

CHAPTER 4

RESULTS

4. RESULTS

4.1 TLR gene frequencies among four different populations

The carrier or observed frequencies (OFs) and the estimated gene frequencies of the 10 TLR genes in our four studied populations have been shown in **Table 1**. All the 10 TLR loci have been detected in each and every population. The observed frequencies of all known TLR genes estimated in Gurkha, Muslim, Rabha and in Rajbanshi populations respectively are represented in **Table 5**. It has been observed that the frequency of TLR4 was very high among the Rabhas, while TLR5 was found to be the least frequent among the studied genes. It has also been observed that among the 10 TLR genes, TLR4 has the highest frequency among the Gurkha's (0.968) while TLR5 (0.971) was found to be the highest among the Muslims (**Fig. 14**). Apart from TLR2, TLR5 gene also showed low frequency in the Rabha population. Another interesting observation reported from the study was the low frequency of the TLR2 gene in all the studied populations. The gene frequencies of all the 10 TLR genes in the three populations were also calculated and presented in **Table 5**. It has been observed that in Rajbanshi population, the frequency of TLR8 (0.894) was highest followed by TLR6 (0.882) and TLR9 (0.882) respectively.

Table 5: Observed carrier frequencies (OFs) and the estimated gene frequencies of the 10 TLR genes in the four study populations.

	RAJBANSHI(RA)	GURKHA(G)	RABHA(R)	MUSLIM
TLR1	0.847	0.928	0.760	0.886
TLR2	0.176	0.608	0.600	0.571
TLR3	0.729	0.856	0.740	0.943
TLR4	0.788	0.968	0.900	0.557
TLR5	0.847	0.928	0.460	0.971
TLR6	0.882	0.896	0.640	0.907
TLR7	0.870	0.864	0.680	0.943
TLR8	0.894	0.824	0.700	0.829
TLR9	0.882	0.784	0.820	0.793
TLR10	0.376	0.832	0.540	0.793

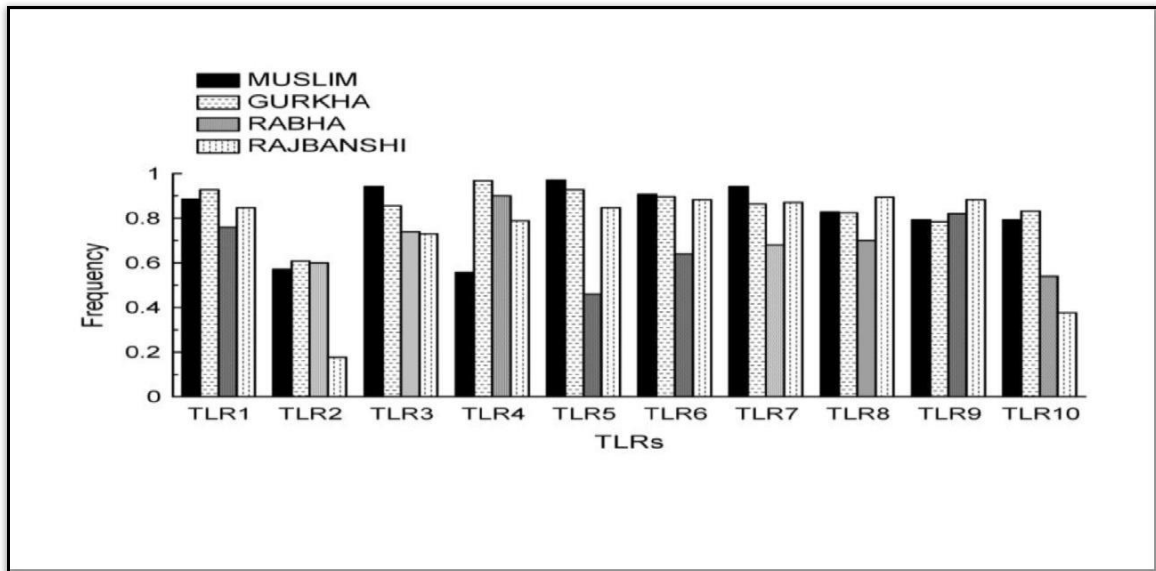


Fig.10- Frequency graph of ten TLR genes of the four populations in the North Bengal region (Kyplot ver-2.0).

