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Original article

# Role of toll like receptors in bacterial and viral diseases – A systemic approach

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ABSTRACT

*Background:* Toll like receptors are key-receptors of the innate immune system, but their role against bacterial and viral infections are yet to be understood.
*Aim:* The present study is aimed to investigate the diversity and frequency distribution of 10 TLR genes among typhoid fever and HIV+ patients. In this study, 44 samples were taken from typhoid patients and 55 samples from HIV+ patients.
*Patients and methods:* Widal test positive samples (>1:80) in case of typhoid and the percentage of CD4+ count in case of HIV+ patient were considered for the PCR-SSP analysis.
*Results:* We found that the frequencies of TLR1 and TLR6 were highest in typhoid patients, whereas the frequencies of TLR8 and TLR9 displayed higher among HIV+ patients. Chi-square values were significant for TLR8 and TLR10 in the case of typhoid patients, whereas in HIV patients significant values were considered for TLR2, TLR4, TLR8 and TLR9 respectively. The odds ratio calculated highest for TLR1 and TLR6 among typhoid patients. TLR4 and TLR9 calculated were highest odd for HIV+ patients. A door line association of TLRs with the disease was found when the relative risk was calculated for TLR2 (1.72), TLR3 (1.21) and TLR10 (1.98) in bacterial infection, whereas in case of viral infection relative risk was calculated for TLR4 (1.62), TLR8 (1.18) and in TLR9 (1.16).
*Conclusion:* This study reports the frequency distribution and association of human TLR genes with the bacterial and viral infection in the North Bengal region of India for the first time. It also signified the gene- disease- environment association study in case of infectious diseases and also the risk factors of bacterial and viral infections in this region. It also depicts the role of TLRs in the recognition of the pathogens.


## 1. Introduction

Free-living organisms have the ability to cope up with the new environment by modifying their gene expression patterns [1]. Extensive variations at the genomic level made the analyses of gene-disease association and their susceptibility possible [2]. The frequency of genes and their alleles vary between different populations in case of different diseases [3]. Till date, slow progress has been observed in the field of genome-wide association studies for the infectious disease in comparison to other diseases. However, some studies involving bacterial and viral diseases contribute substantially to the growing knowledge of the host genetic variations and treatment of the diseases [4,1]. Toll like receptors that regulate both innate and adaptive immune response and polymorphism in the TLR genes has been investigated in case of various diseases [5].

Enteric fever has become an alarming infection nowadays among populations in some areas of India [6]. *Salmonella enterica* serotype *typhi* (S. typhi) is a gram-negative bacterium, restricted in human and cause a wide range of food- and water-borne diseases ranging from self-limiting gastroenteritis to systemic typhoid fever [7]. The occurrence of typhoid fever is less in developing and industrialized countries, but high in the countries of South-East Asia including India [6]. According to Crump et al. (2004) typhoid fever caused over 20 million illnesses and over 200 thousand deaths during the year 2000 [8]. Poor sanitation, lack of safe drinking water supply and low socioeconomic conditions

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has amplified the disease in India, which increases the morbidity and mortality [9].

Primarily, TLR4 and TLR5 play major roles in the activation of immune responses against LPS and flagellin. TLR4 polymorphisms among the Asian Malay population confer a higher risk for typhoid infection in case of *S. typhi* [10]. Genetic association study among the Vietnam population has proven no association of TLR5392STOP stop codon with typhoid fever [11]. Binding site modulation of TLR gene receptors against the lipopolysaccharide (LPS), flagellin or other antigens of *Salmonella typhi* evokes the host immune response during typhoid fever [12].

On the other side susceptibility to the human immunodeficiency virus (HIV) infection and disease progression are variable among populations [13]. A small percentage of 0.2% of the HIV-1 sero-positive patient is able to control the HIV-1 infection over 10 years. The adult HIV prevalence at national level has 0.26% in 2015 [14]. It means that they can maintain a viral load of fewer than 50 copies of HIV-1 RNA per ml [15] more of HIV-1 RNA will accelerate the prevalence of the disease. Infection with human immunodeficiency virus (HIV) results in progressive deterioration of the immune system in untreated patients [16]. Different TLRs expressed on different cell types in the human immune system and up-regulated by the effect of cytokines like IFN-y induces the expression of TLR4 in peripheral blood monocytes [17].

