ASSOCIATION OF GENETIC VARIANTS OF PARTIAL EXON 3 REGION OF TLR4 GENE WITH MASTITIS IN SAHIWAL CATTLE

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Received: 02-04-2011 Accepted: 01-11-2011

ABSTRACT

AluI restriction digestion exhibited three AA, AB and BB of partial exon 3 region of TLR4 gene in Sahiwal cattle. It was found that Sahiwal cattle with BB genotype were susceptible (60.00 %) to mastitis. Association between all the genotypes combinations of partial exon 3 region of TLR4 gene was found to be non-significant with mastitis, except between AA and AB genotype.

Key words: TLR4 gene, Mastitis, Sahiwal cattle.

The annual losses in the dairy industry due mastitis was approximately 2 billion dollars in USA and 526 million dollars in India. (Varshney et al., 2004). Goldammer et al. (2004) found that mastitis strongly increased mRNA expression, thereby suggesting that TLR4 gene might be related with mastitis. Similarly, Ogorevc et al. (2009) suggested TLR4 gene as a strong candidate for inclusion in cattle breeding programs to augment the accuracy of selection for mastitis resistance. Therefore, an attempt was made to study the association of partial exon 3 region of TLR4 gene with mastitis in Sahiwal cattle.

Mastitis data was collected from 1994-2008 (14 years) of 188 Sahiwal cattle maintained at cattle yard of National Dairy Research Institute, Karnal. The Pair of primers were designed from partial exon 3 region of TLR4 gene (Forward - 5’ AGACAGCATTTCACTCCCT 3’ and Reverse - 5’ ACCACCCGACACACTGATGAT 3’) and PCR-RFLP method was performed using AluI restriction enzyme which showed presence of 3 genotypes i.e. AA, AB and BB genotypes. Gene and genotypic frequencies were estimated by the gene counting method (Falconer and Mackey, 1996). Association study of partial exon 3 region of TLR4 gene variants with mastitis and not mastitis Sahiwal cattle was calculated using x² test (Snedecor and Cochran, 1994) using the formula x² = (observed - Expected)² /Expected.

The genotypic frequencies were 0.01, 0.61 and 0.37 for AA, AB and BB genotype respectively and the allelic frequencies of A and B alleles were 0.32 and 0.68 respectively in Sahiwal cattle. In the present study, the null hypothesis was that there is no significance difference between AA, AB and BB genotypes with respect to mastitis, whereas, alternative hypothesis was that there is significant difference between AA, AB and BB genotypes with respect to mastitis. To test the hypothesis chi square test was applied [x² = (observed - Expected)² / Expected]. The calculated x² value (32.86) was found to be more than tabulated value (3.84). So the alternative hypothesis at 5% level of significance was accepted i.e. AA, AB and BB genotypes differ significantly regarding mastitis incidence. Animals with BB genotype showed more incidences of mastitis than the animals of AA and AB genotypes.

Associations were also carried out between two genotypes combinations i.e: AA and AB; AB and BB and AA and BB genotype and it was found that association between AA and BB and AB and BB genotypes with mastitis was non-significant, whereas, association between AA and AB genotype with mastitis was significant (Table 1). Similar association study between the SNP and somatic cell score (SCS) in the Chinese Simmental, Holstein and

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Sanhe cattle breeds showed that the SCS of individuals with a CC genotype was significantly lower than that of the TT genotype (Wang et al., 2008). In contrast to a combined linkage and linkage disequilibrium method was used to investigate possible associations between the TLR genes and mastitis susceptibility in the Norwegian Red cattle population. The analysis did not detect any significant association between TLR4 gene and mastitis in Norwegian Red cattle (Opsal et al., 2008). In Chinese Holstein, Sanhe cattle and Chinese Simmental the effect of the TLR4 polymorphism on somatic cell score was analyzed the results indicated that the somatic cell score were significantly affected by lactation month and the type of breeds (P < 0.05), but not by different genotypes (P > 0.05) (Wang et al., 2006).

### CONCLUSION

The partial exon 3 region of TLR4 gene when digested with AluI restriction enzyme produced different variants which were obtained which were associated with mastitis in Sahiwal cattle. It was observed that AB genotype of TLR4 gene was comparatively better than BB genotype in selection of mastitis resistant Sahiwal cattle. The association between AA and BB genotype with mastitis incidence was non-significant may be due less numbers of animals in AA genotype as compared to AB and BB genotype.

### REFERENCES


### TABLE 1: Incidence of mastitis in different genetic variants of partial exon 3 region of TLR4 gene in Sahiwal cattle.

<table>
<thead>
<tr>
<th>TLR4 genotype</th>
<th>Animals affected with Mastitis</th>
<th>Animals not affected with Mastitis</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
</tr>
<tr>
<td>AA</td>
<td>1</td>
<td>50.0a</td>
<td>1</td>
</tr>
<tr>
<td>AB</td>
<td>60</td>
<td>51.7a</td>
<td>56</td>
</tr>
<tr>
<td>BB</td>
<td>42</td>
<td>60.0a</td>
<td>28</td>
</tr>
<tr>
<td>Total</td>
<td>103</td>
<td>54.8</td>
<td>85</td>
</tr>
</tbody>
</table>

(Similar superscript indicate non-significant difference between two genotypes at P >0.05).