ABSTRACT

Host defense against the invading microbial antigens is recognized by the immune system. The immune system consists of two major components, innate immunity an acquired immunity. Two different types of immunity that is interrelated recognize invading microorganisms as non-self molecules which triggers the immune responses to eliminate them from the body. At the beginning of the 21th century, Toll protein was shown to be an essential receptor for host defense against the fungal infection in Drosophila, which only helps in innate immunity. Thereafter, a mammalian homolog of the Toll receptor (TLR4) was shown to induce expression of genes involved in inflammatory responses. After that one point mutation in the region of TLR4 gene has been observed in a mouse strain that is unresponsive to Lipolysaccharide. These studies have made TLR genes a very promising subject of research.

Human Toll like receptor genes comprise a large family consisting of at least 11 members. TLR1–9 is very much conserved between the human and mouse. However, TLR10 is functional in the human, whereas C-terminal half of the mouse TLR10 gene is substituted to an unrelated and non-productive sequence and indicating that mouse TLR10 is non-functional. Similarly, mouse TLR11 is functional, but the stop codon present in the human TLR11 gene, which results in lack of production of human TLR11. All the TLR genes are located in diverse chromosomes in mammalian genome. TLR receptors of innate immune system known as pattern recognition receptors (PRRs) recognizes a conserved molecular pattern, also known as Pathogen associated molecular patterns (PAMPs). TLRs signaling pathway occur via different signaling molecules like MyD88, IRAK, and IRF3 etc. which helps in the production of various kinds of cytokines for the inflammatory and other types of diseases. TLRs are mainly dependent on environmental pathogen for their recognition of diverse pathogens. Extensive variation among the TLR genes helps to understand the gene- disease- environment associations against various kinds of diseases and also their frequency distribution in different populations.

The present study was aimed to estimate the frequency distribution and phylogenetic relationship of ten human TLR genes among the studied four populations namely, Rajbanshi, Rabha, Gurkha and Muslim in the North Bengal region of India where different types of tribal populations reside. Present study has also aimed to study the frequency and distribution patterns of TLR

genes in the patients of Rheumatoid arthritis, Typhoid fever and in HIV and their association with the TLR genes if any.

Blood samples were collected from the volunteers with prior informed consent. In both population- based and disease related study, three generation pedigree of the volunteers were taken into account and only unrelated individuals were included in the study. DNA samples were extracted using standard protocol. Ten TLR genes were analyzed using PCR-SSP typing. Different available software was used for the analyzing of the data.

It has been documented from population based section that TLR8 and TLR9 are having very high frequency among Rajbanshi in respect to the other three populations. In Gurkha population TLR4 and TLR5 are showing highest frequencies. On the other hand, in Rabha population, frequency of TLR4 is highest, whereas in Muslim population TLR3, TLR5 and TLR7 are showing the highest frequencies.

The present study has also documented the phylogenetic relationship of the above mentioned four populations and found that Gurkha and Muslim are very close to each other, whereas Rabha is distantly related. Rajbanshi is close to Gurkha population as evident from the Nei‘s genetic distance analysis. Principal component analysis demonstrated the relationship and genetic structure of Gurkha and Muslim population. Rabha is more distantly related from the other three populations. It may occur due to the environmental pathogens present in their surroundings and that may have the direct relationship with the change of the frequency pattern of the TLR genes among the four studied populations. It is inferred from the results that Rajbanshi population is susceptible for various kinds of viral chronic diseases. On the other hand in Gurkha and Rabha population the frequency of TLR4 is high. TLR5 is also very high in Gurkha. They may probably susceptible for bacterial diseases and various gastro-insetinal diseases. High frequencies of some of the endosomal TLRs in Muslim population suggested that they are probably susceptible to get infected with viral and entero- bacterial diseases. The study also gives a light on the convergent evolution/selection pressure of the TLR genes among the population. Convergent evolution has occurred in TLR genes among the above-mentioned populations due to the sharing of similar environmental conditions. It is quite interesting to observe that although the Rajbanshi, Gurkha and Rabha populations have shared ancestry due to their emergence from a common East-Asian stock, but there is no similarity in the distribution of TLR genes as has been recorded in the

present study. However, there exist considerable similarities in the distribution of TLR genes between the Muslim and the Gurkha population who share the same environment but differ considerably in their ethnicity. This striking observation may depict the impact of environmental selection on the distribution of TLR genes. Such influences of the environment on TLR distribution may depend on the constant presence of specific pathogens in respective environment. Thus, it may be assumed that TLR genes play a significant role in shaping the genetic ancestry of the above mentioned populations from North Bengal region of India as well as in determining the exposure of the diseases in these populations.

