Original Article

J Babol Univ Med Sci
Vol 20, Issu 7; July 2018. P: 47-54

Immunization Evaluation of Type III Secretion System Recombinant Antigens and Shiga Like Toxin Binding Subunit of E. coli O157:H7

M. Taheri (BSc)[1], Sh. Nazarian (PhD)*[2], F. Ebrahimi (PhD)[2], M. Bakhshi (PhD)[3], J. Fathi (BSc)[1]

1.Faculty and Research Institute of Basic Sciences, Imam Hussein University, Tehran, I.R.Iran

2.Biology Research Center, Faculty and Research Institute of Basic Sciences, Imam Hussein University, Tehran, I.R.Iran

3.Nanobiotechnology group, Faculty and Research Institute of Basic Sciences, Imam Hussein University, Tehran, I.R.Iran

J Babol Univ Med Sci; 20(7); July 2018; PP: 47-54

Received: Nov 14th 2017, Revised: Mar 14th 2018, Accepted: Mar 26th 2018.

ABSTRACT

BACKGROUND AND OBJECTIVE: *Escherichia coli* enterohemorrhagic (EHEC) causes diarrhea, hemolysis colitis, and uremic hemolytic syndrome. Due to the dangers of antibiotic treatment in the EHEC O157: H7 infection, vaccines offer a promising way to prevent infection. The purpose of this study was to investigate the immunity of recombinant proteins Intimin, EspA and Stx2b against infection of *E. coli* O157 H7.

METHODS: In this research study, expression of recombinant Intimin, EspA and Stx2b proteins *E.coli* BL21 DE3 was induced with IPTG. Recombinant proteins were examined by Western Blot and purified using the affinity Ni-NTA chromatography. Subcutaneous and intraperitoneally immunization of mice was performed and the antibody titer was determined by ELISA. The mice were orally infected with bacteria and the bacterial loss and mortality of the mice were examined.

FINDINGS: SDS-PAGE analysis showed expression of proteins with 11,17 and 34 kDa molecular weight. The recombinant proteins were confirmed by Western Blot. ELISA end point titer for humoral response to EspA, Intimin and Stx2b was 25600. The loss of bacteria in mice was 10^2 cfu / ml and mortality rate were reduced to 60 Percent.

CONCLUSION: The results showed that immune response against EspA, Intimin and Stx2b proteins protects animals against challenges using *E. coli* O157: H7.

KEY WORDS: *Enterohaemorrhagic Escherichia coli, Shiga like toxin, Type III secretion system, Recombinant protein, Immunization*

Please cite this article as follows:

Taheri M, Nazarian Sh, Ebrahimi F, Bakhshi M, Fathi J. Immunization Evaluation of Type III Secretion System Recombinant Antigens and Shiga Like Toxin Binding Subunit of E. coli O157:H7. J Babol Univ Med Sci. 2018;20(7):47-54.

***Corresponding Author: Sh. Nazarian (PhD)**
Address: Biology Research Center, Faculty and Research Institute of Basic Sciences, Imam Hussein University, Tehran, I.R.Iran
Tel: +98 21 77104934
E-mail: nazarian56@gmail.com

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

Introduction

During pregnancy and childbirth, tremendous changes have taken place in women's spirits, which make them very sensitive to psychological stimuli and sometimes cause problems (1). The process of coping with maternal delivery is naturally challenging (2). Premature infants (less than 37 weeks of gestation) constitute 11.4% of the births of the newborns (3). The premature birth of an infant affects the physical and emotional-psychological well-being of parents (4) and presents them with many problems, and can negatively affect the mood of their mothers and their reciprocal patterns of behavior with their infants. (5).

Among the factors influencing the mental health of mothers after delivery is the health status of the born infant. (6). The results of the researches show that 40-76% of mothers with preterm and low birth weight infants have an experience of depression and other psychological symptoms including incompatibility (8,7). The need to care of a premature infant puts mothers in an unpredictable and unexpected condition (9).

The hospitalization experience of these infants in the intensive care unit is scary for mothers (10). The longer period and the duration of care for the infants in the Neonatal Intensive Care Unit (NICU), results in the greater anxiety and stress for mothers, and the mental health of mothers will be further affected (11). Usually, an incubator or warmer device is used to care for these infants. While these devices often unnecessarily remove infants from their mothers and deprive them of skin-to-skin contact, hence, Kangroo Mother Care (KMC), in which the infant is in skin contact with mother, is introduced as an appropriate alternative to incubation care of premature infants who overcome the initial problems. So today, this type of care is recommended in accordance with the recommendations of the WHO and the Neonatal Health Department, which is to be implemented continuously.

