

Serum Tumor Necrosis Factor - Alpha Alterations of Dry Cows Vaccinated with Inactive Coronavirus, Rotavirus, *E. coli* Combined Vaccine

Zafer Mecitoglu^{1*}, Gulsah Demir Akgul¹, Serkan Catik¹, Duygu Udum² and Hasan Batmaz¹

¹ Department of Internal Medicine, Uludag University, Faculty of Veterinary Medicine, 16059, Gorukle, Bursa, TURKEY

² Department of Biochemistry, Uludag University, Faculty of Veterinary Medicine, 16059, Gorukle, Bursa, TURKEY

Received: 08.02.2017; Accepted: 05.04.2017; Published Online: 16.05.2017

ABSTRACT

Tumor Necrosis Factor alpha (TNF- α) is a proinflammatory cytokine, closely associated with insulin resistance and related diseases such as type II ketosis and fatty liver of cows. Aim of the presented study was to investigate circulating levels of TNF- α after vaccinating dry cattle with inactive rotavirus, coronavirus and E.coli combined vaccine. 14 cattle were randomly assigned to two groups; vaccination group was vaccinated on days 0 and 21 of the study and control group not. Blood samples were collected on days 0, 1, 5, 15, 21, 22, 26, 36 of the study for evaluation of TNF- α and total leukocyte counts. Although not significant, TNF- α levels decreased in vaccination group after the second application. Total Leukocyte counts did not differ between and within groups. Results of the study demonstrates that vaccinating cattle with inactive rotavirus, coronavirus and E.coli combined vaccine in dry period does not induce alterations in circulating TNF- α levels.

Keywords: TNF- α , Vaccination, Rotavirus, Coronavirus, *E. coli*, Dry Period

Abbreviations: TNF- α : tumor necrosis factor alpha

INTRODUCTION

TNF- α or cachexin is a proinflammatory cytokine, primarily produced by macrophages and to a lesser extent by lymphocytes, kupffer cells and adipocytes (Beutler and Cerami, 1989; Yoshioka et. al., 1998). TNF- α has immunological roles such as antimicrobial activity, antitumor activity and mediation of immunity (Alaaddine et. al., 1999). Viral diseases, other cytokines and endotoxin lipopolysaccharide causes elevation of TNF- α levels (De Forge et. al.,1990). Vaccination, especially with live vaccines is also reported to increase blood TNF- α levels for a period as long as a month (Hacker et. al., 1998; Pukhalsky et. al., 2003).

Type II ketosis and related fatty liver syndrome of cows is a disease complex of dairy cattle in early days of lactation. Insulin resistance, plays a important role in ethiopathogenesis of both diseases (Holtenius and Traven, 1990; Holtenius, 1993). Recent studies demonstrated the relationship between inflammation, TNF- α and insulin resistance (Hotamisligil, et. al., 1995; Hotamisligil, 1999). However, to our knowledge, relation between post vaccination cytokine levels and insulin resistance in cattle is not studied before.

Vaccinating cattle in their dry period is a widely used strategy to increase specific antibody levels in colostrum. As rotavirus, coronavirus and *Escherichia coli* (*E.coli*) are among the most important pathogens involved in scours of calves, especially vaccination of cattle against these pathogens is a common practice in almost every dairy herd.

As mentioned above TNF- α is closely related with insulin resistance and metabolic diseases of dairy cows. Thus alterations in circulating TNF- α levels around parturition could be associated with increased insulin resistance. Aim of the presented study is to investigate serum TNF- α levels of cattle after vaccination with rotavirus, coronavirus and E.coli combined inactive vaccine.