The HIV disease progression can be estimated by measuring marker expression in the course of the disease. The degree of CD4+ T-cell depletion is the most important marker for the detection of HIV [18]. Indeed, the most characteristic feature of HIV is the depletion of the CD4+ T-helper-inducer subset of T cells. The other markers that are also reliable for estimating HIV disease progression include b2m, neopterin, IgG, IgM, anti-p24, anti-gp120, TNF etc. [18].

Several association studies have been reported in case of TLRs with HIV. It has been reported that depletion of CD4+(Th2) cells in HIV positive individuals releases bacterial components that directly activates TLR4 [19]. According to Baenziger et al. (2009), the chronic activation of TLR7 leads to immune dysregulation in murine model which is similar to human [20]. Several other TLRs are also associated with HIV disease progression.

## 2. Subjects and methods

### 2.1. Selection of patients for typhoid fever

Typhoid patients were diagnosed by expert doctors of North Bengal Medical College and Hospital, Shushrutnagar, Siliguri (latitude & longitude 26.7271°N, 88.3953°E) on the basis of specific symptoms of typhoid fever. Screening of the typhoid patients were based on the positive results of the Widal test [21]. The serum agglutination test was done against S. typhi "O" and "H" antigens using a Salmonella antigen kit (Beacon diagnostic Pvt. Ltd, Navsari India). The test was performed according to the manufacturer's instruction. The serum antibody titer of 1:80 or above was considered positive for the typhoid fever.

### 2.2. Selection of patients for human immunodeficiency virus

Fifty-five HIV-infected patients (including 33 women, 22 men and, median age-34) and 70 healthy individuals (47 women, 23 men, a range of 20–52) were included in this study (Table 1). Individuals under any sorts of medication were excluded from the control group (n = 70) in our study. Positive HIV patients were selected based on the viral infection and counting of CD4+ cells within the range of 156–756 $\times$ $10^6$ cells/L. Laboratory values for patients who did not receive anti retroviral therapy (ART) had 117–730 $\times$ $10^6$ cells/L CD4+ cells per litre, but CD4+ count became 142–890 $\times$ $10^6$ cells/L after receiving ART.

**Table 1**
Demographic characteristics of Typhoid fever patients and HIV+ patients and Healthy donors.

| Sex | Typhoid patients | Healthy Donors |
|---|---|---|
| Male | 18 (40%) | 27 (38%) |
| Female | 26 (60%) | 43 (62%) |
| **Total** | **44** | **70** |
| Sex | HIV+ patients | Healthy Donors |
| Male | 22(40%) | 23(32%) |
| Female | 33(60%) | 47(67%) |
| **Total** | **55** | **70** |

HIV-Human Immunodeficiency Virus.

### 2.3. Sample collection

3 mL of venous blood was collected from Forty-four typhoid patients between December 2014 to June 2016 from Siliguri and adjoining areas of West Bengal and Seventy healthy control subjects were taken after screening by the doctors. A detailed clinical report was taken from the patients who were admitted to the hospitals and primary health care of Siliguri and adjacent areas with gastro-intestinal problems.

The demographic characteristics of Fifty-five HIV+ patient and seventy healthy donors are represented in Table 1. 5 mL of blood samples were collected from each individual who attended the District Hospital with prior informed consent. Simultaneously, samples were collected from healthy donors after proper examination by the doctors.

The samples were stored in EDTA at −20 °C until use. The investigation was approved by the Human ethics committee of the Department of Zoology, University of North Bengal (Zoo/4133/2011) and performed in accordance with the Declaration of Helsinki, 1975.