Rheumatoid arthritis is a chronic inflammatory disease that affects the joints. It is an autoimmune disease by producing different types of autoantibodies in the serum. 110 numbers of patients were selected for association study and the numbers of healthy control samples were 100. Patients were selected by evaluating the diagnostic result of anti-CCP and RF titre assay and compared with the control group. The patients were also selected on the basis of American college of Rheumatology (ACR) and European League against Rheumatism (EULAR) criteria 1987. It has been found that the median range of anti-CCP is 182.7 (16.5 to 504.93) compared to the control group which is 10.8(8.2 to 13.1), whereas the RF titre concentration is 142.3 (41.20 to 198.0) in case of rheumatic patients and 16.8 (12 to 20.5) for control group.

Molecular typing of ten human TLR genes was performed in the rheumatoid arthritis patients and the control group. Significant associations are found in case of TLR2, TLR4, TLR5, TLR8 and TLR9. The frequency of TLR1, TLR6 and TLR8 are highest among patients. Relative risks are also high for TLR4, TLR6, TLR7, TLR8 and TLR9. Door line association has been found in case of TLR1 and TLR3.

Odd ratio is very high for TLR1, TLR4, TLR6, TLR8 and TLR9. Low odd has been found in case of TLR2, TLR5 and TLR10. The sensitivity for the positive association with the disease was also calculated. High sensitivity has been observed for TLR1, TLR3, TLR6 and TLR8, whereas low sensitivity has been observed in case of TLR2, TLR5 and TLR10.

In conclusion, the results may help to find out the association of TLR genes with the rheumatoid arthritis which was not previously studied in this region. The genetic profile study of the TLR genes in human and their association with the RA has got immense importance. It has been

found that some of the TLRs those are highly associated with the disease, need further investigation to establish their role in case of RA as the toll like receptors are also regulated by the environmental factors.

Another study with typhoid fever caused due to *Salmonella typhi*, a gram- negative bacterium, was carried out. It is restricted in human and causes a wide range of food- and water-borne diseases ranging from self-limiting gastroenteritis to systemic typhoid fever. Typhoid patients were screened by Widal test positive result carried out by serum agglutination test. The serum antibody titre of 1: 80 or above was considered positive for the typhoid fever.

Molecular documentation of ten human TLR genes in typhoid patients and the control group were performed. Significant associations are found for TLR8. TLR10. TLR1, TLR5 and TLR6 are highly up-regulated among typhoid patients. It proves that the flagellin protein and other antigens from *S. typhi* up- regulate the TLR genes. Positive association are found for TLR1 (5.54) and TLR6 (4.77) in respect to their odd ratio. Door line association of TLRs with the disease has been observed when the relative risk was calculated for TLR2 (1.72), TLR3 (1.21) and TLR10 (1.98). The result indicates the higher association in case of some of the TLRs with typhoid fever in the region of Siliguri. It also signifies the risk factor of typhoid fever with TLR genes.

Human immunodeficiency virus infection is characterized by a gradual dysfunction, mainly in cell-mediated immunity but also in humoral immunity. It is also characterized by CD4+ cell depletion and leading to high levels of HIV RNA and development of opportunistic disease. Positive HIV patients were selected based on the viral infection and CD4+ cell count results (CD4+ count range- 156- 756 x 10^6 cells/L).

Molecular typing was performed for all ten TLR genes for patients and control group. It has been observed that the gene frequency of TLR8 (0.809) and TLR9 (0.865) are very high. Chi-square analyses ($\chi 2$) were performed to compare the differences in carrier frequencies (OF) of TLR genes among the patients and control samples. Significant differences are found in case of TLR2, TLR4, TLR8 and TLR9. Odd ratio is observed very high in case of TLR4 (9.56), TLR8 (6.04) and TLR9 (10.06), increased multiple times between patients and control group. Risk ratio was also high for TLR8 and TLR9 which manly recognize viral RNA and dsDNA. Furthermore, this

study suggested the significant association between the clinical parameters and the role of TLR genes in occurrence of HIV. It also deciphers that the viral RNA/DNA, which is present in the endosomal compartment of the cell, activates the TLRs in course of the disease.

It can be concluded that the results will not only help to understand the genetic background of the studied population in respect to their TLR genes, but also the gene- environment interaction in the northern part of West Bengal. This study also helps in illustrating the convergent evolution of TLR genes in respect to their ethnic background. Furthermore, this study will help to understand the degree of association of TLR genes with the disease.