MATERIALS AND METHODS

Multiparous Holstein Fresian cows (n:14) in the beginning of the dry period with BCS between 3.25 - 3.50 from the same herd were selected for the study. Mean lactation of the selected animals was $2.9 \pm 0,3$; and mean milk yield in the previous lactation was $9,130 \pm 66$ kg (305d) per cow were used in the presented study. All cows were from the same herd with management and feeding conditions. 14 animals were selected according to the

* Corresponding author: zmecitoglu@uludag.edu.tr

results of clinical and hematological examinations. 7 animals randomly selected were designated as vaccination group (VG) and other 7 as control group (CG). VG received, rotavirus-coronavirus- E.coli toxoid combined commercial vaccine (Scourguard 4K[®], Zoetis) on days 0 and 21 of the study. CG group did not receive any vaccinations. Animals did not receive any other vaccine or medication during the study period. Blood samples were collected by jugular venipuncture from each cow into 10 mL evacuated tubes containing (Ethylenediaminetetraacetic acid) EDTA and tubes without anticoagulant. After clotting, the samples were centrifuged at 1000 rpm for 15 min and sera were immediately separated and stored at -20°C until analyses. Samples of all animals were collected on day 0, before first vaccination and on day 1, 5 and 15 after the first vaccination. Second dose of the vaccine was administered on day 21 to VG group and sera samples were collected again from both groups before booster vaccination and on day 1, 5 and 15 (days 22, 26 and 36 of the study) after second vaccination. Animals were clinically examined before every sample collection. Serum TNF- α levels were measured by using commercial bovine TNF- α ELISA kit as per the manufacturer's instructions (CSB-E 1202B, CUSABIO Biotech Co., Wuhan, China). Total leukocyte counts were measured by using an automatic analyzer (Vetscan HM-5, Abaxis, California, USA).

Statistical analyses of the results were performed by using Sigma Plot 12 software (Systat Software Inc., San Jose, California, USA). The mean values within the groups were compared using one-way repeated measures analysis of variance (one-way RM ANOVA). The mean values between the groups were compared using Student t-test for every day of the study. For all analyses, $P \leq 0.05$ was considered significant

RESULTS

TNF- α levels of cows in both groups did not differ significantly during study period (Figure 1). A non significant increase till the second and then a decrease in TNF- α levels were detected in VG. Total leukocyte counts did not differ between and within groups (Table 2). None of the animals used in the presented study show any adverse reactions due to vaccination. Metabolic diseases such as clinical ketosis, fatty liver and hypocalcaemia were not observed in any of the animals during the study period.

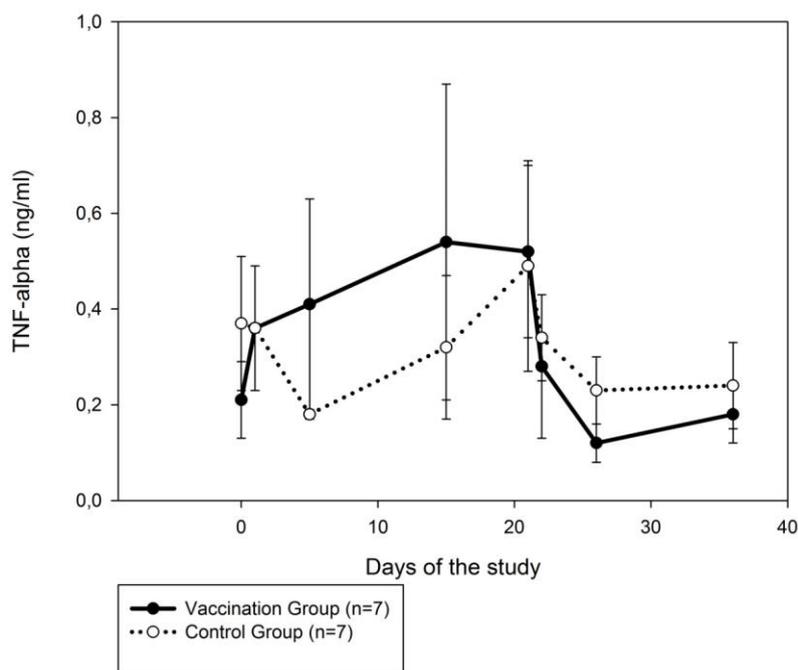


Figure 1. Mean serum TNF- α levels (\pm SEM) of vaccinated group (VG) and control (CG) group.

Table 2. Mean total leukocyte count (\pm SEM) of vaccinated group (VG) and control (CG) group.