### 2.4. DNA extraction and PCR-SSP typing

Genomic DNA was extracted from the blood samples using the standard Phenol-Chloroform extraction method with slight modifications. DNA integrity was checked in UV-transilluminator. O.D value was taken with 260/280 nm. Value of 1 or above was found as good quality of the DNA (Fig 1a). PCR-SSP typing was done for all the 10 TLRs. The TLR primers were designed using NCBI BLAST tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) (Table 2) [22] and procured from the Integrated DNA Technologies, Inc, Iowa, USA. Each 25 μl PCR reaction mixture contained 5X PCR buffer (Promega Corporation, Agora, Fitchburg Center, Fitchburg, Wisconsin), 5 μL of 10 mM dNTPs, 1.5 μL of 25 mM MgCl2, 1.5 μL of primers, and 1–1.5 U of Taq DNA polymerase. 1.5–2 μl of 100 ng DNA samples were then added to the PCR mixture. The reaction conditions for PCR consisted of an initial denaturation step of 94 °C for 3 min followed by 30 cycles of 94 °C for 30 s, 56.9 °C for 50 s and 72 °C for 1 min, followed by a single final extension of 72 °C for10 min. Slight modifications in the annealing temperatures of different primer sets were made as per the requirement. The PCR products were analyzed using ethidium bromide prestained 1% agarose gel electrophoresis. Samples were then visualized on UV transilluminator. All the lanes of the product loaded gel showed a control band, except for the negative control lane. The reactions were repeated to avoid false reactions where no control bands were found (Fig 1b).

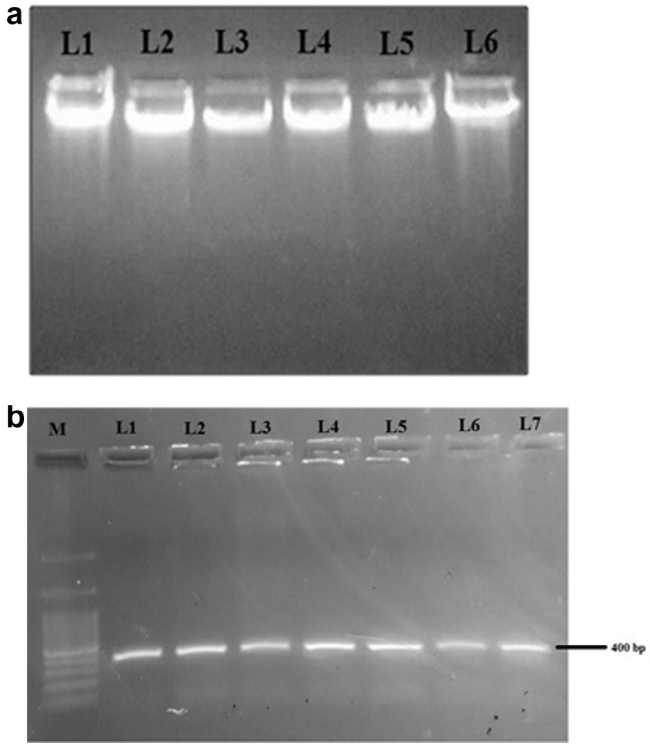


**Fig. 1.** a-Agarose gel electrophoresis showing results of DNA isolated from typhoid patient's blood samples (L1-L6). b-Agarose gel electrophoresis results showing PCR amplification of TLR5 in HIV+ samples (M-100 bp DNA ladder, L1-L7 positive samples).

## 3. Statistical analyses

All statistical data were analyzed using SPSS (Ver-16.0) (Armonk, New York, USA), Kyplot (ver-2.0) and MS-Excel programme (Redmond, Washington, USA). Statistical significances were determined using the chi-square test or the Fisher's exact test with p-value <0.05 being considered significant.

## 4. Results

### 4.1. Analysis of typhoid patients

Observed frequencies of ten TLR genes from 44 typhoid patients are represented in Table 3a. It has been observed from the table that in case of typhoid patients, both TLR1 and TLR6 have the highest frequency of 0.977, which were followed by TLR 4 and TLR5, having the frequency of 0.909 and 0.931 respectively (Table 3) (Fig 2a). Chi-square analyses ($\chi^2$) were performed to compare the differences in carrier frequencies (OF) of TLR genes among the patients and control samples. Among the 10 TLR loci, significant differences are observed only in case of TLR8 and TLR10 (Table 3). ANOVA was also performed for study the significant differences among control and patient groups which was found 0.0007 ($p < 0.001$) and in case of two-tailed *t*-test, the value was 0.521 for patient and control group.

