ABSTRACT

Cluster of Differentiation (CD14) protein was identified as a differentiation marker on the surface of monocytes and macrophages and was characterized as a receptor for bacterial endotoxin (LPS). It is regarded as the first described pattern-recognition receptor (PRR) and one of the excellent candidates for mastitis resistance in cattle. Present study was carried out with the objective to characterize and identify genetic polymorphism of promoter region of CD14 gene and its association with clinical mastitis in lactating Karan Fries cattle maintained at NDRI Karnal. Twelve SNPs were found in the complete sequence of 553 base pairs of promoter region of CD14 gene of Karan Fries cows. Cows were also screened using PCR-RFLP with Hpy188I restriction enzyme which revealed three genotypes AA, AB and BB. All three genotypes differed significantly regarding mastitis incidence.

Key words: CD14, Karan-Fries, Mastitis, RFLP, SNP.

INTRODUCTION

Infectious diseases like mastitis, the inflammation of parenchyma of mammary glands, have a high detrimental consequence to animal health that leads to reduced longevity, productivity and causing economic loss to livestock industry. It can be classified into subclinical, clinical and chronic forms on the basis of its severity while its degree dependents on the nature of causative pathogen and on the age, breed, immunological health and lactation state of the animal. In the era of “omics”, with much advancement in proteomics and genomics, efforts are directed towards identification of markers for mastitis so that ensuing mastitis can be detected at an early stage or a mastitis resistant animal can be selected for further breeding.

CD14 (Cluster of Differentiation 14) gene is one of the excellent candidates for mastitis resistance in cattle (Ogorevc et al., 2008 and 2009). It has been mapped on bovine chromosome number 7 (Ogorevc et al., 2009) spanning 2630 bp region and comprises of promoter region and 2 exons. Total coding sequence is 1122 bp long with open reading frame of 1119 bp which encodes 373 amino acids (Ibeagha et al., 2008). The protein encoded by this gene is a component of the innate immune system which is the first line of defence against microbial infection (Janeway et al., 2002). It is pattern recognition receptor in some types of immune system cells such as monocytes, macrophages and dendritic cells and binds mainly with lipopolysaccharide of gram negative bacteria, lipoarabinomannan of Mycobacteria, mannuronic acid polymers of Pseudomonas species and to peptidoglycans of Staphylococcus aureus, and thus releases various cytokines which act for body’s defence against a wide range of pathogens including gram negative and gram positive bacteria (Pal et al., 2011). Few reports are available so far for genetic polymorphism of CD14 gene in dairy animals (Ibeagha et al., 2008, Kumar, 2012, Pal et al., 2011). Since there is no report on CD14 gene polymorphism in Karan Fries cattle (Bos indicus x Bos taurus) which is one of the important cross breed cattle in India. Therefore, present study was carried out with the objective to characterize and identify genetic polymorphism of promoter region of CD14 gene and its association with clinical mastitis.

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MATERIALS AND METHODS

Blood samples were collected from randomly selected 100 Karan Fries cows maintained at cattle yard of National Dairy Research Institute, Karnal. Cows with history of incidences of clinical mastitis (affected ≥ once) and also non-affected cows were selected. Genomic DNA isolation was done by phenol-chloroform method as described by Sambrook and Russel (1989) with minor modifications. Quality of genomic DNA was checked on 0.6% agarose gel electrophoresis. Quality and quantity of DNA was also estimated by Nanodrop spectrophotometer method. Polymerase chain reaction (PCR) was carried out using forward (5’ACACACCTGGAGAAGGCAA3’) and reverses (5’TCCAAGGGCTAGTTCCAGAG3’) gene-specific oligonucleotide primers as reported by Kumar (2012) to amplify 553 base pair (177-729 nt) of promoter region of bovine CD14 gene in Karan Fries cows. The PCR reaction mixture was incubated in thermal cycler initially at 94°C for 2 minutes followed by 34 cycles of 94°C for 30 seconds, 59°C for 30 seconds, 72°C for 40 seconds, and a final extension of 72°C for 10 minutes. The amplified PCR products were checked on 2% agarose gel. The PCR amplified target product was custom sequenced (M/s. SciGenom Labs Pvt Ltd.), edited (BioEdit) and ClustalW analysis was done to align edited sequence with reported Bos taurus sequence (EU148610.1).

RESULTS AND DISCUSSION

The targeted 553 bp of promoter region of bovine CD14 gene was successfully amplified by PCR using genomic DNA of selected Karan Fries cows (Fig 1). DNA sequencing revealed twelve nucleotide changes when compared with reference sequence of Bos taurus (EU148610.1) at positions G269A, T271C, C273A, G276C, G277A, T281C, G357T, A418C, G431A, C458G, A615D, and -616A (Fig 3 and 4). Three SNPs at position 357, 431 and 458 were similar to that observed by Kumar (2012) in Sahiwal (Bos indicus) cows while remaining nine SNPs observed in Karan Fries cows are new compared to reference Bos taurus (EU148610.1) and Bos indicus (Kumar, 2012).

Association analysis of CD14 gene promoter with clinical mastitis: Digestion of 553 bp PCR product with Hpy188I restriction enzyme showed total four fragments of 305, 248, 138 and 110 bp. These fragments revealed three polymorphic patterns for promoter region of CD14 gene in Karan Fries cows. Cows with band patterns 305 and 248 bp were assigned genotype AA while those with 305, 248, 138 and 110 bp as AB and 305, 138 and 110 bp as BB genotype (Figure 2). The AA genotype was found with highest frequency of 0.41 than AB (0.33) and BB genotype (0.26) while allele A was having

FIG 1: PCR Product of promoter region of CD14 gene in Karan fries cows.

| Lane 1-19 | PCR product (553bp) |
| M | 50bp DNA ladder |

FIG 3: Chromatogram showing nucleotide change at position 458 (C>G)

Karan Fries ACCTAGTAGCAGCAGGAG 461
Bos taurus ACCTAGTAGCAGCAGGAG 461

***458***
higher frequency of 0.58 than allele B (0.42). These finding are similar to the observations of Kumar (2012) for same region of CD14 gene in Sahiwal CD14 gene wherein genotype AA and allele A were having highest frequency.

Since the calculated $\chi^2$ value for genotypes (7.14) was more than tabulated value (5.99) the alternative hypothesis at 2 degree of freedom and 5% level of significance was accepted. Hence, AA, AB and BB genotypes of Karan Fries cattle differed significantly regarding mastitis incidence. Within AA genotype 51.22% of cows were in mastitis non-affected group whereas those in AB and BB genotypes 78.78% and 65.38%, respectively were mastitis affected. Therefore, it can be inferred that allele A could be having important role in less susceptibility against mastitis in Karan Fries cows. Because animals with allele A might have higher percentage of leucocytes expressing CD14 molecules on their surface. However higher expression of CD14 molecules on surface of leucocytes may lead to increase the speed of response to pathogen attack (Paape et al., 2000; Akira et al., 2006).

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REFERENCES