Days	Mean Total Leukocyte Count ($10^9/L$)								P
	0	1	5	15	21	22	26	36	
VG (n:7)	8,74 \pm 0,93	8,38 \pm 0,54	9,57 \pm 1,21	6,94 \pm 1,42	8,57 \pm 0,55	7,30 \pm 0,53	8,16 \pm 1,14	7,73 \pm 0,54	0,49
CG (n:7)	8,91 \pm 0,86	9,81 \pm 1,01	8,77 \pm 1,47	8,06 \pm 0,87	8,85 \pm 0,67	7,21 \pm 0,91	7,98 \pm 1,36	7,98 \pm 1,36	0,22
P	0,61	0,19	0,68	0,51	0,77	0,93	0,86	0,59	

DISCUSSION

A study conducted on cows suffering fatty liver revealed that insulin response is decreased when severity of fatty liver increases (Ohtsuka et. al., 2001). In the same study is also concluded that increased TNF- α activity is positively correlated with insulin resistance. Relationship between inflammatory cytokines like TNF- α and diseases such as obesity and diabetes mellitus which are associated with insulin resistance is well documented in humans and murine models (Hotamisligil, et. al. 1995; Hotamisligil, 1999). Similar relationship was also reported in cattle, as subcutaneous injection of recombinant bovine TNF- α induced elevated plasma insulin levels and inhibited insulin stimulated glucose utilization in heifer steers (Kushibiki et. al., 2000). Exact mechanism of TNF- α related insulin resistance is unclear however Hotamisligil et. al. (1996) reported that insulin receptor substrate (IRS) could play a major in TNF- α related insulin resistance.

Attenuated live vaccines against measles and yellow fever are demonstrated to induce production of cytokines including TNF- α in human (Hacker et. al., 1998; Pukhalsky et. al., 2003). Similarly vaccination of cattle with Brucella RB51 vaccine resulted with increased expression of TNF- α from trophoblastic epithelial cells (Palmer et. al., 1998). Although such reactions are not reported in cattle vaccinated with inactive vaccines, vaccination of mice with subcutaneously inactivated coronavirus is reported to result with increased cytokine production including TNF- α (Takasuka et. al., 2004). However In the presented study blood TNF- α levels and total leukocyte counts did not show significant differences between samplings during the study period. On the other hand, TNF- α show a slight decrease after booster vaccination (Figure-1). This finding was also reported after vaccination of humans with inactivated influenza vaccine and decreased serum TNF- α levels days after vaccination were related with cytokine uptake or redistribution of immune cells to other compartments (Ramakrishnan et. al., 2012).

Along with viral antigen, adjuvants used in vaccines could also be a cause for increased TNF- α production. Adjuvant in the vaccine used in the presented study was quil-A. However studies conducted on cytokine responses after administration of quil-A did not detect any increase in TNF- α levels (Valensi et. al., 1994; Rothel et. al., 1998).

Based on the findings of the presented study it could be concluded that vaccination against coronavirus, rotavirus and E.coli did not significantly affect circulating levels of TNF- α in dry cows. However further studies conducted in dry period with vaccines against different diseases could elicit increased TNF- α levels.

ACKNOWLEDGEMENTS

This study was financially supported by the Research Fund of Uludag University.

ANIMAL RIGHTS STATEMENT

This study was approved by the Uludag University Animal Research Local Ethics Committee

REFERENCES

- Alaaeddine N, Battista JAD, Pelletier JP, Kiansa K, Cloutier JM and Pelletier JM (1999). Differential effects of IL-8, LIF (pro-inflammatory) and IL-11 (anti-inflammatory) on TNF- α -induced PGE₂ release and on signalling pathways in human OA synovial fibroblasts. Cytokine 11(12): 1020–1030.