The experience of implementing such a project, despite the provision of a KMC package has been seriously implemented in a limited number of university centers, and is still not fully institutionalized at the national level (12). Reports demonstrated that in the KMC method, mental stress, stress and doomof mothers' conscience are less than the conventional one, and mothers prefer to use skin contact. They have expressed more self-confidence, higher self-esteem and a sense of satisfaction and ability to do positive work for their premature infants (13, 14). Findings of the study by Faramarzi et al showed that maternal care using KMC method had increased maternal compatibility with existing problems. In other words, this method of care will help mothers to have a better performance in infant care in stressful conditions due to the birth of premature and low birth weight infants. In another study, De Macedo and colleagues reported that KMC plays an important role in mood changes and reduction of depression in mothers (15).

Kangroo Mother Care (KMC) also improves the health and well-being of infants through more effective control of body temperature, success in breastfeeding, infection prevention, and closer relationship between mother and child (16), and prepare them to establish a to-way and coordinated interactive pattern (17). A review of the literature showed that most research focused on the effect of Kangroo Mother Care on the physical condition of the infant (19,18), and in the few studies, mothers with premature infants were as target population.

Since the underlying psychological basis of the individual comes from the very early childhood and infancy, primary support of the mother is very important in future interactions between them (20), and on the other hand, given the broad and valuable view of prevention of mental health problems, in this study, the effect of Kangroo Mother Care on the mental health of mothers with premature infants was investigated.

Methods

This quasi-experimental study was carried out using available random sampling method after obtaining permission from the Ethics Committee of Babol University of Medical Sciences with number: Mubabol.REC.1394.119. This study was performed on 60 mothers with premature infant admitted to the Neonatal Intensive Care Unit at the Medical and Educational Center of Ayatollah Rouhani affiliated to Babol University of Medical Sciences in 2012-2013 in two groups of control and intervention. Mothers with premature infants (less than 37 weeks of gestational age) admitted to the neonatal intensive care unit who had a tendency to perform Kangroo Mother Care and

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

their infants had a stable physiological status and weight of between 1000 and 2500 grams, at least reading and writing skills in Persian, singleton, pregnancy with tendency, no history of depression and birth of premature infants, no abnormalities in the infant and no adverse events were included in the study. Discharge earlier than one week after starting the Kangroo Mother Care was exclusion criteria of the study.

In this research, with 95% confidence, the test power of 80% and d = 0.65 (21), the sample size was estimated to be 30 in each group. After cooperation of mothers to participate in the study and following all ethical considerations in the research (confidentiality of information, obtaining written consent from mothers, voluntary participation in the study) demographic information questionnaire and Goldberg & Hiller questionnaire with 28 questions were used as a tool for data collection (20).

The mothers were trained in the intervention group to put the infant naked in the middle of their chest to make skin contact, and provide maternity care for at least 2 hours, 3-4 times a day for one week. Monitoring the mother's performance was continuously and effectively performed by the researcher to ensure that the intervention was carried out.

However, the mothers in the control group were in the process of ongoing care. The mental health of mothers first and after one month after admission was considered based on the questionnaire. The General Health Questionnaire, a 28-item questionnaire, was developed by Goldberg and Hiller in 1979, which includes four levels of physical symptoms, anxiety and sleep disorders, impaired social function, and severe depression (20).

Of the 28 items of the questionnaire, items 1 to 7 are related to the scale of physical symptoms, 8 to 14 symptoms of anxiety and sleep disorders, and 15 to 21 items are related to the assessment of social function symptoms and ultimately 22 to 28 items measure the symptoms of depression. The questionnaire, as a screening tool, spends around 10-12 minutes, (22) examines the person's mental status in a recent month (23). In this study, 28 questions were used in the standard form and responses were coded using Likert score (0-1-2-3).

The cut-off point in this study was that individuals with a score of 22 and below were enrolled in the healthy group and those with a score of 23 and higher were categorized in the group of people with a disorder (25, 24). Noorbala et al., in evaluating the validity of the general health questionnaire with 28- items, based on traditional scoring, reported cut off score of 6 and based on simple Likert score, reported the cutoff point of 22, and the reliability coefficient of the questionnaire was 91% (26).

In this study, the reliability coefficient was 89%. The questionnaire was completed two times, before the starting of Kangroo Mother Care at the beginning of the infant admission and after one month of infant admission. This study was performed by comparing the questionnaires completed before and after the intervention by the mother. After collecting the final data, obtained data were analyzed using SPSS 14 software and multivariate covariance analysis (MANCOVA), $p < 0.05$ was considered significant.