4.2. Study of heterogeneity among the local population(s).

4.2.1 Chi-Square Test

Chi-square analyses (χ^2) have been performed to compare the differences in carrier frequencies (OF) of TLR genes among the four populations (**Table 6**). Significant differences were observed in case of 9 out of 10 TLR loci among all the four populations except for TLR9. No significant difference was found for TLR9 in any of the comparisons among four populations. Mean unbiased genetic diversity of TLR genes in the three populations was calculated to be 0.240 ± 0.038 for Gurkhas, 0.258 ± 0.049 for Muslims and 0.410 ± 0.033 for Rabhas. It is observed that there are no significant differences between Rajbanshi and Gurkha when compared with the other two populations. There are no significant differences found for TLR1 and TLR9 when compared between the Rajbanshis and other three populations. When compared Rajbanshi with other three populations it was found that non significant cases were observed only for TLR1 and in TLR9 but significant results has been found in case of other TLRs.

Table 6. Chi square (χ^2) values of different populations and their comparison (Kyplot beta ver-2.0).

	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***	1.820	45.051***	66.880***
TLR6	0.007	0.135	9.822**	0.009	14.349 ***	17.349
TLR7	0.004	2.700	6.005*	3.927*	6.745**	20.745***
TLR8	1.458	1.330	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**

* P< 0.05, ** P< 0.01, *** P< 0.001 N.S- Non significant

4.2.2 Principal Component Analyses

Principal Component Analyses (PCA) was performed based on the OFs of the ten TLR genes to investigate the genetic relationship as well as the structure of our studied populations (**Fig. 15**). Frequencies of ten TLR genes were considered in the analyses. The score plot shown in **figure 1** was computed based on first two components, wherein the first and the second component accounted for 56.17% and 31.94% variability respectively. It was evident from the positions occupied by different populations based on their TLR gene frequencies were in accordance with their geographical proximities.

From the Principal Component Analyses it is evident that the four populations occupied three different quadrants of the score plot, whereas the Muslims occupied the upper right quadrant, Gurkhas occupied the same upper right quadrant but lower than Muslim and the Rabhas occupied the lower right quadrant of the plot. Rajbanshi population occupied the upper left quadrant position in the plot. Gurkha and Muslim remain quiet closer to each other in the PCA plot.