Fischer's exact test for probability showed significant association for TLR8 (p = 0.022, <0.05) and TLR10 (p = 0.0005, <0.001). When the odd ratio and 95% confidence interval [23–25] for ten different TLRs in typhoid patients were calculated, it has been documented that TLR2 (odd- 2.02, CI- 0.82–4.97, p- 0.12), TLR4 (odd- 2.5, CI- 0.76–8.16, p- 0.12), and TLR5 (odd- 2.01, CI- 0.51–7.89, p- 0.31) showed high associations, whereas TLR7 (odd- 0.59, CI- 0.17–1.97, p- 0.39), TLR8 (odd- 0.26, CI- 0.08–0.82, p- **0.02**) and TLR9 (odd- 0.58, CI- 0.19–1.80, p- 0.35) showed lower association among the patients and control samples (Table 4). The relative risks for different TLRs were calculated. The relative risks for TLR7 (RR- 0.94, p- 0.41), TLR8 (RR- 0.83, p- 0.03) and TLR9 (RR- 0.93, p- 0.37) are very low, whereas door line association is found in case of TLR1 (1.10, p- **0.04**), TLR5 (1.06, p- 0.27) and TLR6 (1.08, p- 0.07). On the other hand a little bit of higher associations are observed in cases of TLR2 (1.72, p- 0.12), TLR3 (1.21, p- 0.03), TLR4 (1.13, p- 0.09) and TLR10 (1.98, p- 0.0004) (Table 4).

The disease prevalence was estimated using diagnostic tests based on Bayer's theorm. The sensitivity was found to be very high in case of TLR1 (97.73), TLR4 (90.91), TLR5 (93.18) and TLR6 (97.73) (Table 5). Low sensitivity was reported in cases of TLR2, TLR8, and TLR10 which signified the low prevalence of the disease in the patients.

### 4.2. Analysis of HIV patients

Observed frequency data of ten different TLR genes from 55 HIV positive patients were analyzed. It is observed that the gene frequency of TLR8 (0.809) and TLR9 (0.865) are very high (Table 6) (Fig 2b). 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. No significant differences have been observed among the other TLRs (Table 6). ANOVA was also performed for significant difference among the control and patient groups which was 0.04 ($p < 0.05$) and in case of two-tailed *t*-test, the value was 0.93 for patient and control group.

Fischer's exact test for probability showed significant association for TLR4 (p = 0.00001, >0.001) and TLR8 (p = 0.01, <0.01) and TLR9 (p = 0.01, <0.01). When odd ratio and 95% confidence interval [22–24] for ten different TLRs in HIV patients were calculated, high associations are observed in case of TLR4 (odd- 9.56, CI-3.11–29.37, p- < 0.0001), TLR8 (odd-6.04, CI- 1.30–28.05, p- 0.007), and TLR9 (odd- 10.06, CI- 1.25–80.60, p- 0.005), whereas TLR2 (odd- 0.07,

**Table 2**
List of primers for the 10 TLR alleles in human.

| Genes | Forward Primers (5′-3′) | Reverse Primers (3′-5′) | Product size(bp) | GC content (%) |
|---|---|---|---|---|
| **TLR1** | TCAACCAGGAATTGGAATAC | AGTTCCAGATTTGCTACAGT | 382 | 40 |
| **TLR2** | GGATGGTTGTGCTTTTAAGTACTG | AAGATCCCAACTAGACAAAGACTG | 2671 | 41.67 |
| **TLR3** | ATTGGGTCTGGGAACATTTCTCTTC | GTGAGATTTAAACATTCCTCTTCGC | 792 | 44/40 |
| **TLR4** | TTCTTCTAACTTCCTCTCCTGTG | TTAGCTGTTCGGCTCTACTATGG | 1087 | 43/47 |
| **TLR5** | CATTGTATGCACTGTCACTC | CCACCACCATGATGAGAGCA | 446 | 45/55 |
| **TLR6** | ACAACCCTTTAGGATAGCCACTG | AAACTCACAATAGGATGGCAGG | 398 | 47.83 |
| **TLR7** | AGTGTCTAAAGAACCTGG | CTTGGCCTTACAGAAATG | 545 | 44 |
| **TLR8** | CAGAATAGCAGGCGTAACACATCA | AATGTCACAGGTGCATTCAAAGGG | 637 | 45.83 |
| **TLR9** | TCTAGGGGCTGAATGTGACC | ACAACCCGTCACTGTTGCTT | 1106 | 55 |
| **TLR10** | GTCGAAGACCCAATATACAG | ATTAAGCAATAGAACCGATG | 954 | 45/35 |
| **Growth Hormone (Positive control)** | CTTCCCAACCATTCCCTTA | CGGATTTCTGTTGTGTTTC | 424 | 47/42 |

