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Toll like Receptors: An Insight Role against the Pathogen

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Abstract

Host defense against the invading microbial antigens are recognized by the immune system. The present study focuses on the frequency distribution and phylogenetic relationship of ten human TLR genes among four populations in the North Bengal region of India and also aimed to study the frequency and distribution of TLR genes in the patients of Rheumatoid arthritis, Typhoid fever and in HIV. It has been documented from population based study that TLR8 and TLR9 are having very high frequency among Rajbanshi in respect of other three populations. In Gurkha population TLR4 and TLR5 are showing the highest frequencies. On the other hand, in Rabha population, the frequency of TLR4 is highest, whereas in Muslim population TLR3, TLR5 and TLR7 are showing the highest frequencies. This study has also documented the phylogenetic relationship of the four populations and found that convergent evolution has occurred among the population in respect of their TLR genes.
In case of the rheumatoid arthritis frequency of TLR1, TLR6 and TLR8 are highest among patients. Relative risks are also high for TLR4, TLR6, TLR7, TLR8 and TLR9. Odd ratio is very high for TLR1, TLR4, TLR6, TLR8 and TLR9.
TLR1, TLR5 and TLR6 are highly up-regulated among typhoid patients whereas TLR8 (0.809) and TLR9 (0.865) are very high among HIV patients. Odd ratio is observed very high in case of both typhoid and HIV increased multiple times between patients and control group. It can be concluded that the results will not only help to understand the genetic background of the studied ethnic populations in respect to their TLR genes, but also shade light on the association with the TLR genes with the above mentioned diseases.

Keywords: Convergent Evolution; Rheumatoid Arthritis; Typhoid; HIV

Introduction

The Indian subcontinent is located between 8 degree N to 37-degree N latitude and 68 degree to 97 degree longitude. This country assembled over 100 million people in the country with all the different populations and their different cultural and linguistic background [1]. The populations reside in this country are mixed between the western Caucasians and the Oriental in the East [2-6]. Genetic diversity study among the present human populations can be very useful in reconstructing concepts regarding population diversity and migration routes and also in identifying the ancestral populations. The subcontinent not only exhibits enormous morphological, cultural, and linguistic diversity but also stands only second to Africa in its genetic richness [1]. Therefore, people of the Indian continent have been and continue to be of interest for investigation in different areas, all aimed at exploring their vast genetic wealth. Moreover, the continent has served as a major corridor for the dispersal of modern humans that started from Africa about 10,000 years ago [7]. Thus, India occupies a center stage in human evolution.

Innate Immunity plays a vital role in recognition of the diverse set of foreign pathogens via some receptors and generates immune responses [8]. They recognize it through some molecular receptors and send some signals through which different cell type's release different cytokines by which they counteract with the pathogens. Various families of molecular markers are there for the recognition of pathogens of which TLRs or Toll like Receptors are the most important. Toll like receptor genes were first identified in

Drosophila sp. TLRs are group-1 membrane glycoproteins that are conserved from C. elegans to human [9]. They are also known as the pattern recognition receptors (PRRs) or Danger associated molecular pattern (DAMPs). These receptors mainly act in innate immunity. There are mainly ten types of TLRs present in human and mouse, of which some are represented as pseudogenes due to the evolutionary constraints [10].

These ten TLRs are located in different chromosomes. TLR 1, 2, 3, 6 and 10 are present in chromosome number 4, TLR4 in chromosome number 9, TLR7 and 8 in X chromosome and TLR9 in chromosome number 3. These receptor genes are not like that of Human Leukocyte Antigen (HLA) and Killer cell immunoglobulin like receptor (KIR) because they are locus specific, present in a single chromosome. So linkage study is much more complicated in case of TLR genes.

Structural studies of TLR-ligand complexes have become an attractive area of research as it is critical in understanding the innate immunity as well as designing the novel drugs [11]. TLRs are type-I transmembrane glycoproteins composed of extracellular, transmembrane and intracellular signaling domains [12]. The extracellular domain contains leucine-rich repeat (LRR) and is responsible for binding the so-called pathogen associated molecular patterns, PAMPs [13,14]. The extracellular domains of all the TLR family proteins contain 16-28 LRRs [15]. On the basis of their sequences and structural patterns, LRR family proteins can be classified into seven subfamilies such as RI-like (ribonuclease inhibitor-like),CC (cysteine containing), PS (plant specific), SDS22-like, bacterial, and TpLRR (Treponema pallidum LRR) [15,16]. TLRs, typical subfamily proteins, have LRR modules of 24 amino acids with the conserved motif of xLxxLxxLxLxxNxLxxLPxxxFx.

The activation pathway of TLR signalling originates from the cytoplasmic TIR domains. The downstream signaling pathway via TIR domain, a TIR domain-containing adaptor and MyD88, was first characterized to play a crucial role. In addition, recent accumulating evidences indicate that TLR signaling pathway consists of a MyD88-dependent pathway that is common to all TLRs, and a MyD88-independent pathway, restricted to the TLR3- and TLR4 [17,18]. There are other similar pathways present which help in TLR signaling.