Results

In this study, 60 mothers were enrolled. Most of the participating mothers had a high school diploma (65.7%) and 76.6% were housekeeper (Table 1).

Table 1. Distribution of absolute and relative frequency of some demographic characteristics of mothers in two groups

Group	variable	intervention N(%)	control N(%)	X^2
Education	Under diploma	2(3.4)	8(13.4)	4.49
	Diploma	17(28.4)	13(21.3)	
	Bachelor	5(8.3)	2(3.4)	
	Higher than diploma	6(10)	7(11.8)	
Occupation	House keeper	25(41.6)	21(35)	2.79
	Employee	5(8.4)	9(15)	

The mean of the most subscales of the intervention group after the implementation of the maternal care program was reduced, so that the overall mental health score in the intervention group before and after maternal care was 28.77±13.09 and 13.66±6.51, respectively and in the control group was changed from 30.86±15.97 to 20.99±8.89 ($p < 0.01$) (Table 2). The intervention group compared with the control group after receiving maternal care was significantly reduced in the sub

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

variables of the mental health scale, except for the symptoms of depression in the intervention group. In other words, the reduction of mean in variables of the mental health scale for the intervention group by maternal care was statistically significant. The results of the multivariate covariance analysis test for subscales of mental health test after removing the effect of pre-test by hoteling test showed that in the sub variables of the mental health scale, there was a significant decrease in the mean of depression scores in the intervention group (Table 3). Two groups were similar and did not have a significant difference in terms of interventional variables such as infant's age, infant's disease during admission, birth rate and age of mothers ($p < 0.05$).

Table 2. Comparison of mental health variables in two groups of intervention and control

Variable	Intervention group			Control group		
	Pre-test Mean±SD	Post-test Mean±SD	p-value	Pre-test Mean±SD	Post -test Mean±SD	p-value
Symptoms of anxiety and sleep disorders	4.62±8.23	1.50±3.70	<0.001	5.31±8.83	3.39±5.67	<0.001
Social function	3.31±9.32	2.58±5.90	<0.001	2.38±10.16	2.20±7.13	<0.001
Symptoms of depression	1.90±3.87	1.30±1.03	<0.001	2.79±3.77	2.31±2.70	<0.002
Physical symptoms	4.27±7.73	1.58±2.93	<0.001	4.85±8.10	3.31±5.20	<0.002
mental health	13.09±28.77	6.51±13.56	<0.001	15.97±30.86	8.89±20.90	<0.001

*** Significant difference in the level of 0.05 between the two groups**

Table 3. Summary of single-variable covariance test results

Variable	SS	df	MS	F	Sig
Symptoms of anxiety and sleep disorders	47.87	1	47.87	6.99	0.01
Social function	18.78	1	18.78	3.86	0.04
Symptoms of depression	4.88	1	4.88	1.23	0.17
Physical symptoms	57.35	1	57.35	7.17	0.01
mental health	442.52	1	442.52	8.15	0.01

Discussion

The results of this study showed that Kangroo Mother Care had a significant effect on improving the mental health of mothers in both the whole scale and some of its subscales. There was a significant difference between the mean scores of the subjects in the intervention and control groups at the post-test stage in the whole scales of mental health and its subscales, including physical symptoms, insomnia and anxiety and social function. In confirmation of our findings, the study by Badiee et al. also showed that Kangroo Mother Care had a positive effect on the mental health of mothers with low-birthweight infant (11). Palizvan et al. also found that kangaroo mother care was effective on the general health of mothers with a neonate (term) and without history of hospitalization (27). Along with the results of our study in the anxiety subscale, Keshavarz et al. showed that skin contact is effective on the positional anxiety of mothers admitted to the cesarean section (28). However, in our study, more intervention days and effect of KMC for a longer period (one month after admission of infant) have been investigated and evaluated. The study of Karimi et al. showed the effect of mother and infant skin contact on the attachment and anxiety of mothers regarding the health status of the

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

newborn (17). Faramarzi et al. in their study acknowledged that close proximity to the skin and the contact of the skin to the mother and the baby caused a greater sense of comfort, health and compatibility in mothers (2).

In the study of Feldman et al., mothers showed more positive emotions with skin contact with their infants and became more compatible with their infant. According to their findings, the rate of depression in mothers was also reduced. In three months of infancy, parents showed more sensitivity to caring for the child and provided a better home environment for them. and in the six-month follow up of mental development, these children developed significantly on the basis of the Bayley development test of infants and the psycho-motor development index (29). Nyqvist et al., in a study in Sweden regarding the effect of Kangroo Mother Care, found that this intervention reduced the symptoms of depression in mothers and made parents more sensitive to the baby. In addition, it increases the breastfeeding duration of mothers and leads to better interaction between parents (30).

