Short Communication

Genetic Association of Bovine TNF-α Gene Polymorphism with Clinical and Sub-clinical Mastitis in Sahiwal Cows

Huma Sattar1, Sehrish Firyal1,*, Ali Raza Awan1, Habib-Ur Rehman2, Muhammad Sajid Hasni3 and Amjad Islam Aqib4*

1Institute of biochemistry and biotechnology, University of Veterinary and Animal Sciences, Lahore, 54000
2Department of Physiology, University of Veterinary and Animal Sciences, Lahore, 54000
3Department of Epidemiology and public health, University of Veterinary and Animal Sciences, Lahore, 54000
4Department of Medicine, Cholistan University of Veterinary and Animal Sciences, Bahawalpur, 63100

ABSTRACT

One major problem of dairy cattle industry is the high prevalence of mammary gland infection. Despite significant technological developments in animal husbandry, udder infection is still widespread; particularly in large herds that causes high production losses to the dairy industry of Pakistan. Identification of molecular marker sequences that are directly related to immunity against mastitis might be helpful for animal selection trait and prevention of this disease. The aim of the present study was to assess the effect of polymorphism in bovine tumor necrosis factor alpha (TNF-α) gene on immune function and its role in mastitis susceptibility in Pakistani Sahiwal cows. In this study, a total of 150 Sahiwal cows were selected suffering from clinical (n=50), subclinical (n=50) mastitis along with non-mastitic (n=50) cows from different dairy farms of Punjab. TNF-α gene was amplified with specific primer and sequenced to get the full-length sequence of this gene. A total of nine changes including transition (n=6) and transversion (n=3) were found at the different position of this gene. Due to these changes, the amino acid is changed that leads to significant change in the folding of 3D protein structure of clinical sample, while in subclinical samples, showing the same variation in overall 3D protein structure analysis of TNF-α gene. The association between polymorphism identified within the TNF-α gene with mastitis reported in this study revealed that SNPs has potential to serve as a molecular marker for screening of mastitis resistant and susceptible Sahiwal cows.

One of the most prevalent disease of the dairy industry is mastitis that playing a devastating role for economic loss in the affected farms (Aqib et al., 2019b). Economic loss includes poor milk quality, low milk production, cost of treatment, labor charges, premature culling and increased risk of other diseases (Fourichon et al., 2005; Ali et al., 2011). Despite considerable technological advancement, it is still very challenging to control mastitis because several factors (environmental and genetic) are involved in etiology of mastitis (Carvajal et al., 2013; Aqib et al., 2019a; Mahboob et al., 2018). Mastitis resistance and susceptibility is a complex trait influenced by genetic variation. Among these variations, the polymorphism in immunity genes associated with mastitis are primary key factors in defensive mechanism of mammary gland (Rupp and Boichard, 2003; Ibeagha-Awemu et al., 2008).

Dairy cattle are more susceptible to mastitis because of poor hygiene conditions and weak mammary gland defense mechanism (Sordillo and Streicher, 2002). Various cellular and soluble immune components are involved in protecting the mammary gland from infectious diseases. The mammary gland tissue is protected by innate and acquired immune system (Mesquita et al., 2012). A vast variety of cytokines associated with acquired immune system, among which tumor necrosis factor alpha (TNF-α) gene is a candidate factor that has been proven to play important role in mastitis susceptibility and resistance.

TNF-α is main pro-inflammatory adipokine that is a member of cytokine group of systematic immune...
defense. It is involved in proliferation, differentiation, and activation of many immune system cells including B lymphocytes, NK (natural killer) along with the stimulation and release of other cytokines (Wojdak-Maksymiec and Mikolajczyk, 2012). It is 17 KDa molecule consist of 212 amino acids arranged in stable homotrimer and composed of two β-pleated sheets and β-strands, joined together antiparallel (Tang et al., 1996). TNF-α gene has BTA23q22 chromosomal position, having four exons and three introns (Bannerman, 2009; Moyes et al., 2009). This gene is involved in different biological functions of host defense system, hence polymorphism in the TNF-α gene could be used as a tool for selection of mastitis resistant animal selection (Parameswaran and Patial, 2010).

The present study is designed to investigate the polymorphism in bovine TNF-α gene and its effect on change in amino acid sequence leading to change in the 3D structure of a protein in Pakistani Sahiwal dairy cattle. A subordinate objective of the study is to find out the TNF-α gene-based molecular marker to differentiate between mastitis susceptible and resistant Sahiwal cows to promote the inheritance of resistance trait.

Materials and methods

For the identification of polymorphism within TNF-α gene, a total of 150 Sahiwal cows (clinical mastitis n=50; subclinical mastitis n=50; non-mastitis n=50) were selected. For subclinical mastitis test, Surf Field mastitis test (Muhammad et al., 1995) was performed at the animal site. For this purpose, 3% solution of household detergent (Lever Brothers, Surf Excel, and Arial) was mixed with quarter foremilk sample in equal volume and was swirled for 15-20 seconds and looked for thickening of the mixture (i.e. gel formation). About 5-10 mL blood sample was collected from jugular vein aseptically in blood vacutainer tubes. Organic DNA extraction method was used for DNA extraction from blood samples (Sambrook et al., 2001).

