Research Paper

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# Expression Study of Interleukin 8 in Deoni Cattle by Real Time Polymerase Chain Reaction (Rt-Pcr)

Biotechnology

**KEYWORDS :** Subclinical Mastitis, Interleukin 8, Real Time PCR, Gene Expression

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## ABSTRACT

*Interleukin- 8 (IL 8), also known as Neutrophil Activator Agent, is a chemokine molecule involved in the activation and extravasation of neutrophils during an inflammatory response. A remarkable property of IL 8 is its variable expression in different cells under different conditions. In the present study, quantitative real time PCR was used to analyse the expression of IL 8 gene in milk somatic cells from subclinical mastitic cows in relation to healthy cows of Deoni breed. It was found that IL 8 expression was significantly increased in response to subclinical mastitis. The change in the expression profile of IL 8 in subclinical mastitis shows the significant role played by this cytokine in mediating the inflammatory reactions during mastitis in Deoni cattle. This study reveals the importance of IL 8 as valuable biomarker of subclinical mastitis and the scope of manipulation of IL 8 expression in controlling mastitis in cattle.*

**Introduction**

Mastitis is one of the major production diseases of dairy industry all over the world. Subclinical mastitis is a serious form of mastitis in India, with prevalence varying from 20-83% in cows and 45% in buffaloes (Sharma *et. al.*, 2012) and results in tremendous economic loss. Subclinical form of mastitis is difficult to detect as the animal appears healthy with apparently healthy udder and normal colour and appearance of milk (Laevens *et al.*, 1997). However, the bacterial and the somatic cell count of the milk may increase. The subclinical mastitic animals are the greatest reservoirs of infection to healthy animals.

Cytokines are regulatory proteins that play a central role in immune system and involved in every facet of immunity and inflammation, including antigen presentation, bone marrow differentiation, cellular recruitment, activation and adhesion of molecules of the immune system etc (Balkwill and Burke, 1989). The cytokines predominantly produced by monocytes include Tumor Necrosis Factor (TNF) and many interleukin molecules like IL 6, IL 8, IL 12, IL 15, IL 18 and IL 23. Chemokines are group of small 8- 12KD molecules that are capable of inducing chemotaxis in a variety of cells including neutrophils, monocytes, lymphocytes, eosinophils, fibroblasts and keratinocytes. IL 8 is a well known chemokine molecule, also named as Neutrophil Activator Agent or CXCL8.

A remarkable property of IL 8 is its variation of expression in different cells under different conditions. In healthy tissues, IL 8 expression is neglible but on stimulation by infections due to bacteria, virus etc or other cellular stresses, it's concentration increases rapidly by 10-100 folds. Maximum IL 8 expression is generated by a combination of three different mechanisms: de-repression of the IL 8 gene promoter, transcriptional activation of gene nuclear factor-kB and JUN-N-terminal proteineases pathway and by stabilization of IL 8 mRNA by p38 mitogen activated proteinkinase pathway (Hoffmann *et al.*, 2002).

The present study was conducted at National Institute of Veterinary Epidemiology and Disease Informatics (NIVEDI), Bengaluru, to detect the change in expression profile of IL 8 in the milk somatic cells of subclinical mastitic and healthy animals of Deoni cattle of India.

**Materials and method**

**Collection of milk samples**

The milk samples were collected from Deoni cattle maintained at Sothern Regional Station (SRS), National Dairy Research Institute (NDRI), Bengaluru. The samples were collected aseptically in 50ml centrifuge tubes, after cleaning and washing the udder with potassium permanganate solution (1:1000) and swabbing with 70% ethanol.

**Milk somatic cells counting**

Somatic cells count (SCC) was done using NucleoCounter SCC-200 (ChemoMetec, USA) and on the basis of SCC, healthy and subclinical mastitic animals were selected.

**Isolation of somatic cells from the milk samples**

75µl of 0.5mM EDTA was added to 15ml of milk and centrifuged at 2000g for 15 mins at 4°C. Supernatant was discarded and the fat layer formed was removed with alcohol soaked cotton. To the pellet, added 20ml of PBS-EDTA solution and centrifuged at 2000g for 15 mins at 4°C. Supernatant was discarded and the process repeated 2-3 times until a clear supernatant formed. Added 10ml of PBS to the pellet and continued centrifugation at 2000g for 15 mins at 4°C. The pellet formed was resuspended in 2ml of PBS, transferred to the 2ml centrifuge tubes and centrifuged at 2000g for 15 mins at 4°C. Supernatant discarded, final resuspension of somatic cell pellet was done in 500µl PBS and stored at 4°C.

**Extraction of mRNA and conversion to cDNA**

mRNA was extracted from somatic cells using RNA isolation kit, RNeasy Mini Kit (Qiagen, USA) as per the manufacturers protocol. The concentration of extracted RNA was determined using the NanoDrop 2000/2000C spectrophotometer (NanoDrop Technologies) and 260/280 nm ratio was calculated to evaluate the purity of RNA. Reverse transcription was performed and obtained cDNA using QuantiTect Reverse Transcription Kit (Qiagen, USA) as per the manufacturers protocol.

**Quantification of IL-8 gene by real time PCR**

Real-time PCR was performed by Taqman assay in the Light Cycler 480 Real Time PCR System (Roche, USA). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as endogenous control. The primer pairs used by Lee *et al.*, 2006 were used in the present study. 20µl of reaction volume was prepared with 2µl of cDNA template and loaded on to 96 well plate. PCR was performed with intial denaturation at 95°C for 5 mins followed by 45 cycles of denaturation at 95°C for 10 sec, 58°C annealing at for 30s, and extension at 72°C for 1s.