North Bengal, northern part of West Bengal, region has got full of cultural and linguistic variations. Presence of various ethnic populations in this region signifies the variation in gene pool of the populations. In the present study such four ethnic populations namely Rajbanshi, Gurkha, Muslim, Rabha were chosen to reveal the gene-environment interactions of ten human TLR genes among the populations.

Populations of North Bengal

Rajbanshis are highly diversified ethnic community with rich cultural, linguistic and social background. They account for 18.4% of the total Scheduled Caste population of West Bengal (26° 20'- 27° 03' N and 88° 18'- 89° 29' E) as per 2001 Census of India. Although distributed dispersedly throughout the state, the Rajbanshis are mainly inhabitant of Terai and Dooars region of West Bengal, especially in the districts of Jalpaiguri and Coochbehar. They have an Indo-European linguistic background. Beside their own dialect they also speak Bengali, Assamese and some other minor languages.

Rabha is a very little known small endogamous scheduled tribe community of India with a conserved gene pool [19-21]. In West Bengal, they are mainly distributed in forest villages of dooars region of Jalpaiguri and Coochbehar districts. Historically they are considered as the primitive inhabitants of the region who remained isolated from other neighboring populations due to their strict endogamy. According to H.H. Risley, Rabhas belong to Indo-mongoloid stock [22], having a unique genetic constitution.

Indian Gurkhas constitute a community of Nepali speaking people, populating in the Eastern and North-Eastern states like West Bengal and Sikkim [23] with sizeable populations in the states of Meghalaya, Nagaland, Manipur, Tripura, Mizoram, and Arunachal Pradesh as well as in Assam. In West Bengal, they are distributed in Terai and Dooars as well as in the hilly regions of the northern part of the state. Nepali language has become the common binding thread of all the Gurkha castes and clans.

Populations				
	Gurkha	Muslim	Rabha	Rajbanshi
Sample size	125	140	50	85
Total	400 sample			
Mean Age (Yr)	31 yrs	34 yrs	29 yrs	28 yrs
Linguistic Family	Tibeto- Burman	Indo- Aryan	Tibeto- Burman	Indo- Aryan

Table 1: Demographic profile of the studied populations.

Bengali Muslims inhabiting in West Bengal represent the second-largest ethnic Muslim community in the world, after Arabs [24] native to modern-day Bangladesh and the eastern states of India including West Bengal and Assam. They speak Bengali dialects and have strong cultural similarities with the Bengali Hindus, thereby increasing the cultural richness of West Bengal. They are the second-largest community and also the largest minority group of the state. Bengali Muslims comprises 27.01% of the total population of West Bengal [20]. The demographic profile of the studied populations has been mentioned in Table 1.

The Major Findings from the Population based Study

Selection and convergent evolution are the two main forces which shape the TLR genes in their respective environment. Populations, which share their environment and inhabited in a particular region face a strong selection pressure due to the presence of pathogens in their surrounding environment. Also the Muslim population, very close to Gurkha in respect of the presence of TLRs, faces the same environmental condition. As a result, selection of specific TLR genes of the immune system shaping the population in respect of their ethnicity. On the other hand, convergent evolution of TLR genes has occurred due to the sharing of similar environment. Although Gurkha and Muslim belong to two different lineages, but their TLR distribution is same due to the sharing of similar environmental conditions (Figures 1-3). Rajbanshi, Gurkha and Rabha belong to the same East-Asian lineage, but considerably differ in their TLR distribution.

This striking observation may infer the impact of environmental selection in 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 of North Bengal region of India as well as in determining the disease exposure in these populations.

	RAXG	RAXM	RAXR	MXG	GXR	RXM
TLR1	2.723	0.401	1.056	0.9314	8.020**	3.687
TLR2	36.63***	32.22***	23.54***	0.229	0.005	0.034
TLR3	4.385*	18.52***	0.004	4.691*	2.549	13.427***
TLR4	15.569***	11.33***	2.047	57.426***	2.134	17.499***
TLR5	2.723	9.99**	20.80***	1.82	45.051***	66.880***
TLR6	0.007	0.135	9.822**	0.009	14.349 ***	17.349
TLR7	0.004	2.7	6.005*	3.927*	6.745**	20.745***
TLR8	1.458	1.33	6.841**	0.0041	2.591	2.986
TLR9	2.728	2.364	0.562	0.0006	0.1057	0.0424
TLR10	44.031***	37.81***	2.789	0.4299	14.6650***	10.612**

Table 2: Chi square (χ^2) values of different populations and their comparison (Kyplot beta ver-2.0) [25].
* P< 0.05, ** P< 0.01, *** P< 0.001 N.S- Non significant.