For amplification of TNF-α gene, six sets of primer were designed using already reported sequence of B. taurus from NCBI (NCBI GenBank; Accession no. AC_000180.1) (Table I). Optimization of primers was carried out with following temperature profile; initial denaturation-95°C for 5 min, denaturation-94°C for 30 s, annealing of TNF1 at 56°C, TNF2 at 60°C, TNF3 at 55°C, TNF4 at 52°C, TNF5 at 56°C and TNF6 at 58°C for 30 s, extension-72°C for 60 s, final extension-72°C for 10 min, repeat step 1-3 for 30 cycle.

Purified amplicon was subjected for commercial sequencing. The sequencing result was analyzed manually using BioEdit software followed by sequence alignment through NCBI BLAST. Protein analysis was done using ExPasy translate tool along with the development of 3D structure using PAYMOl software.

Results

In this present study, the TNF-α gene of Pakistani Sahiwal cow was sequenced for identification of polymorphism. Comparative sequence analysis with reference sequence revealed SNPs at different position of the gene. In TNF-α gene sequence of clinical and subclinical mastitic cows, six transition SNPs were identified at location 268 (A>G), 383 (G>A), 750 (C>T), 2075 (A>G), 2444 (T>C), 2511(C>T); Three changes were identified at position 538 (G>C), 1139 (T>G) and 2512 (T>G) that show transversion polymorphism (Table II). The protein analysis shows missense mutation in amino acid sequence i.e., Leucine is changed with Tryptophan at 215 (Fig.1).

Table I.- Details of primers used to investigate the polymorphism within TNF-α gene

| Primer Name | 5’-3’ Sequence | GC (%) | Product size (bp) |
|-------------|----------------|--------|------------------|
| TNF1 F      | CTTCCCTTTCTCCAGCTCCT  | 55     | 692              |
| TNF1 R      | GAGACACAGGAGAAGCCTGTGG  | 60     | 692              |
| TNF2 F      | CCACAAGGCTCCTGCCTGTTC  | 60     | 484              |
| TNF2 R      | TGCTTACTCTGATCGTCCAACA  | 43     | 43               |
| TNF3 F      | GTTTGGACGAATAGAATAAGCA  | 55     | 55               |
| TNF3 R      | ACCCTGACGCTCCTGCTGCT  | 60     | 690              |
| TNF4 F      | CTGGGTCATGAAAGGACAGAG  | 53     | 53               |
| TNF4 R      | TAGTCCGCGGAGCTGATCTCA  | 55     | 55               |
| TNF5 F      | GAGATCAAACCTGCGGACTA  | 55     | 55               |
| TNF5 R      | GCAAACATAAACACAGAGGATGTT  | 44     | 44               |
| TNF6 F      | AACCTCCCTCTGCTGCCAAT  | 50     | 50               |
| TNF6 R      | AAAGGCCCCATGCAAGATTA  | 45     | 480              |

Fig. 1. Deduced amino acid sequence of TNF-α gene of normal and mastitic Sahiwal cows.
Table II. SNPs identified in TNF-α gene sequence in clinical & subclinical mastitic Sahiwal cows.

| Position | Reference | SNPs | Region | Type of mastitis          | Results    |
|----------|-----------|------|--------|---------------------------|------------|
| 268      | A         | G    | Intron | Clinical & Subclinical    | Transition |
| 383      | G         | A    | Intron | Clinical & Subclinical    | Transition |
| 538      | G         | C    | Intron | Clinical & Subclinical    | Transversion |
| 750      | C         | T    | Exon   | Clinical & Subclinical    | Transition |
| 1139     | T         | G    | Intron | Clinical & Subclinical    | Transversion |
| 2075     | A         | G    | Intron | Clinical & Subclinical    | Transition |
| 2444     | T         | C    | Exon   | Clinical & Subclinical    | Transition |
| 2511     | C         | T    | Exon   | Clinical & Subclinical    | Transition |
| 2512     | T         | G    | Exon   | Clinical & Subclinical    | Transversion |

Discussion

SNPs were identified in TNF-α gene in both clinical and subclinical cases but not in non-mastitic samples so, this candidate gene are associated with cow immune response against invading microorganism. A total of nine changes including transition (n=6) and transversion (n=3) were found at different positions (exon and intron) of this gene. The polymorphism at position 111 (C/T), 209 (C/T) and 308 (A/G) in exon 4 of TNF-α were reported by Shirasuna (2011) and Maksymiec (2013) in Holstein-Friesian cows and Firyal (2018) in Sahiwal cows (Shirasuna et al., 2010; Wojdak-Maksymiec et al., 2013; Firyal et al., 2018) but the other SNPs are not reported.

The protein analysis performed indicates that there is a change in amino acid from Leucine to tryptophan at amino acid position 215. These mutational changes in polar groups produce a change in the overall 3D protein structure of TNF-α gene (Fig. 2).

All these changes were found in both clinical and subclinical mastitis samples of Sahiwal cows at different intronic and exonic region. A comparison of the TNF-α gene in various type of mastitis in the present study indicates variations within the TNF-α gene that is responsible for mastitis. So, TNF-α is a potential candidate gene for the screening of the mastitis susceptible and resistant dairy cows.

Conclusion

The present investigation demonstrates the presence of nucleotide changes at various positions in bovine TNF-α gene that leads to change in amino acid sequence. Our result suggests that these SNPs may be used as a potential genetic marker for screening of mastitis resistant and susceptible Sahiwal cows. This can be useful in the selective breeding of cattle for an enhanced immune response as a tool to improve inherent animal health.

Statement of conflict of interest

The authors declare no conflict of interest.

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