second had mean antibody titre of 0.03 IU/ml (SD ± 0.02), 40 were in third and had mean antibody titre of 0.05 IU/ml (SD ± 0.03). In fifth group, 4 women belonging to second trimester had antibody titre 0.05 IU/ml and 16 women to third trimester had mean antibody titre 0.066 IU/ml (SD ± 0.03). The protective level against tetanus is 0.01 IU/ml.

In our study, 72 women had antibody titre below protective level, 300 women had antibody equal to protective level. Out of 1064, 692 women had antibody titre above protective level. Only 6.7% women had antibody titre below protective level, 28% had developed protective antibody titre and 65% women had high antibody titre after vaccination. The protective level of antibody titre against tetanus in study population with respect to their pregnancy trimester is explained in Table 7. In this, women were divided into two major groups < 30 years and > 30 years of age. In this study, 1336 women were in < 30 years group and 156 were in > 30 years group. 448 women were of < 30 group in 2nd trimester and had mean antibody titre of 0.034 IU/ml and 504 women were in 3rd trimester and had mean antibody titre 0.1 IU/ml. The 44 women from > 30years were in 2nd trimester and had mean antibody titre of 0.04 IU/ml and 56 women of this group were in 3rd trimester had mean antibody titre 0.001 IU/ml. Overall antibodies titre of 3rd trimester is higher than antibody titre of 2nd trimester. It also compares with control group in which 384 women

Table 5. Vaccination status and sero-surveillance of study population.

Age Group	No. of samples	% of vaccination	Mean antibody titre	Standard deviation (SD)	Control	Mean antibody titre (unit/ml)
15-20	228	164 (71.9%)	0.033	±0.027	64	0.001
21-25	624	412 (66%)	0.039	±0.022	212	0.001
26-30	496	388 (78.2%)	0.042	±0.052	108	>0.001
31-35	128	80 (62.5%)	0.040	±0.023	48	>0.001
36-40	28	20 (71.4%)	0.043	±0.038	8	0.001

INJ: Injection, titre in unit/ml.

Table 6. Trimester wise distribution of antibody titre of study population.

Age groups	TRIMES (Ist)	Mean Antibody titers/SD (unit/ml)	TRIMES (2^{nd})	Mean antibody Titers/SD (unit/ml)	TRIMES (3^{rd})	Mean antibody titers/SD (unit/ml)
15-20	4	0.01	84	0.023±0.02	76	0.05±0.030
21-25	NIL	NIL	216	0.022±0.03	196	0.042±0.02
26-30	8	0.005	148	0.052±0.02	232	0.05±0.06
31-35	NIL	NIL	40	0.023±0.02	40	0.05±0.030
36-40	NIL	NIL	4	0.038±0.01	16	0.066±0.03

Table 7. Mean antibody titre according to age and trimester of pregnancy.

Age group	No. of samples	Trimes (2^{nd})	Trimes (3^{rd})	Control
>30	1336	448 (0.034 IU/ml)	504 (0.1 IU/ml)	384 (0.00 IU/ml)
<30	156	44 (0.041 IU/ml)	56 (0.081 IU/ml)	56 (0.001 IU/ml)

Table 8. Distribution of samples with respect to protective level of antibody titre.

Protective level (0.01 unit/ml)	Below protective level	Protective level	Higher than protective level	Control	P-value
Number of samples	72	300	692	440	
Percentage of samples (%)	6.76	28	65	29	0.000
Mean antibody titre (unit/ml)	0.006	0.01	0.05	0.001	0.000

were > 30 years and 56 were in > 30 years and protective titre is higher than protective level by 65%, which is significantly higher (p = 0.000) and protective level is 28% (Table 8). Antibody titre of control group is almost negligible. A negligible antibody titre was found in few women of control group which may be possible due to the exposure to environment.

DISCUSSION

Pakistan being a country with low per capita income, low literacy rate and due to male dominating society people use to keep their women within four walls of their home and are not visiting centre's of basic health facilities. 70% of population resides in rural area, and having no access to health care facilities. National Health Survey of Pakistan also states that 60% of birth by high economic status mothers were catered and attended by Government or private doctors as compared to only 10% of births to low pregnant women of low socio-economic status (NHS, 1998). Midwives (Dais) are most common birth attendants for low and middle socio-economic status pregnant women. The majority of rural mothers had deliveries assisted by a Dais.