**Table 3**
Observed frequencies of the 10 TLR genes in the control and typhoid patients. $\chi^2$ values were also mentioned where each gene was compared with controls and patients for any statistical differences.

| Patients controls | | | $\chi^2$ | Relative risk | p value |
|---|---|---|---|---|---|
| **TLR1** | **0.977** | 0.885 | 1.982 | 1.10 | 0.04 |
| **TLR2** | 0.295 | 0.171 | 1.757 | 1.72 | 0.12 |
| **TLR3** | 0.886 | 0.728 | 3.153 | 1.21 | 0.03 |
| **TLR4** | 0.909 | 0.8 | 1.667 | 1.13 | 0.09 |
| **TLR5** | **0.931** | 0.871 | 0.503 | 1.06 | 0.27 |
| **TLR6** | **0.977** | 0.9 | 1.429 | 1.08 | 0.07 |
| **TLR7** | 0.863 | 0.914 | 0.296 | 0.94 | 0.41 |
| **TLR8** | 0.772 | 0.928 | 4.459* | 0.83 | 0.03 |
| **TLR9** | 0.840 | 0.9 | 0.413 | 0.93 | 0.37 |
| **TLR10** | 0.681 | 0.342 | 11.128*** | 1.98 | 0.0004 |

*P < 0.05, **P < 0.01, ***P < 0.001.

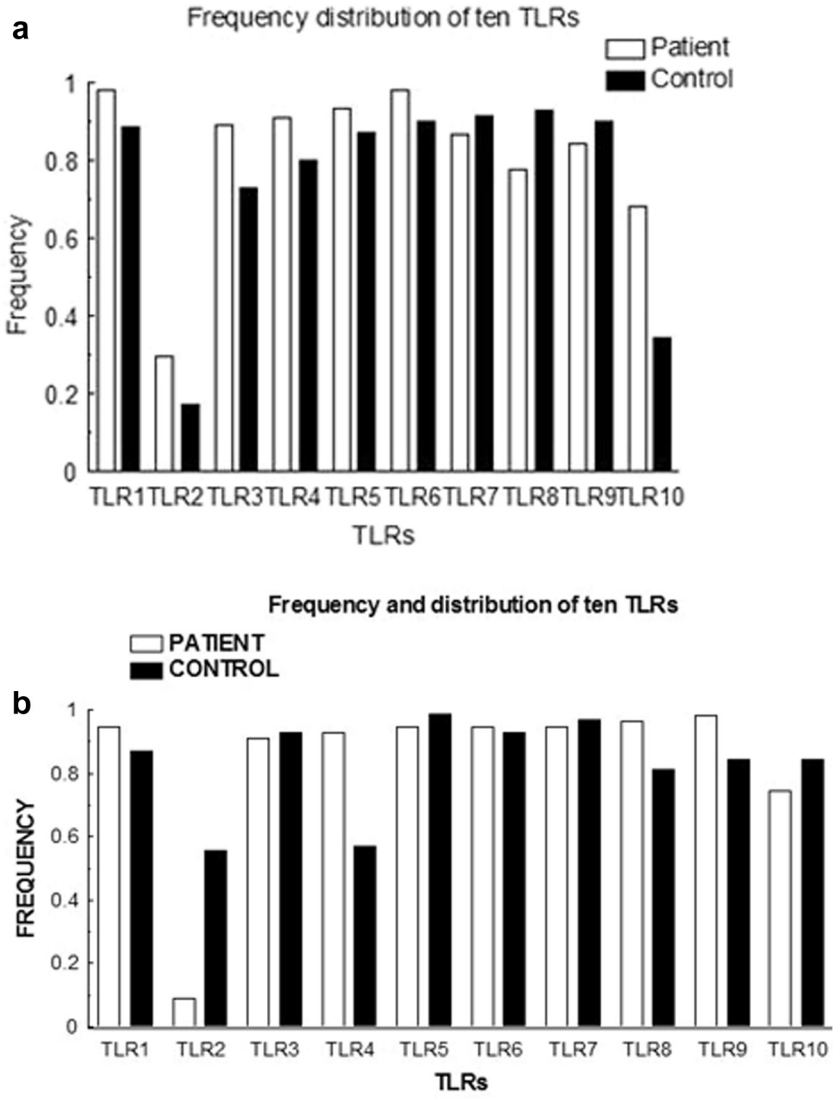


**Fig. 2.** a-Frequency graph of ten TLR genes was constructed using Kyplot (ver-2.0) of typhoid patients. b-Frequency graph of ten TLR genes was constructed using Kyplot (ver-2.0) of HIV positive patients.