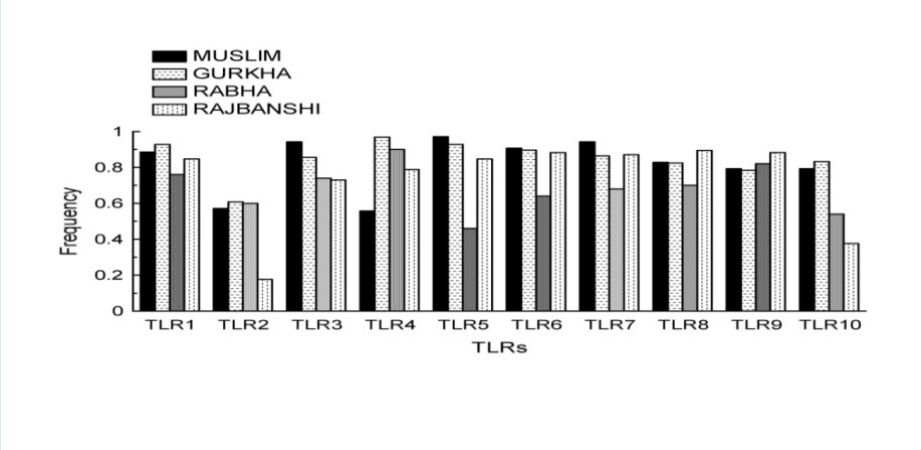

Figure1: Frequency graph of ten TLR genes of the four populations in the North Bengal region (Kyplot ver-2.0) [25].

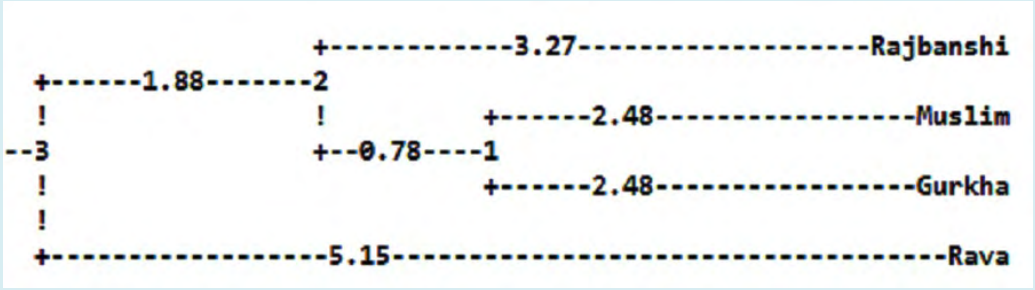

Figure 2: Neighbour joining tree was constructed using POPGENE (ver-1.32) and Phylip (ver-3.5) showing relationship among Rajbanshi and three other populations.

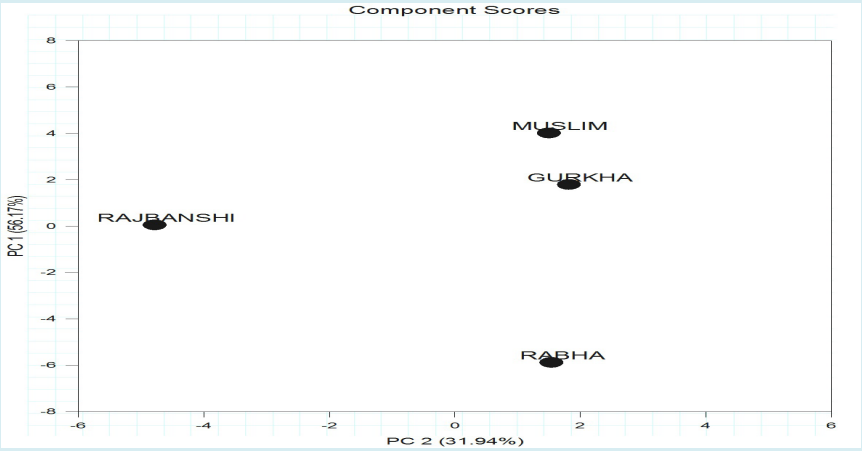

Figure 3: Principle Component Analyses (PCA) based of the 10 TLR genes in the four ethnic populations of North Bengal (Minitab ver-6) [25].

Among Rajbanshi population, high frequency of TLR8 and TLR9 is observed. So, it can inferred that this population, mainly inhabited in the tea garden areas of Terai and Dooars region of North Bengal, may be susceptible to some viral diseases. In Gurkha population, mainly inhabited in the hilly region of North Bengal, TLR4 and TLR5 are present with high frequency. A survey report on this population conveys their susceptibility for bacterial infections. On the other hand, TLR3, TLR5, and TLR7 are present with high frequency (Figure 1 & Table 2). Probably they get infected both with bacterial and viral diseases as we have found that occurrence of HIV infection among Muslim population is much higher than other populations in this region. In Rabha population the frequency of TLR4 is highest, which indicates that the population is susceptible to LPS and other bacterial antigens. A report has already been published out of the present work by these authors on the susceptibility of chronic gastro-intestinal diseases among Rabha population.