Relative quantification method was used for the analysis of real time PCR data. The Cp value for IL 8 and for GAPDH was determined and the expression of IL 8 was calibrated with respect to GAPDH and converted to relative expression ratio. Quantification of gene expression was measured using Comparative CT method or $2^{(-\Delta\Delta CT)}$ method and expressed as fold change in gene expression.

**Results and Discussion**

Somatic cell count (SCC) in milk is considered as a rapid and reliable indicator of subclinical mastitis, worldwide. In the present study, SCC was conducted in the milk samples collected from Deoni cattle, based on which two groups of cattle (n=8) were identified, healthy group and subclinical mastitic (SCM) group with mean value of SCC as 0.414±0.102 and 10.73±3.17 respectively ( fig 1).

Cytokines are key regulatory molecules involved in modulating the innate as well as specific immunity in mastitis (Sordillo, *et al.,* 1997) and IL 8 molecules are chemokines that mediate the activation and extravasation of neutrophils in bacterial invasion (Kehrli and Harp, 2001). Here we used real time PCR to measure the relative expression of IL 8 in the milk somatic cells of normal and subclinical mastitic animals. The milk somatic cells were isolated and total mRNA extracted from cells. The concentration and purity of mRNA was examined by NanoDrop analysis. The mRNA had concentration 4.22±0.37 ng/µl and absorbance of 1.84±0.08 at 260/280 nm indicating the purity of the sample. cDNA was synthesized from mRNA and quantified the relative expression of IL 8 gene using Taqman assay. GAPDH gene was used as reference gene or house keeping gene as control for error between samples.

The mean Cp value for target (IL 8) and reference gene (GAPDH) were 29.32±0.90 and 28.57±0.70 respectively with the range 22.77 to 35.48 for the target and 24.47 to 32.55 for the reference gene ( fig 2). The fold change in gene expression of IL 8 in the healthy group and SCM group was calculated using $2^{(-\Delta\Delta CP)}$ method (Kenneth and Thomas, 2001). The mean fold change in gene expression was significantly (0.70±0.028) higher in milk somatic cells of SCM group and lower (0.13±0.03) in healthy group ( fig 3). This data indicates that the IL 8 expression is significantly increased in subclinical mastitis condition and this finding reaffirms the chemotactic activity of IL 8 at times of bacterial invasion. The epithelial and endothelial cells of mammary gland play pivotal role in chemotaxis and produce higher level of IL 8 in conditions like coliform mastitis (Mc Clenahan *et al.*, 2006).

Peli *et al.*, 2004, have reported upregulation of IL 8 in milk samples with higher SCC from Italian Friesian dairy cows. Also cytokine expression profile studies in the milk samples from Kankrej, Gir and the crossbred cattle of India, revealed higher levels of cytokines including IL 8 indicating its importance in mammary gland immunity (Bhatt *et al.*, 2012). Deoni cattle are the native breed of Maharashtra, Karnataka and Andhra Pradesh. They are dual purpose animals and the cows of this breed are moderate milk producers and contribute to the valuable genetic resource of the nation. This study of IL 8 expression in Deoni cattle together with the previous studies in other important genetic breeds indicate that IL 8 can be used as a valuable biomarker of subclinical mastitis and reinforces the importance of further studies in understanding its potential role in preventing mastitis

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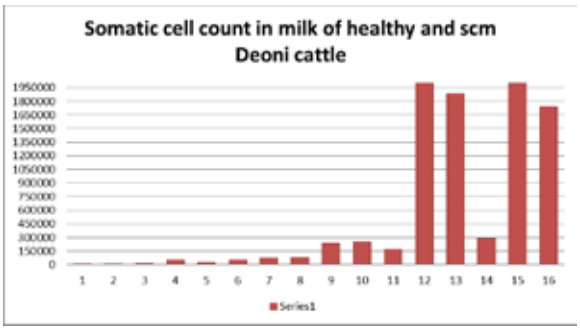
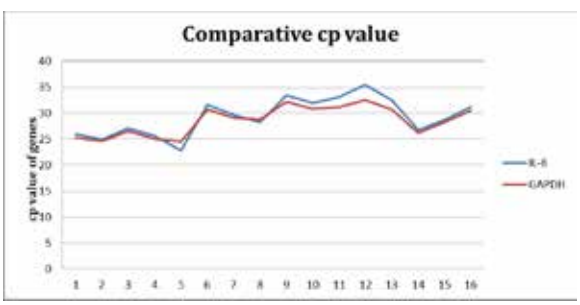
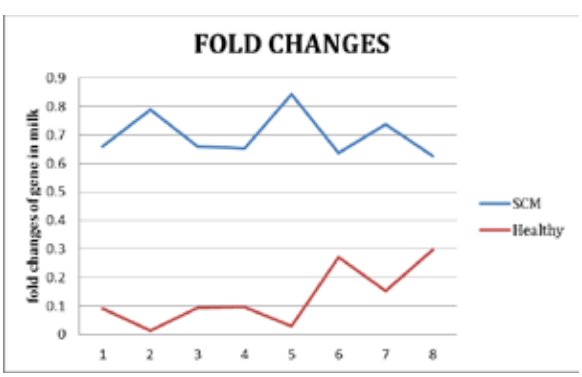
**1. Milk Somatic Cell Counts In Healthy (1-8) and SCM (9-16) animals of Deoni cattle**




**2. Cp value of IL-8 and GAPDH gene expression in milk somatic cells of subclinical mastitis (SCM) and healthy group of Deoni cattle**




**3. Fold change in gene expression of IL-8 gene in subclinical mastitis (SCM) and healthy animals of Deoni cattle**



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