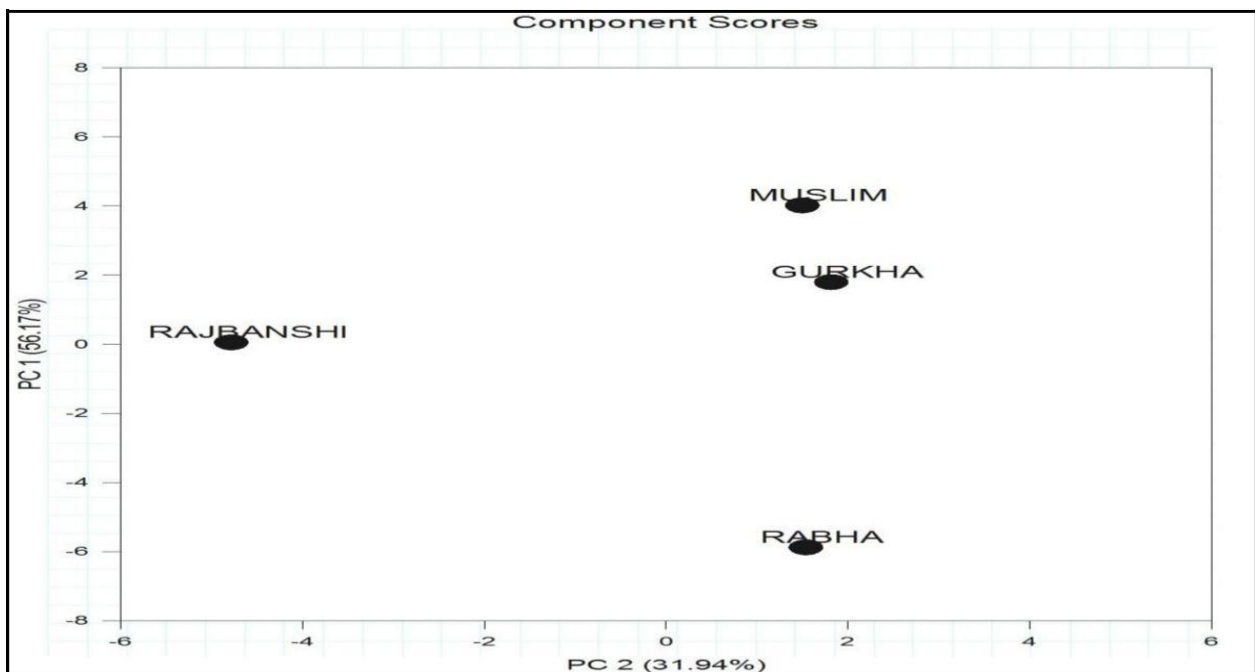


Fig. 11- Principle Component Analyses (PCA) based of the 10 TLR genes in the four ethnic populations of North Bengal (Minitab ver-6).

4.2.3 Genetic distance calculation

Genetic distances represented the measures of Nei genetic distances of the studied populations in the region of North Bengal areas. Nei's genetic distance was also calculated between the populations (**Table 7**) and it is observed that the Gurkha-Muslim genetic distance (0.052) was considerably lesser than the Gurkha-Rabha genetic distance (0.0973). The genetic distance is found to be the highest among the Muslims and the Rabhas (0.1557). The genetic distance between Rajbanshi- Gurkha is 0.0685, between Rajbanshi- Muslim is 0.0694, and between Rajbanshi- Rabha is 0.0745. When considered the Rajbanshi population, least genetic distance is observed among Rajbanshi and Gurkha and greater among Rajbanshi and Rabha (**Fig. 16**).

Table 7: Nei's Genetic distances of four populations using POPGENE (Ver- 1.32) software.

POP ID	RAJBANSHI	RABHA	MUSLIM	GURKHA
RAJBANSHI	0.000			
RABHA	0.0745	0.000		
MUSLIM	0.0694	0.1557	0.000	
GURKHA	0.0685	0.0973	0.0526	0.000

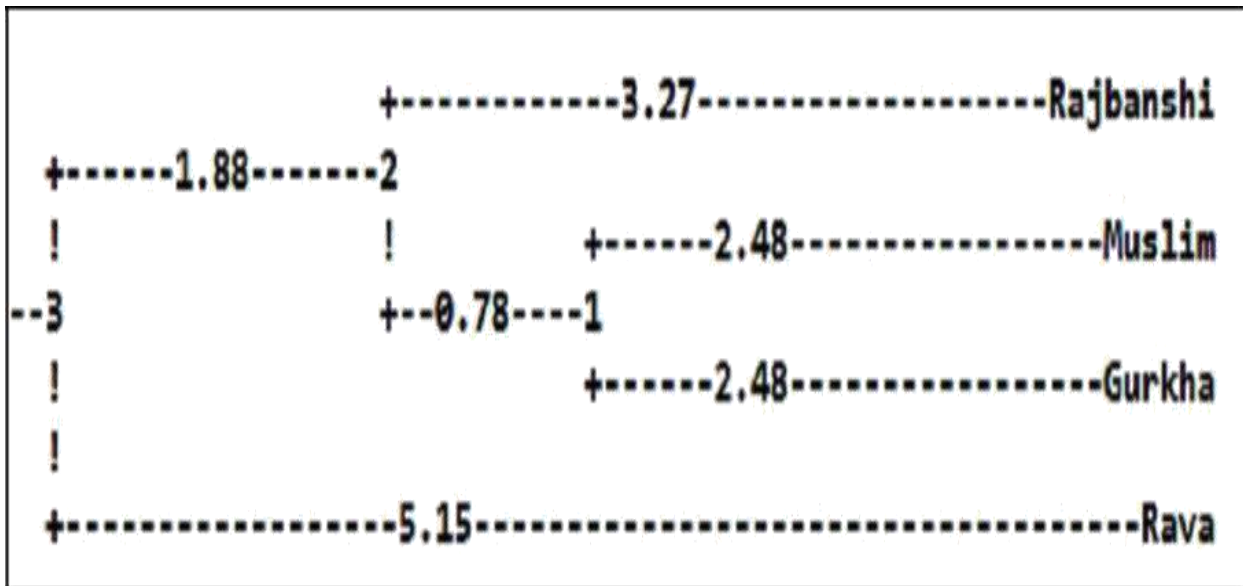


Fig. 12- Neighbour joining tree showing relationship among Rajbanshi and three other populations (POPGENE ver- 1.32 and Phylip ver-3.5)

Frequency, Distribution and Association study of TLR genes among Rheumatoid arthritis, Typhoid fever and Human Immunodeficiency Virus patients

4.3 Association of TLR genes among Rheumatoid arthritis patients

4.3.1 Diagnostic and Demographic profile

Demographic profile of patients and control group has been compared. Out of 110 positive samples of rheumatoid patients, 28 were men and 82 female patients. The healthy control samples were also collected from the Siliguri area with proper consent from the individuals. The average age of the patients group was 47 years whereas in case of control group it was 44 years.