Phylogenetic and genetic distance assessment based on ten human TLR loci revealed that Muslim and Gurkha population are close to each other. Rabha is distantly related with the other three populations. Genetic distance analysis also proves the closeness of Gurkha and Muslim. The distances between Rajbanshi -Gurkha and Rajbanshi-Muslim are also close (Figures 2 & 3).

Conclusively, the combined picture represented the result of the analysis of TLR genes infer that:

1. The studied populations now-a-days is very much a mixed population in this region.
2. Substantial variation of the studied populations has been documented in respect of their TLR genes.
3. Due to the sharing of the same environmental conditions it is evident that they come closer not due to their ethnicity but in respect to their TLR genes. This is very interesting and reported for the first time.
4. On the other side, it is also interesting to note that convergent evolution occurs among the four populations in this region. Convergent evolution of TLR genes gives the shape of the population irrespective of their ethnicity.
5. Gurkha, Muslim and Rabha belong to the Mongoloid

origin as it has been reported in various studies based on other markers like HLA and KIR. The deviation of this finding occurs in respect of the TLR genes, because TLRs are considered as the main markers of the innate immunity. It also depends on the environmental pathogen for their proper functioning. This is the main reason why the populations, irrespective of their ethnicity merge into a common line.

Genetic structure of Muslim population of this region has received the gene flow from the neighboring country, Bangladesh but there is also a considerable admixture of Tibeto Burman element in Muslim population of this region and that differentiates from the other Muslim population of India. In Gurkha population, gene flow occurs between Nepali speaking Gurkha and the population of other region. The admixture of Rajbanshi of this region with the local Bengali population cannot be ignored. The Rabha population is restricted in some particular areas of North Bengal region and not mixed with the other local population. So, the gene pool is restricted because of their endogamous character.

The present study on these four populations unveiled the curtain of the frequency and distribution in respect of their TLR genes. This study is one of the primary and first hand report on population based study of TLR genes. So, further study is needed to reveal their genetic background in respect of their TLR genes and to know how TLR genes act on them in different conditions.

Disease Based Study

In the present study we have also focused on the different diseases that are found among the populations of North Bengal region. We have selected Rheumatoid arthritis, and bacteria and virus related diseases like Typhoid and AIDS.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation of synovial membrane of the joints caused by the infiltration of activated immune cells including CD4+ T cells, B cells, and antigen-presenting cells. It typically results in warm, swollen, and painful joints, thereby causing disability, deformities, premature deaths, and economic loss. Estimates of heritability suggested that genetic factors contribute more or less 50% to the risk of developing RA. Indeed, the development of autoimmune diseases like rheumatoid arthritis (RA) depends on the interaction between the genetic background and surrounding environment. The criteria for the selection of RA patients were based on ACR and EULAR criteria (Table 3).

	Risk ratio	Odd ratio	Confidence intervals (95%)	p value
TLR1	1.07	2.36	1.21-6.49	0.09
TLR2	0.67	0.46	0.99-3.20	0.006
TLR3	1.01	1.11	1.33-9.40	0.81
TLR4	1.57	5.86	1.06-3.84	<0.0001
TLR5	0.67	0.21	0.24-0.75	<0.0001
TLR6	1.14	2.8	0.85-6.55	0.01
TLR7	1.2	1.78	0.26-0.80	0.05
TLR8	1.13	3.55	0.45-2.69	0.01
TLR9	**1.18**	**2.02**	2.90-11.81	0.03
TLR10	0.62	0.43	0.10-0.43	0.003

Table 3: Risk ratio and odd ratio for ten different TLRs in association with Rheumatoid arthritis.

During the onset of the disease, various serological factors like CRP, anti-CCP, rheumatic factor (RF) are produced and identified as markers of the disease. C- Reactive proteins are produced in the hepatocytes of RA patients due to the influences of certain cytokines like IL-6, TNF-α etc. [26]. Anti-ccp in combination with RF factor is very much sensitive for the diagnosis of RA. Anti-CCP is another well-known marker of RA pathogenesis. It is sensitive and specific than RFs. Citrullinated proteins, a non-standard amino acid, are produced by post translation modification of arginine by peptidylarginine deaminase (PADI) enzymes. The apoptotic cells also activate the enzyme. So, when apoptotic cells are not cleared properly, the level of this protein and enzyme are raised in the inflamed area [27]. These proteins are mainly found in the form of filaggrin and cyclic citrullinated proteins. Autoantibodies are produced by the immune system against

this altered peptide in case of RA in the synovial tissue and increase the severity of the disease. Anti-CCP is locally produced in RA joints and very high sensitivity and specificity for the diagnosis of the disease [28]. The antibody titer has a prognostic value in destruction of the joints in this disease with 88% sensitivity and 98% specificity Table 4 & Figure 4.