CI- 0.02–0.22, p- < 0.0001), TLR5 (odd- 0.25, CI- 0.02–2.48, p- 0.23) and TLR7 (odd- 0.50, CI- 0.08–3.16, p- 0.47) showed lower association with the disease (Table 7). The relative risks for different TLRs were calculated (Table 6). The relative risks for TLR2 (RR- 0.16), TLR5 (RR- 0.95) and TLR10 (RR- 0.88) are found to be very low, whereas door line associations has been found in case of TLR4 (1.62, p- < 0.0001), TLR8 (1.18, p- 0.007) and TLR9 (1.16, p- 0.005).

The prevalence of the disease in the patients was estimated diagnostically using Bayer's theorem. The sensitivity values are

**Table 4**
Risk ratio and odd ratio for ten different TLRs in association with typhoid fever.

| | Risk ratio | Odd ratio | Confidence intervals |
|---|---|---|---|
| **TLR1** | 1.10 | **5.54** | 0.66–45 |
| **TLR2** | 1.72 | 2.02 | 0.82–4.97 |
| **TLR3** | 1.21 | 2.90 | 0.99–8.46 |
| **TLR4** | 1.13 | 2.5 | 0.76–8.16 |
| **TLR5** | 1.06 | 2.01 | 0.51–7.89 |
| **TLR6** | 1.08 | **4.77** | 0.56–40 |
| **TLR7** | 0.94 | 0.59 | 0.17–1.97 |
| **TLR8** | 0.83 | 0.26 | 0.08–0.82 |
| **TLR9** | 0.93 | 0.58 | 0.19–1.80 |
| **TLR10** | 1.98 | **4.10** | 1.83–9.17 |

**Table 5**
Diagnostic test values for typhoid patients based on bayer's theorm.

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| **TLR1** | 97.73 | 11.43 | 40.95 | 88.89 |
| **TLR2** | 29.55 | 82.86 | 52.00 | 65.17 |
| **TLR3** | 88.64 | 27.14 | 43.33 | 79.17 |
| **TLR4** | 90.91 | 20.00 | 41.67 | 77.78 |
| **TLR5** | 93.18 | 12.86 | 40.20 | 75.00 |
| **TLR6** | 97.73 | 10.00 | 40.57 | 87.50 |
| **TLR7** | 86.36 | 8.57 | 37.20 | 50.00 |
| **TLR8** | 77.27 | 7.14 | 34.34 | 33.33 |
| **TLR9** | 84.09 | 10.00 | 37.00 | 50.00 |
| **TLR10** | 68.18 | 65.71 | 55.56 | 76.67 |

PPV-Positive predicted value, NPV-Negative predicted values.

**Table 6**
Observed frequencies of the 10 Human TLR genes in the control and HIV+ patients. $\chi^2$ values were also mentioned where each gene was compared with controls and patients for any statistical differences and measurement of relative risk.

| | Patients | Controls | $\chi^2$ | Relative risk | P value |
|---|---|---|---|---|---|
| **TLR1** | 0.766 | 0.641 | 1.185 | 1.08 | 0.14 |
| **TLR2** | 0.046 | 0.334 | 27.343*** | 0.16 | <0.0001 |
| **TLR3** | 0.698 | 0.732 | 0.004 | 0.97 | 0.69 |
| **TLR4** | 0.730 | 0.345 | 17.939*** | **1.62** | <0.0001 |
| **TLR5** | 0.766 | 0.880 | 0.573 | 0.95 | 0.23 |
| **TLR6** | 0.766 | 0.732 | 0.0002 | 1.01 | 0.69 |
| **TLR7** | 0.766 | 0.830 | 0.076 | 0.97 | 0.47 |
| **TLR8** | 0.809 | 0.569 | 5.168* | **1.18** | **0.007** |
| **TLR9** | 0.865 | 0.603 | 5.345* | **1.16** | **0.005** |
| **TLR10** | 0.495 | 0.603 | 1.268 | 0.88 | 0.19 |

*P < 0.05, **P < 0.01, ***P < 0.001.