Diagnostic data were also collected for the conformation of the positive samples for the rheumatoid arthritis. The selected diagnostic tests were anti-CCP and RF titre . The median range of anti-CCP in the patients is 182.7 (16.5 to 504.93) but in case of control group the range is very low 10.8(8.2 to 13.1). In case of RF titre the median range is 142.3 (41.20 to 198.0) for RA patients. But low value has been detected for the control group 16.8 (12 to 20.5). These two diagnostic tests were selected as major criteria for the positive samples in case of RA (**Table 8**).

Table 8: Demographic data and clinical characteristics of RA patients and control groups

		RA group (n=110)	Control group (n=100)
Gender n (%)	Male	28 (25.5)	57 (57%)
	Female	82 (74.5)	43(43%)
Age (Years)	Median (Range)	47 (39-54.5)	44(38-50)
Disease duration (Years)	Mean (SD)	5.6± 2.4	-----
Anti CCP (IU/ml)	{Median (IQR)}	182.7 (16.5 to 504.93)	10.8(8.2 to 13.1)
	Positive cases	N (%)	84 (76.4 %)
	Negative Cases	N (%)	26 (23.6%)
RF titre (IU/ml)	{Median (IQR)}	142.3 (41.20 to 198.0)	16.8 (12 to 20.5)
	Positive cases	N (%)	86 (78.2)
	Negative Cases	N (%)	24 (21.8)

(Guha *et. al.*, 2018)

4.3.2 Gene frequency and Chi-square analysis

Observed frequency data of ten TLR genes from 110 rheumatoid arthritis patients were analyzed. It has been found that in rheumatoid patients TLR1 (0.95) and TLR8 (0.95) are highly up regulated (**Fig. 17**). It has also been found that TLR3, TLR6 and TLR9 are also very high with the frequency of 0.90, 0.92 and 0.82 (**Table 9**). Chi- square analyses for significant data were observed for TLR6 and TLR8 and TLR9 (for 95% significant). In case of other TLRs like TLR2, TLR4, TLR5, and TLR10 have been found some significant results for the association with the disease. On the other hand TLR1 and TLR7 have showed the non-significant result.

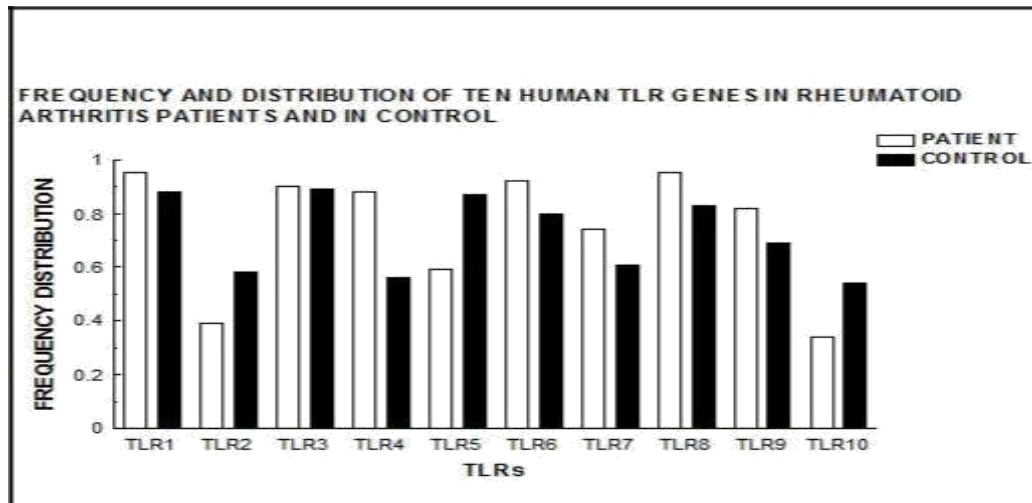


Fig 13. Frequency graph of ten TLR genes of rheumatoid arthritis patients (Kyplot ver-2.0).