	Sensitivity	Specificity	PPV	NPV
TLR1	94.55	12	54.2	67
TLR2	39.09	42	42.6	39
TLR3	90	11	52.7	50
TLR4	88.18	44	63.4	77
TLR5	59.09	13	42.8	22
TLR6	91.82	20	55.8	69
TLR7	73.64	39	57	57
TLR8	94.55	17	55.6	74
TLR9	81.82	31	56.6	61
TLR10	33.64	46	40.7	38

Table 4: Diagnostic test values for Rheumatoid arthritis patients based on bayer's theorm.

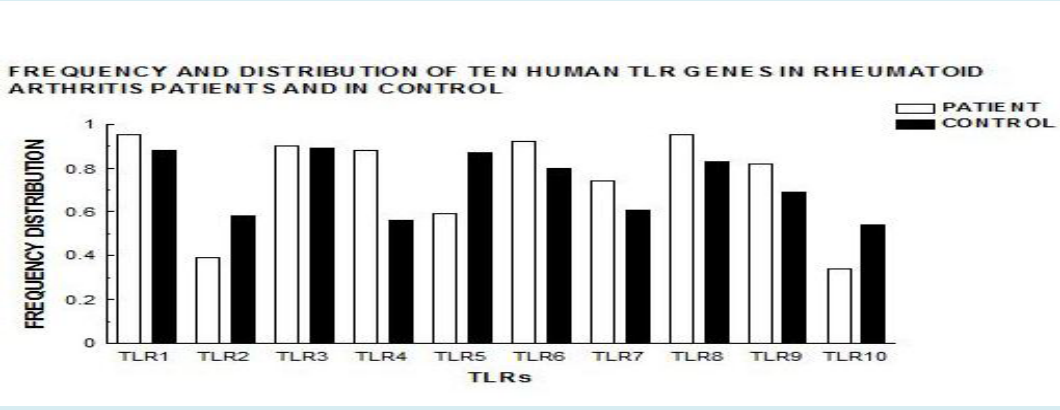

Figure 4: Frequency graph of ten TLR genes of rheumatoid arthritis patients (Kyplot ver-2.0).

Typhoid Fever

Enteric fever is a common infection among the populations in endemic countries like India [29]. *Salmonella enterica* serotype *typhi* (*S. typhi*) is a gram negative bacteria that is restricted to human and causes a wide range of food and water-borne diseases ranging from self-limiting gastroenteritis to systemic typhoid fever [30]. The occurrence of typhoid fever is less in developing and industrialized countries, but it is high prevalent in countries like India and South- East Asia [29]. According to Crump *et. al*., typhoid fever caused 21,650,974 illnesses and 216,510 deaths during the year 2000 [31]. The Poor sanitation, lack of a safe drinking water supply, unhygienic condition and low socio economic conditions have amplified the disease progression in India which increased the morbidity and mortality among population [32]. Salmonella genus is divided into two distinct species, Salmonella bongori and Salmonella enterica. The serotype typhi and paratyphoid A, B and C is present in human and in other higher primates [33]. Salmonella produces multiple PAMPs like flagella, fimbrae, LPS (Vi antigen), and bacterial DNA and develop survival mechanism from the host cells by producing superoxide dismutase, salmonella containing vacuole, type I secretion system etc. [34] (Figure 5). These virulence factors have been recognized by the pattern recognition receptors like TLRs [33] which initiate an immune response and form a link between the innate and adaptive immunity [9] (Tables 5 & 6).

	Risk ratio	Odd ratio	Confidence intervals
TLR1	1.1	5.54	0.66-45
TLR2	1.72	2.02	0.82-4.97
TLR3	1.21	2.9	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	4.1	1.83-9.17

Table 5: Risk ratio and odd ratio for ten different TLRs in association with typhoid fever [35].

	Sensitivity	Specificity	PPV	NPV
TLR1	97.73	11.43	41	88.9
TLR2	29.55	82.86	52	65.2
TLR3	88.64	27.14	43.3	79.2
TLR4	90.91	20	41.7	77.8
TLR5	93.18	12.86	40.2	75
TLR6	97.73	10	40.6	87.5
TLR7	86.36	8.57	37.2	50
TLR8	77.27	7.14	34.3	33.3
TLR9	84.09	10	37	50
TLR10	68.18	65.71	55.6	76.7

Table 6: Diagnostic test values for typhoid patients based on baye's theorm [35].
PPV- Positive predicted value, NPV- Negative predicted value.