In a study by Saatsaz et al., the daily contact between mother and the infant for 20-30 minute days for 10 days reduced the severity of postpartum mourning in mothers (31). De Alecar and colleagues in their study emphasized that Kangroo Mother Care would have an effect on the improvement of postpartum depression, feeling of strength, happiness, and speed of action in the mother (32). In another study, De Macedo and colleagues reported that Kangroo Mother Care plays an important role in mood change and reduction of depression in mothers (15).

In our study, the subscales of depression symptoms did not show a meaningful relationship between the two groups, although the mean scores of this area decreased after intervention. As mentioned, effective components in mental health are symptoms of depression and people with depression symptoms have low self-confidence and self-esteem (33). Therefore, interventions that lead to improving the level of depression in mothers are considered important. In this regard, Lee et al. suggested that Kangroo Mother Care, in addition to fixing the physiological symptoms of the baby, it also increases the self-esteem of the mothers (13). However, Arzani et al showed that there was no significant difference in maternal self-esteem score before and after KMC interventions and at the time of discharge (34), which could be related to the type of instrument used to measure the level of self-esteem, the duration of the KMC in a day or the time of completing the self-esteem questionnaire (during the discharge of the baby, which is an anxious and stressful time for parents).

Considering the birth rate of the premature infant in the country and the complications of the illness and the admission of the infant to the mother, it is recommended to provide a suitable and non-prescriptive approach that is easy to do and requires no equipment and high cost. Therefore, it is desirable to provide this care method for mothers with premature infants in the very early days of the infant's birth, and the health care staff and nurses to carry out and promote this care continuously (24 hours), because the mothers are responsible for care and maintenance of the infants and this is something that nurses must understand and prioritize.

The limitations of this study are the cultural and social characteristics of the community, which requires people to be conservative in many areas such as mental health, especially in our research, which is used by self-report scales to measure this structure. Therefore, it is suggested that in future research, other appropriate tools be used to measure more accurately the mental health of individuals.

Our research showed that Kangroo Mother Care can provide more mental health for mothers, leading to reduced physical symptoms, insomnia and anxiety, social ineffectiveness and depression in mothers with preterm infants. Therefore, it is recommended that the result of this research be of interest to maternal and neonatal health care providers in order to continually use this care method in neonatal intensive care units because the mother is in direct interaction with the newborn and, of course, the quality of this relationship and interaction is also effective on the process of infant growth. In addition, the findings of this study can help nursing managers to improve the knowledge of nurses through the provision and holding of workshops, panel discussions and discussions on Kangroo Mother Care.

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

Acknowledgment

Hereby, we would like to thank to the Vice-Chancellor for Research and Technology of Babol University of Medical Sciences for financial support and from the colleagues of the neonatal intensive care unit of the Ayatollah Rohani Hospital in Babol which helped us in the process of implementation of this research.

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

References

1.Nazarian S, Gargari SLM, Rasooli I, Alerasol M, Bagheri S, Alipoor SD. Prevalent phenotypic and genotypic profile of enterotoxigenic Escherichia coli among Iranian children. Jpn J Infect Dis. 2014;67(2):78-85.

2.Bakhshi M, Ebrahimi F, Nazarian S, Zargan J, Behzadi F, Gariz DS. Nano-encapsulation of chicken immunoglobulin (IgY) in sodium alginate nanoparticles: In vitro characterization. Biologicals. 2017;49:69-75.

3.Mousavi SL, Rasooli I, Nazarian S, Amani J. Simultaneous detection of Escherichia coli O157: H7, toxigenic Vibrio cholerae, and Salmonella typhimurium by multiplex PCR. Arch Clin Infect Dis. 2009;4(2):97-103.

4.Saeedi P, Yazdanparast M, Behzadi E, Salmanian AH, Mousavi SL, Nazarian S, et al. A review on strategies for decreasing E. coli O157:H7 risk in animals. Microb Pathog. 2017;103:186-95.

5. Mirhoseini A, Amani J, Nazarian S. Review on pathogenicity mechanism of enterotoxigenic *Escherichia coli* and vaccines against it. Microbial Pathogenesis. 2018;117:162-169.

6.Cramer J. Enterohemorrahagic *Escherichia coli* (EHEC): Hemorrhagic Colitis and Hemolytic Uremic Syndrome (HUS). Emerg Infect Dis. Clinical Case Studies (Northem Germany). 2014:213-27.