In the light of above conditions and background, this study was conducted with the objective to find the status of tetanus vaccination and practice in lower socio-

economic group on one hand and urban rural population on the other hand. The women enrolled in the study for sample collection ranged from 15 to 40 years. This age range is main reproductive age and childbearing age, as similar study conducted in Dhaka enrolled more or less the same age group (Perry et al., 1998). A study was carried out in Turkey to determine the tetanus vaccination status for pregnant women, and to examine the effects of various factors on tetanus toxoid (TT) vaccination coverage during pregnancy in reproductive-age women. Current vaccination rates with TT during pregnancy were found to be well below universal levels and there is need to launch effective mass media campaigns that target urban and suburban populations, and inform and motivate women to request vaccination against tetanus (Maral et al., 2001).

Awareness and practice of tetanus vaccination during pregnancy started in Pakistan in 1982, when tetanus toxoid two doses (TT2) program was introduced by Expanded Program of Immunization (EPI) in Pakistan. The percentage of Tetanus TT2 coverage in pregnant women has increasing trend from 1982 to 2000 (Gentili et al., 1985). Study population is estimated with respect to their pregnancy trimester; the women with 2nd and 3rd trimester of pregnancy stage (Table 2) are scheduled for tetanus vaccination, followed by 7th and 8th month of pregnancy (EPI, 2007). The trend of antenatal check up is higher in advance stage of pregnancy, as 123 women belong to 2nd trimester of pregnancy and 140 women belong to 3rd trimester of pregnancy in this study (Table 2).

Characteristics which affect vaccination practice and immune response are age, previous exposure, parity and available facilities (Vasanti and Raman, 1980). This study shows that with age, conception increase in turn parity has increased as shown in Table 4. The increasing trend of multiple pregnancies is from > 25 years to 40 years. It indicates that this is the main reproductive and child bearing age where average number of conception rate is 4 to 5 times. Antibody titre is increased with age, as number of conception increases, which in turn increased number of injections, or exposure of individuals which boosts up the immune response as the same finding was obtained in New Guinea (Barile et al., 1970). High immunity levels were found in population having more education in Turkey and also reported by others as well (Ozturk et al., 2003; Dundar et al., 2005).

Insufficient tetanus immunity among childbearing age has also been reported in recent studies in Turkey (Berrin et al., 2007). A higher birth rate has shown to decrease the use of antenatal care and associated with reduced tetanus toxoid immunization uptake (Navaneetham and Dharmalingham, 2002; Thind, 2005). The reasons observed for this includes the experience women gain after each pregnancy and childbirth, time and cost associated with larger families which in turn decreases antenatal care utilization. Maternal educations also plays major role in the utilization of TT immunization (EPI, 2007). Percentage of vaccination is lower in rural (67%) women than in urban (74%) women. The rural women expected to have received less vaccination due to shortage of resources, lack of awareness and distance from EPI centers substantiating the work done in India (Navaneetham and Dharmalingham, 2002). The vaccination percentage is higher in women of 26 to 30 years (Table 5) as was also studied in Dhaka, Bangladesh (Perry et al., 1998). Younger rural population having better exposure is due to prevalent EPI programs, so comparatively higher vaccination percentage and higher immune response is obtained in this group. Maximum practice of tetanus vaccination was in women of 26 to 30 years, the increasing trend was found in < 25 and decreasing trend in women from > 30 years, and was similar to study conducted in New Guinea (Barile et al., 1970).

The 65% of women had antibody titre higher than 0.01 IU/ml and 28% had antibody titre equal to protective level that is 0.01 IU/ml. Only 6.7% had antibody titre below the protective level (Table 2). When study population is divided into two major groups < 30 years and > 30 years, a higher number of population was found in < 30 group than in > 30 group with same distribution of population in control group. As the schedule for tetanus toxoid vaccination is between 7th and 8th month of pregnancy, the large population lies in 3rd trimester than in 2nd trimester of pregnancy. The antibody titre is higher in women of 3rd trimester than in women of 2nd trimester. Antibody titre of control group is obviously negligible (Table 7). Our study conforms to earlier studies carried out in Turkey and India where antitetanus antibodies levels are well within the protective levels (Thind, 2005; Berrin et al., 2007).

This finding shows that vaccination among population with the existing schedule is most effective and appropriate. However, to provoke the representative population of our country Expanded Program of Immunization should be more active and effective for further coverage in this field; as tetanus is not a communicable disease and thus may not be protected by herd immunity. Immunity is to be acquired by individual women who are going to give birth to children and these women should be encouraged to receive antenatal care from qualified personals and adopt good hygiene practices during delivering child and this will help reach Tetanus elimination goal.

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