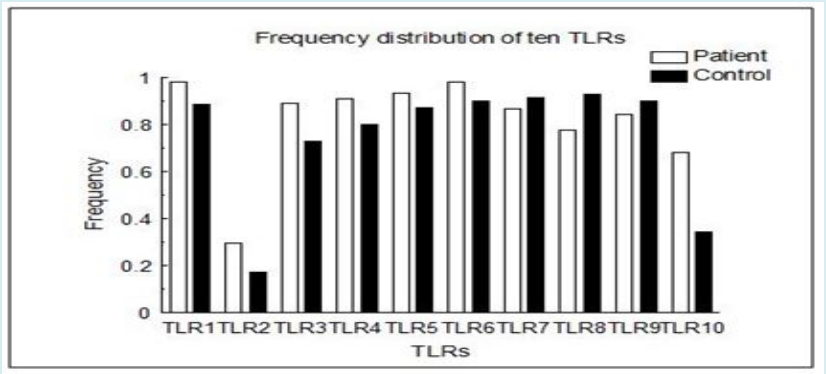

Figure 5: Frequency graph of ten TLR genes was constructed using Kyplot (ver-2.0) of typhoid patients [35].

Human Immunodeficiency Virus

Toll like receptors regulate both the innate and adaptive immune response. Polymorphism in TLR genes has been investigated in case of various diseases [36]. Susceptibility to HIV infection and disease progression are variable

among different populations and also it has been genetically determined [37]. A small percentage (0.2%) of HIV-1 sero-positive patients is able to control the HIV-1 infection over several years (Figure 6 & Tables 7,8). The adult HIV prevalence at national level has 0.26% in 2015 [38]. It defines that they can maintain a viral load which is fewer than 50 copies of HIV-1 RNA per ml [39]. Different TLRs expressed on different cell types in the human immune system and up regulated by cytokines. IFN- γ also induces the expression of TLR4 in peripheral blood monocytes [40].

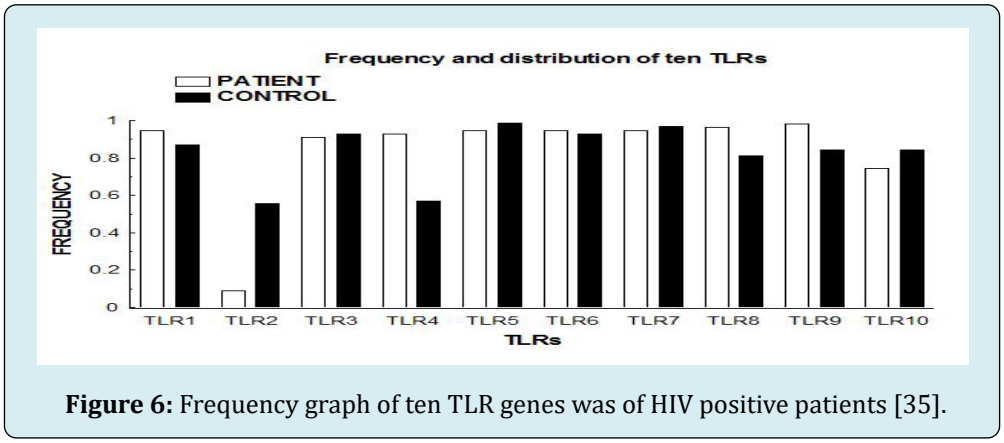

Figure 6: Frequency graph of ten TLR genes was of HIV positive patients [35].

	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	1E-05
TLR5	0.25	0.02-2.48	0.9592	0.31
TLR6	1.33	0.30-5.84	1.0182	0.73
TLR7	0.5	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

Table 7: Risk ratio and odd ratio for ten different TLRs in association with HIV patient [35].

	Sensitivity	Specificity	PPV	NPV
TLR1	94.55	12.86	46	75
TLR2	9.09	44.29	11.4	38.3
TLR3	90.91	7.14	43.5	50
TLR4	92.73	42.86	56	88.2
TLR5	94.55	1.43	43	25
TLR6	94.55	7.14	44.4	62.5
TLR7	94.55	2.86	43.3	40
TLR8	96.36	18.57	48.2	86.7
TLR9	98.18	15.71	47.8	91.7
TLR10	74.55	15.71	41	44

Table 8: Diagnostic test values for HIV patients based on baye's theorm [35].
PPV- Positive predicted value, NPV- Negative predicted value

Sample Size and Selection

Rheumatoid Arthritis

A total number of 110 Rheumatoid Arthritis patients were included in this study. Samples were collected from an authorized diagnostic laboratory of Siliguri and from North Bengal Medical College and Hospital (NBMCH, Sushrutnagar, West Bengal, India) under the guidance of medical practitioners (Table 9). The diagnosis of RA was made by the physician based on the medical and clinical history, physical examinations and symptoms of the disease and most importantly, their fulfillment of the American College of Rheumatology criteria 2010 [41]. For the positive conformation of the disease, anti-ccp and RF titre assay were performed. The cutoff value for anti-ccp estimation was ≥17 U/ml and for RF titre assay it is 20 IU/ml.

Selected disease			
	Rheumatoid Arthritis	Typhoid	Human Immunodeficiency Virus
Sample size	110	44	55
Control size	100	70	70
Total sample size	209		

Table 9: Demographic profile of the Disease samples.