**Table 7**
Risk ratio and odd ratio for ten different TLRs in association with HIV patient.

| | Odd Ratio | Confidence interval | Risk ratio | P value |
|---|---|---|---|---|
| **TLR1** | 2.55 | 0.65–9.94 | 1.0849 | 0.22 |
| **TLR2** | 0.07 | 0.02–0.22 | 0.1632 | 5.22 |
| **TLR3** | 0.76 | 0.21–2.80 | 0.979 | 0.74 |
| **TLR4** | **9.56** | 3.11–29.37 | **1.6227** | 0.00001 |
| **TLR5** | 0.25 | 0.02–2.48 | 0.9592 | 0.31 |
| **TLR6** | 1.33 | 0.30–5.84 | 1.0182 | 0.73 |
| **TLR7** | 0.50 | 0.08–3.16 | 0.9733 | 0.65 |
| **TLR8** | **6.04** | 1.30–28.05 | **1.1834** | 0.01 |
| **TLR9** | **10.06** | 1.25–80.60 | **1.1649** | 0.01 |
| **TLR10** | 0.54 | 0.22–1.32 | 0.8844 | 0.25 |

found to be very high in cases of TLR8 (96.36), andTLR9 (98.18); however similar sensitivity values have been reported in cases of TLR1, TLR5, TLR6 and TLR7 (Table 8). On the other hand, low specificity values were found in case of TLR5 (1.43), TLR7 (2.86) and little bit of higher specificity in case TLR2 (44.29) are found.

**Table 8**
Diagnostic test values for HIV patients based on bayer's theorm.

| | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| **TLR1** | 94.55 | 12.86 | 46.02 | 75.00 |
| **TLR2** | **9.09** | 44.29 | 11.36 | 38.27 |
| **TLR3** | 90.91 | 7.14 | 43.48 | 50.00 |
| **TLR4** | 92.73 | 42.86 | 56.04 | 88.24 |
| **TLR5** | 94.55 | 1.43 | 42.98 | 25.00 |
| **TLR6** | 94.55 | 7.14 | 44.44 | 62.50 |
| **TLR7** | 94.55 | 2.86 | 43.33 | 40.00 |
| **TLR8** | **96.36** | 18.57 | 48.18 | 86.67 |
| **TLR9** | **98.18** | 15.71 | 47.79 | 91.67 |
| **TLR10** | 74.55 | 15.71 | 41.00 | 44.00 |

PPV-Positive predicted value, NPV-Negative predicted value.

## 5. Discussion

Bacteria and viruses have the peculiar ability to overcome species barriers and can adapt in new hosts. This concept helps us to understand the underlying mysteries behind the origin and emergence of infectious diseases. The TLR based genetic analysis in Typhoid and HIV patients may serve as a powerful model for studying mechanisms of host adaptation, because the pathogens responsible for these diseases are physiologically well characterized and lend themselves to genetic analysis in different populations in the world [5,26].

Typhoid is a major human enteric fever caused by bacterial infection in India. Although not common in urbanized countries, but the disease remains an important and persistent health problem in developing nations like India. Hospital-based surveys and reports from different parts of the country indicate that enteric fever is a major public health problem, with *Salmonella enterica* serovar t*yphi* (*S. typhi*) being the most common pathogenic agent [9]. Various risk factors such as sanitation problems, lack of safe drinking water supply and low socio-economic conditions amplify the rate of evolution of multidrug-resistant salmonellae with reduced sensitivity to different drugs have been reported in India [9,1].

The role of TLRs in typhoid fever patients has not been extensively studied in India, especially in the northern part of West Bengal where the health problems become the major issues among the tea garden workers. Some studies have documented the association of the TLRs with typhoid fever in India [12,27]. An association based study among the Malay population on TLR4 polymorphism confers a higher risk factor for typhoid infection [10]. According to Dunstan et al. (2005) premature stop codon of TLR5 polymorphism suggested no association with typhoid fever caused by *S. typhi*. TLR5 might not play an important role in TLR-stimulated innate immune responses during infection with *Salmonella enterica serovar typhi*. Initiation of these responses may rely on other TLRs that recognize different bacterial ligands [11].