7.Larrie-Bagha SM, Rasooli I, Mousavi-Gargari SL, Rasooli Z, Nazarian S. Passive immunization by recombinant ferric enterobactin protein (FepA) from *Escherichia coli* O157. Iran J Microbiol. 2013;5(2):113-9.

8.Gyles C. Shiga toxin-producing An overview. J Anim Sci. 2007;85(13-suppl):E45-62.

9.Amani J, Salmanian AH, Rafati S, Mousavi SL. Immunogenic properties of chimeric protein from espA, eae and tir genes of *Escherichia coli* O157: H7. Vaccine. 2010;28(42):6923-9.

10.Hosseini ZS, Amani J, Baghbani Arani F, Nazarian S, Motamedi MJ, Shafighian F. Immunogenicity of the nanovaccine containing intimin recombinant protein in the BALB/c mice. Clin Exp Vaccine Res. 2018;7(1):51-60.

11.Baranvand M, Honari H. Nasal immunogenicity induced by STxB and STxB-IpaD antigens in laboratory rats. Koomesh. 2015;16(3):397-403. [In Persian]

12. Saberi F, Salmanian AH, Amani J, Jafari M. Production of Chimeric Tir-intimin Protein from *E. coli* O157: H7 in the Tobacco (Nicotiana tobbacum) Plant and its Immunological Evaluation in an Animal Model. Modares J Med Sci. 2012; 15 (3):23-36.[In Persian]

13.Kazemi R, Akahavian TA, Jafari M, Amani J, Mousavi A, Hatef SA. Cloning and expression of the binding subunit of shiga-like toxin type 2 gene and immunization study in an animal model. Modares J Med Sci. 2016;18(4):45-60. [In Persian]

14.Martorelli L, Garbaccio S, Vilte DA, Albanese AA, Mejías MP, Palermo MS, et al. Immune Response in Calves Vaccinated with Type Three Secretion System Antigens and Shiga Toxin 2B Subunit of *Escherichia coli* O157: H7. PLoS One. 2017;12(1):e0169422.

15.Bakhshi M, Ebrahimi F, Zargan J, Nazarian S, Sheikhzade V. Cloning and Recombinant Expression of EspA as a Virulence Factor of *E.coli* O157: H7. J Mazandaran Univ Med Sci. 2014;24(117):12-20.[In Persian]

16. Khalouie F, Mousavi SL, Nazarian S, Amani J, Pourfarzam P. Immunogenic evaluation of chimeric recombinant protein against ETEC, EHEC and *Shigella*. Mol Biol Res Commun. 2017;6(3):101-12.

17.Abdollahi M, Honari H, Nazarian SH. Subcloning and expression of SO6 gene, Saponaria Officinalis plant in *E.coli* and investigation of antibody titer in rats. J Shahid Sadoughi Univ Med Sci, 2017; 24(12): 1024-33. [In Persian]

18. Nazarian S, Mousavi Gargari L. Immunogenicity Evaluation of PLGA Nanoparticles Contains Recombinant CfaB Protein from Enterotoxigenic *Escherichia Coli* . J Babol Univ Med Sci. 2017;19(9):39-44. [In Persian]

19.Cook S, Bach S, Stevenson S, DeVinney R, Frohlich A, Fang L, et al. Orally administered anti-*Escherichia coli* O157: H7 chicken egg yolk antibodies reduce fecal shedding of the pathogen by ruminants. Canadian J Anim Sci. 2005;85(3):291-9.

[DOR: 20.1001.1.15614107.1397.20.7.7.7]
[DOI: 10.18869/acadpub.jbums.20.7.47]

20.Frankel G, Candy D, Everest P, Dougan G. Characterization of the C-terminal domains of intimin-like proteins of enteropathogenic and enterohemorrhagic *Escherichia coli*, Citrobacter freundii, and Hafnia alvei. Infect Immun. 1994;62(5):1835-42.

21.Kühne SA, Hawes WS, La Ragione RM, Woodward MJ, Whitelam GC, Gough KC. Isolation of recombinant antibodies against EspA and intimin of *Escherichia coli* O157: H7. J Clin Microbiol. 2004;42(7):2966-76.

22.Garcia-Angulo VA, Kalita A, Torres AG. Advances in the development of enterohemorrhagic *Escherichia coli* vaccines using murine models of infection. Vaccine. 2013;31(32):3229-35.

[DOR: 20.1001.1.15614107.1397.20.7.7.7]

[DOI: 10.18869/acadpub.jbums.20.7.47]