- Beutler B and Cerami A. (1989). The biology of cachectin/TNF- α primary mediator of the host response. *Annual Review of Immunology* 7: 625–655.
- De Forge LE, Nguyen DT, Kunkel SL and Remick DG (1990). Regulation of the pathophysiology of tumor necrosis factor. *Journal of Laboratory and Clinical Medicine* 116: 429–438.
- Hacker UT, Jelinek T, Erhardt S, Eigler A, Hartmann G, Nothdurft HD and Endres S (1998). In vivo synthesis of tumor necrosis factor- α in healthy humans after live yellow fever vaccination. *Journal of Infectious Disease* 177(3): 774-778.
- Holtenius P (1993). Hormonal regulation related to the development of fatty liver and ketosis. *Acta Veterinaria Scandinavica* 89: 55–60.
- Holtenius P and Traven M (1990). Impaired glucose tolerance and heterogeneity of insulin responses in cows with abomasal displacement. *Journal of Veterinary Medicine Series A* 37: 445-451.
- Hotamisligil GS (1999). The role of TNF- α and TNF receptors in obesity and insulin resistance. *Journal of Internal Medicine* 245: 621–625.
- Hotamisligil GS, Arner P, Caro JF, Atkinson RL and Spiegelman BM (1995). Increased adipose tissue expression of tumor necrosis factor alpha in human obesity and insulin resistance. *The Journal of Clinical Investigation* 95(5): 2409-2415.
- Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF and Spiegelman BM (1996). IRS-1-Mediated Inhibition of Insulin Receptor Tyrosine Kinase Activity in TNF- α - and Obesity-Induced Insulin Resistance. *Science* 271: 665-670.
- Kushibiki S, Hodate K, Ueda Y, Shingu H, Mori Y, Itoh T and Yokomizo Y (2000). Administration of recombinant bovine tumor necrosis factor-alpha affects intermediary metabolism and insulin and growth hormone secretion in dairy heifers. *Journal of Animal Science* 78(8): 2164-2171.
- Ohtsuka H, Koiwa M, Hatsugaya A, Kudo K, Hoshi F, Itoh N, Yokota H, Okada H and Kawamura S (2001). Relationship between serum TNF activity and insulin resistance in dairy cows affected with naturally occurring fatty liver. *The Journal of Veterinary Medical Science* 63: 1021–1025.
- Palmer MV, Elsasser TH and Cheville NF (1998). Tumor necrosis factor-alpha in pregnant cattle after intravenous or subcutaneous vaccination with *Brucella abortus* strain RB51. *American Journal of Veterinary Research* 59(2): 153-156.
- Pukhalsky AL, Shmarina GV, Bliacher MS, Fedorova IM, Toptygina AP, Fisenko JJ, Alioshkin VA (2003). Cytokine profile after rubella vaccine inoculation: evidence of the immunosuppressive effect of vaccination. *Mediators of Inflammation* 12(4): 203-207.
- Ramakrishnan A, Althoff KN, Lopez JA, Coles CL and Bream JH (2012). Differential serum cytokine responses to inactivated and live attenuated seasonal influenza vaccines. *Cytokine* 60(3): 661-666
- Rothel JS, Corner LA, Lightowers MW, Seow HF, Mc Waters P, Entrican G and Wood PR (1998). Antibody and cytokine responses in efferent lymph following vaccination with different adjuvants. *Veterinary Immunology and Immunopathology* 63: 167-183.
- Takasuka N, Fujii H, Takahashi Y, Kasai M., Morikawa S, Itamura S, Ishii K, Sakaguchi M, Ohnishi K, Ohshima M, Hashimoto S, Odagiri T, Tashiro M, Yoshikura H, Takemori T and Tsunetsugu-Yokota Y (2004). A subcutaneously injected UV-inactivated SARS coronavirus vaccine elicits systemic humoral immunity in mice. *International Immunology* 16 (10): 1423-1430.
- Valensi JP, Carlson JR and Van Nest GA (1994). Systemic cytokine profiles in BALB/c mice immunized with trivalent influenza vaccine containing MF59 oil emulsion and other advanced adjuvants. *The Journal of Immunology* 153: 4029 - 4039.
- Yoshioka M., Ito T., Miyazaki S., Nakajima Y. (1998). The release of tumor necrosis factor-alpha, interleukin-1, interleukin-6 and prostaglandin E2 in bovine Kupffer cells stimulated with bacterial lipopolysaccharide. *Veterinary Immunology and Immunopathology* 66: 301– 307.