In case of HIV+ patients, it was reported that polymorphism in TLR3 (Leu412Phe) has a protective role against the disease [28]. Two variants of TLR4 (Asp299Gly, Thr399Ile) which recognizes lipopolysaccharide (LPS) as their ligand are associated with increased infection risk in HIV+ patients [29]. According to Martinelli et al. (2007) pDCs, which normally secretes the IFN-gamma and activates the natural killer cell and also suppressed due to the presence of gp120 viral envelope of HIV virus? The viral envelope protein also inhibits the TLR9 mediated induction of proinflammatory cytokines in pDCs [30]. Thus the presence of the different types of polymorphic variants of TLR genes having susceptibility to HIV susceptibility or diseases depends on the ethnicity of different populations of the world [31].

The frequency and distribution patterns of ten TLR genes were analyzed and compared in case of typhoid fever and HIV+ patients of Siliguri and adjacent areas. It has been observed that the frequencies of some of the TLRs like TLR1, TLR4, TLR5 and TLR6 are very high when compared with healthy controls in case of typhoid patients, whereas the frequencies of TLR8 and TLR9 are the highest in HIV positive patients. These findings are in agreement with the previously reported work [30,32]. LPS and flagellin produced by the Salmonella elevates the frequency of TLR4 and TLR5 in macrophages and also in intestinal epithelium cells. In the contrary, the frequencies of TLR8 and TLR9 are higher in HIV-positive patients. In course of HIV viral infection, small single-stranded RNA/CpG oligonucleotides activate TLR8 and TLR9which are mainly expressed in monocytes and macrophages.

Recognition of different antigens like vi-capsule, flagellin, LPS and others, activate the signaling pathways for the production of different cytokines in the human. The interaction between TLRs and Pathogen-associated molecular patterns (PAMPs) produced from the bacterial and viral antigens increase the formation of inflammosome and other inflammatory products. It brings the neutrophil and macrophages and induces the production of pro-inflammatory cytokines like interleukin (IL)-6, IL-1b, tumor necrosis factor (TNF)-a, and interferon-gamma (IFN)-c [33]. In case of HIV infection Th1 cytokines like interleukin (IL)-2, and antiviral interferon IFN-gamma are generally decreased and the production of Th2 cytokines such as IL-4, IL-10, proinflammatory cytokines and TNF-$\alpha$, are increased [34].

Chi-square analysis reveals the significant values for different TLRs. Significant associations have been found in cases of TLR8 and TLR10 among the patients and the control samples of typhoid fever. In case of viral infection, significant values are found among TLR2, 4, 8 and TLR9. Positive associations with the typhoid fever are found for TLR1 and TLR6. Door line association has been found among the patients in comparison to their relative risk and risk ratio for the *S. typhi* infected patients. It signifies the positive relationship of the disease among typhoid patients in respect to their TLRs. Increased level of TLR1, 4, 5 and TLR6 expression in the cells prove that antigen from *S. typhi* highly increased the frequency pattern of those TLRs in course of the disease progression. Sensitivity test for TLR1, TLR5, and TLR6 are very high in typhoid positive patients which signify the prevalence of the disease in the population. The predictive values of any diagnostic test are related to its disease prediction ability. The Positive predicted values (PPV) are found to be very low in comparison to the negative predicted values (NPV).

Positive and close association of TLR4, TLR8, and TLR9 with HIV are documented in the present study, which strongly supports the previously published reports. Sensitivity test for TLR8 and TLR9 are also very high, which suggests that the disease is detected in most of the patients. The PPV values are low when compared with NPV. It also inferred from the data that the expressions of some of the TLRs are also very high in patients in course of the viral replication.

## 6. Conclusion

TLR regulates the innate immune response and plays a crucial role in the initiation of adaptive immunity in human populations. Recent trends in the fields of genetics highly focused on the role or association of TLRs in case of bacterial and viral diseases. So, the overall frequency and the distribution pattern of TLRs have been focused in the present study in case of bacterial and viral infections. Positive associations are found for cell surface receptors such as TLR1, TLR2, TLR4, and TLR6, which influence the progression and positive risk for the disease in typhoid patients. But, in case of viral infection, endosomal TLRs play a crucial role in resisting the disease. The present study is one of the major first-hand reports on the association of TLRs with bacterial and viral infections caused due to *S. typhi* and for human immunodeficiency virus in North Bengal region of India.

## Conflict of interest

None.

## Acknowledgments

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejmhg.2017.05.001.

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