Table 9: Gene frequencies of the 10 TLR genes among Rheumatoid arthritis patients and with the control

Gene	Patient	Control
TLR1	0.766	0.653
TLR2	0.219	0.351
TLR3	0.683	0.668
TLR4	0.656	0.336
TLR5	0.360	0.639
TLR6	0.713	0.552
TLR7	0.486	0.375
TLR8	0.766	0.587
TLR9	0.573	0.443
TLR10	0.185	0.321

Table 10: Observed frequencies of the 10 TLR genes in the Rheumatoid arthritis patients and control.

	PATIENTS	CONTROLS	χ^2	Relative risk	p value
TLR1	0.95	0.88	2.089N.S	1.07	0.09
TLR2	0.39	0.58	6.764**	0.67	0.007
TLR3	0.90	0.89	0.0001	1.01	0.81
TLR4	0.88	0.56	25.829***	1.57	<0.0001
TLR5	0.59	0.87	19.037***	0.67	<0.0001
TLR6	0.92	0.80	5.193*	1.14	0.01
TLR7	0.74	0.61	3.264N.S	1.20	0.05
TLR8	0.95	0.83	6.024*	1.13	0.01
TLR9	0.82	0.69	4.009*	1.18	0.03
TLR10	0.34	0.54	8.035**	0.62	0.003

* P< 0.05, ** P< 0.01, *** P< 0.001 N.S- Non significant

4.3.3 Relative risk calculation

Relative risk utilizes the probability of an event occurring in one group compared to the other group. The relative risks for different TLRs were calculated and represented in Table 10. The relative risks for TLR4 (RR- 1.57, p- <0.0001), TLR7 (RR- 1.20, p- 0.05) TLR8 (RR- 1.13, p- 0.01), TLR9 (RR- 1.18, p- 0.03) are very high whereas door line association is found in case of TLR1 (1.07, p- 0.09) and TLR3 (1.01, p- 0.81). Low association with the disease is observed in case of TLR2 (RR- 0.67), TLR5 (RR- 0.67) and TLR10 (RR-0.62). Higher value of relative risk (>1) signify the positive association with the disease whereas lower value define the weak association with the disease.

ANOVA test for comparing among the control and patient groups and the p- value is 0.05, two tailed t-test value was assumed to be 0.80.

4.3.4 Odd ratio/ Risk ratio calculation

The odd ratio and 95% confidence interval for ten different TLRs in rheumatoid patients showed that in case of TLR1 (odd- 2.36, CI-1.21-6.49,p- 0.09), TLR4 (odd-5.86, CI- 1.06-3.84,p- <0.0001), TLR6 (odd- 2.80, CI- 0.85-6.55,p- 0.01), TLR8 (odd- 3.55, CI- 0.45-2.69,p- 0.01) and TLR9 (odd- 2.02, CI- 2.90-11.81,p- 0.03) show high associations whereas TLR2 (odd- 0.46, CI- 0.99-3.20,p- 0.006), TLR5 (odd- 0.21, CI- 0.24-0.75,p- <0.0001) and TLR10 (odd- 0.43, CI- 0.10-0.43,p- 0.003) show lower association among the patients and control samples (**Table 11**).

Table 11: Risk ratio and odd ratio for ten different TLRs in association with Rheumatoid arthritis

	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	2.80	0.85-6.55	0.01
TLR7	1.20	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

4.3.5 Sensitivity and Specificity

The prevalence of the disease in the patients was estimated by the diagnostic test based on Bayer's theorem. Sensitivity is the probability that a test will indicate disease among the individuals. Specificity is the fraction of those without disease. The sensitivity is found very high in case of TLR1 (94.55), TLR3 (90.00), TLR6 (91.82) and TLR8 (94.55) (**Table 12**). A low sensitivity is observed in case of TLR2 (39.09), TLR5 (59.09), and TLR10 (33.64) which signified the low prevalence of the disease among the patients. The positive predicted value is the probability that a subject is diseased, given a positive test result. Negative predicted value is the probability that a subject is healthy, given a negative test result. PPV was found highest for TLR4 (63.40), TLR7 (57.04) and TLR9 (56.60).

Table 12: Diagnostic test values for Rheumatoid arthritis patients based on bayer's theorem.