Typhoid Fever

Typhoid patients were selected on the basis of the specific symptoms that had been found during the typhoid fever in Siliguri region. Typhoid patients were screened by Widal test positive result carried out by serum agglutination test. The serum agglutination test was done against *S. typhi* "O" and "H" antigens using Salmonella antigen kit (Beacon diagnostic Pvt. Ltd, India). The serum antibody titre of 1: 80 or above was considered positive result. 44 positive samples were collected for this study [35].

Human Immunodeficiency Virus

HIV patients were selected based on the specific symptoms that had been found during the disease progression in the HIV positive patients from Hospitals of Siliguri region. Positive HIV patients were selected on the basis of their viral infection and counting of CD4+ cells within the range of 156-756 $\times 10^6$ cells/L. Fifty five HIV positive samples were taken for the study [35].

The Major Findings from the Rheumatoid Arthritis Based Study

Toll like receptors are expressed by synovial cells within the joints of RA patients and a variety of endogenous TLR ligands are expressed [42]. TLR1, TLR2, TLR4, TLR5 and TLR6 are highly expressed on the cell surface and recognize the antigens found on the surface of the pathogen. On the other hand TLR3, TLR7, TLR8 and TLR9 found on the endosomal membrane and antigen must be taken up by the cell. Upon binding to the ligand, TLRs interact with the different adaptor proteins and leads to the activation of resulting cytokines. Different toll like receptor proteins are highly expressed in case of RA patients. Expressions of TLR2 and TLR4 on peripheral blood monocytes have been documented in case of RA patients. TLR3 and TLR7 are also expressed in synovial tissue of RA patients. It has also been observed that in case of early as well as longstanding RA patients these two TLRs are highly expressed [42].

It has been found that some of the TLRs like TLR1, TLR3, TLR6 and TLR8 showed high frequency in the patient (Figure 3). TLR1 and TLR6 are present in the cell surface and after recognition of the bacterial, viral or fungal infection induce pro-inflammatory gene expression in the body via MyD88 dependent pathway. These two TLRs mainly recognize the diacyl and triacyl lipopetides as their antigen in the cell surface. On the other hand TLR3 and TLR8 present in the cell compartment which can recognize single and double stranded RNA. TLR3 signalling pathway occurs via TRIF dependent adaptor molecules mainly responsible for the production of interferons. Different small molecules that have been produced during the inflammation are recognized by the TLRs present inside the cell compartment.

It is documented from the previous data that TLR9 is highly expressed due to the autoimmune disorder. This probably causes in case of rheumatoid arthritis patients too. The relative risk for the disease is also high in case of TLR4, TLR7 and TLR9.

High degree of odd ratio for the association with the disease is also found in case of TLR1, TLR4, TLR6, TLR8 and for TLR9. The highest odd has been found in case of TLR4 in the patients of Siliguri and adjoining region (Table 3). It has been shown that in Chinese Han population certain polymorphic variation in the exon region of TLR4 contributed to RA pathogenesis. This finding supports our data. The anti-

CCP positive and RF positive patients with certain mutation in the TLR4 gene associated with the blunted receptor activity and diminished inflammatory response in humans [43]. The TLRs present inside the cell compartment are also responsible for the progression of the disease due to the production of various antigens during disease progression. So, the data also revealed that the different TLRs present in the cell compartment show a very high frequency.

It has been postulated from the different studies that expression of TLR2, TLR3 and TLR7 are significantly up regulated in RA synovial fibroblast tissue in case of RA patients, but high expression of TLR4 has also been detected on macrophages present in the RA synovium [44]. Our data suggested high elevation of TLR4 in the RA patient, but door line association found in case of TLR3 and TLR7. Although TLR7 showing the much higher association rather than TLR3. Here it has also been found that risk ratio for TLR7 is higher than any other TLR except TLR4. So it can be easily predicted that TLR7 plays a vital role for the severity of the disease in RA patients.

In our study, it was observed that door line association has been found in case of TLR1 and TLR6 in association with the risk factor, but the high odd ratio of this two TLR defined their role in case of RA pathogenesis. Presence of different antigens released during the disease condition may increase their expression in the patients. Although no sufficient data have been found for the profound role of TLR1, TLR5 and TLR6 in case of RA, but the frequencies of TLR2 and TLR10 are found very low in the patients. Sensitivity or true positive cases has been found for TLR1 and TLR8. Association with the disease has also been calculated for other cell surface as well as for the endosomal TLR which is also responsible for the disease pathogenesis.

In case of rheumatic patients, certain TLRs play a vital role for the pathogenesis of the disease. Presence of certain antigens released by the different cell types activate the TLR receptors via different signalling proteins produce certain proinflammatory cytokines which induce the disease progression in the patients. Screening of the ten human TLR genes among the patients of North Bengal region tells about the overall scenario of the role of TLRs and their frequency pattern. This helped us to analyze further role of other different TLRs in case of RA.

The Major Findings from the Typhoid Fever based Study

It has been found that the frequencies of some of the TLRs like TLR1, TLR4, TLR5 and TLR6 are very high compare to healthy controls (Figure 4). Different antigens produced by *S. typhi* elevated the TLR expression in typhoid patients. Recognition of different antigens like vi- capsule, flagellin, LPS and other antigens definitely activated the signaling pathways for the production of different cytokines in human. The interaction between TLRs and PAMPs produced from the *S. typhi* increases the formation of inflammosome [33].

Chi- square analysis reveals the significant values for different TLRs which positively associated with the disease. Correlation study also shows the close association with the patient and the control values for all ten human TLRs [35].

Positive association is found for TLR1 and TLR6 with the disease in respect to their odd ratio which was very highly associated with the disease and TLRs. 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, TLR4, TLR5, and TLR6 expression in the cells proves that antigens from *S. typhi* increases the frequency pattern of those TLRs in course of the disease. It has been now established that TLR5, recognizes the flagellin protein present in the bacteria, plays a significant role in case of typhoid fever. During the contamination of bacterial infection, the expression level of this TLR gene becomes maximum in most of the patients. According to Hue *et. al.,* (2009) TLR4 mainly recognizes the LPS, extent genetic variation within the TLR4 gene involved in defense against typhoid fever in Vietnamese population [45].

Sensitivity test for TLR1, TLR4, TLR5, and TLR6 are very high in typhoid positive patients which signify the prevalence of the disease in the population (Table 5). The predictive values of any diagnostic test are related to its disease prediction ability. The low positive predicted values (PPV) are found when compared to the negative predicted values (NPV) (Table 6). It has also been proven that the flagellin protein from the bacteria increases the expression of TLR5 in positive cases and multiplies the disease susceptibility among patients.

The Major Findings from the HIV based Study

In our study, we have found drastic increase of TLR4, TLR8 and TLR9 in HIV+ patients. TLR4 mainly recognizes endotoxin (LPS) as their ligands. HIV is an enveloped retrovirus which uses RNA as their genetic material and used reverse transcriptase and DNA integration in host cells to replicate. The envelope protein complex of HIV-1 is synthesized as a polyprotein (gp160) that is cleaved intracellularly to a heterodimer of surface subunit gp120 and trans-membrane subunit gp41, are non-covalently linked [46]. TLR4 binds to the gp120 protein of HIV and trigger proinflammatory cytokine production via activation

of NF-κB. In this study the higher odd ratio and the relative risk for the disease indicates the ongoing promotion of the disease [47]. On the other hand function of TLR9 has been suppressed by gp120 protein. It also suppresses the function of pDCs cells and IFN-α where TLR9 expresses [48]. TLR9 also expresses in the cell compartment like in endosomal compartment where they successfully recognizes the ssRNA, CpG oligonucleotides and express constitutively [49]. Certain polymorphic variation in TLR9 (1635A/G and +1174G/A) increases the susceptibility for the disease [50]. It has been also found that it is same for higher level of odd ratio and relative risk and thereby it can be suggested that TLR9 constitutively express in the cell. The sensitivity is also very high which is near 100% for TLR9 where the disease is positive for number of samples in our study. Another TLR also important in case of HIV is TLR8 which recognizes single stranded viral RNA and mainly express in myeloid DCs and in monocytes/ macrophages in human. The frequency of TLR8 and TLR9 is very high in case of HIV+ patients (Figure 5). The odd ratio and relative risk are showing the higher values in case of the disease (Table 7). Sensitivity is also very high in case of TLR8. TLRs mainly express during HIV infection, produce type-I interferon cytokines via TLR signaling pathway. Significant data are also found for TLR2, TLR4, TLR8 and TLR9 (Table 8). P value has been considered for significance in case of TLR4, TLR8 and TLR9 which indicates the positive correlation of the disease with the TLR markers.

Conclusion

The review focuses on the two major part of the TLR study. In one hand it describes about the relation of TLR genes with the four major populations in the North Bengal region and on the other hand it also gives a glimpse on the role of TLRs in case of three major diseases.

In the population based study it has been found that environmental selection of some specific TLR genes among different population and convergent evolution shapes the population of this region into a peculiar nature irrespective of their ethnicity. This study is one of the primary and first hand report on population based study of TLR genes. So, further study is needed to reveal their genetic background in respect of their TLR genes and to know how TLR genes act on them in different conditions.

In this present work, the main goal was to determine the genetic predisposition in three main diseases and the role of TLR genes in the disease pathogenesis in multi ethnic populations in Siliguri and adjoining areas. Current findings on the risk factor of three diseases based on TLR gene profile provide a compact knowledge on the genetic basis of the diseases. Further study is needed to illuminate the role of single nucleotide polymorphism of TLR genes and susceptibility/resistant for the diseases in Sub- Himalayan region of West Bengal.

Conflict of Interest: None

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