	SENSITIVITY	SPECIFICITY	PPV	NPV
TLR1	94.55	12.00	54.17	66.67
TLR2	39.09	42.00	42.57	38.53
TLR3	90.00	11.00	52.66	50.00
TLR4	88.18	44.00	63.40	77.19
TLR5	59.09	13.00	42.76	22.41
TLR6	91.82	20.00	55.80	68.97
TLR7	73.64	39.00	57.04	57.35
TLR8	94.55	17.00	55.61	73.91
TLR9	81.82	31.00	56.60	60.78
TLR10	33.64	46.00	40.66	38.06

PPV- Positive predicted value, NPV- Negative predicted value

4.4 Association of TLR genes among typhoid fever patients

4.4.1 Observed frequency and Chi-square analysis

Observed frequency data of ten TLR genes from 44 typhoid patients were analyzed. It has been found that in typhoid patients TLR1 (0.977) and TLR6 (0.977) are highly up regulated (**Fig. 18**). TLR4 and TLR5 are also very high with the frequency of 0.909 and 0.931 (**Table 13**). Chi-square analyses for significant data have been observed for TLR8 and TLR10.

Table 13: Observed frequencies of the 10 TLR genes in the control and patients of typhoid fever.

	PATIENTS	CONTROLS	χ^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 N.S- Non significant

4.4.2 Relative risk calculation

The relative risks for different TLRs were calculated (**Table 13**). 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 observed 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 slightly higher association is observed in case 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).

4.4.3 Odd ratio/ Risk ratio calculation

Fischer's exact test for probability showed significant association for TLR8 ($p= 0.022, <0.05$) and TLR10 ($p= 0.0005, >0.001$). When calculated the odd ratio and 95% confidence interval for ten different TLRs, in typhoid patients it has been found that in case of 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, $p- 0.31$) shows high associations, whereas TLR7 (odd- 0.59, CI- 0.17-1.97, $p- 0.39$), TLR8 (odd- 0.26, CI- 0.08-0.82, $p- \mathbf{0.02}$) and TLR9 (odd- 0.58, CI- 0.19-1.80, $p- 0.35$) shows lower association among the patients and control samples (**Table 14**).

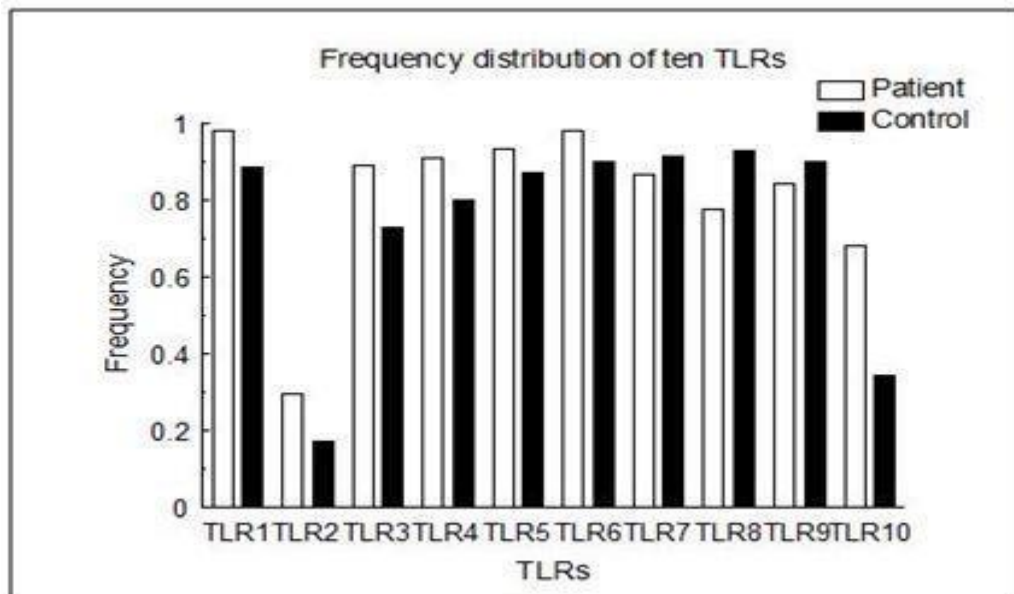


Fig 14. Frequency graph of ten TLR genes of typhoid patients (Kyplot ver-2.0)

The “O” antigen test value for Widal test was considered positive where the values above 1: 80 were marked as a positive sample (**Table 15**). Chi-square analyses (χ^2) were performed to compare the differences in carrier frequencies (OF) of TLR genes among the patients and control samples. Significant differences are observed in case of TLR8 and TLR10. No significant differences are found among the other eight TLRs (**Table 13**). ANOVA test for comparing

among the control and patient groups and the p- value is 0007 (> 0.001), two tailed t-test value was assumed to be 0.521.

Table 14: 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

4.4.4 Sensitivity and Specificity

The prevalence of the disease in the patients was estimated by the diagnostic test based on Bayer's theorem. The sensitivity was found very high in case of TLR1 (97.73), TLR4 (90.91), TLR5 (93.18) and TLR6 (97.73) (**Table 16**). A low sensitivity is estimated in case of TLR2, TLR8, and TLR10 which signified the low prevalence of the disease in the patients.

Table 16: Diagnostic test values for typhoid patients based on baye's theorem.

	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 value

Table 15: Widal test result of the positive typhoid samples

SAMPLE NO.	Age	Sex	Widal test result
1	17	F	—O - 1:160 —H - 1:20
2	32	M	—O - 1:160 —H - 1:40
3	19	F	—O - 1:160 —H - 1:20 —AH - 1:20
4	21	F	—O - 1:160 —H - 1:40

5	32	M	—Ol- 1:160 —Hl- 1:40
6	23	M	—Ol- 1:320 —Hl- 1:40
7	45	F	—Ol- 1:40 —Hl- 1:80 —Ahl- 1:20 —Bhl- 1:20
8	24	M	—Ol- 1:80 —Hl- 1:40 —Ahl- 1:20
9	27	M	—Ol- 1:160 —Hl- 1:80
10	03	F	—Ol- 1:160 —Hl- 1:160
11	26	F	—Ol- 1:160 —Hl- 1:80
12	38	M	—Ol- 1:160 —Hl- 1:80
13	10	M	—Ol- 1:80 —Hl- 1:20
14	19	F	—Ol- 1:160 —Hl- 1:80
15	18	M	—Ol- 1:320 —Hl- 1:160
16	55	M	—Ol- 1:160 —Hl- 1:80
17	22	F	—Ol- 1:320 —Hl- 1:80

18	23	F	—Ol- 1:160 —Hl- 1:80
19	25	M	—Ol- 1:160 —Hl- 1:80
20	74	F	—Ol- 1:80
21	20	F	—Ol- 1:80
22	28	M	—Ol- 1:160 —Hl- 1:40
23	27	M	—Ol- 1:80
24	70	F	—Ol- 1:80
25	30	F	—Ol- 1:20 —Hl- 1:40 —Ahl- 1:160
26	42	F	—Ol- 1:20 —Hl- 1:40 —Ahl- 1:160
27	22	M	—Ol- 1:80 —Hl- 1:20
28	70	M	—Ol- 1:40 —Hl- 1:20 —Ahl- 1:80
29	23	F	—Ol- 1:80 —Hl- 1:20
30	4	F	—Ol- 1:80
31	40	F	—Ol- 1:160 —Hl- 1:80
32	12	M	—Ol- 1:80
33	17	F	—Ol- 1:160 —Hl- 1:80

34	19	M	—Ol- 1:160 —Hl- 1:40
35	21	F	—Ol- 1:160 —Hl- 1:80
36	55	M	—Ol- 1:160 —Hl- 1:320
37	45	F	—Ol-1:160 —Hl-1:80
38	25	M	—Ol-1:80
39	76	M	—Ol-1:80 —Hl-1:240
40	60	F	—Ol-1:160
41	35	F	—AHl- 1:160
42	22	F	—Ol- 1:160
43	42	F	—Ol- 1:80 —Hl- 1:160
44	09	F	—Ol- 1:320 —Hl- 1:160

4.5 Association of TLR genes among HIV+ patients

4.5.1 Observed frequency and Chi-square analysis

Observed frequency data of ten TLR genes from 55 typhoid patients were analyzed. It has been observed that in HIV+ patients, TLR8 (0.809) and TLR9 (0.865) are highly up regulated (**Fig. 19**). TLR1 (0.766) and TLR6 (0.766) are also very high among HIV patients (**Table 17**). Chi-square analyses for significant data are observed for TLR2, TLR4, TLR8 and TLR9. Different samples were confirmed by the CD4+ marker test results (**Table 18**).

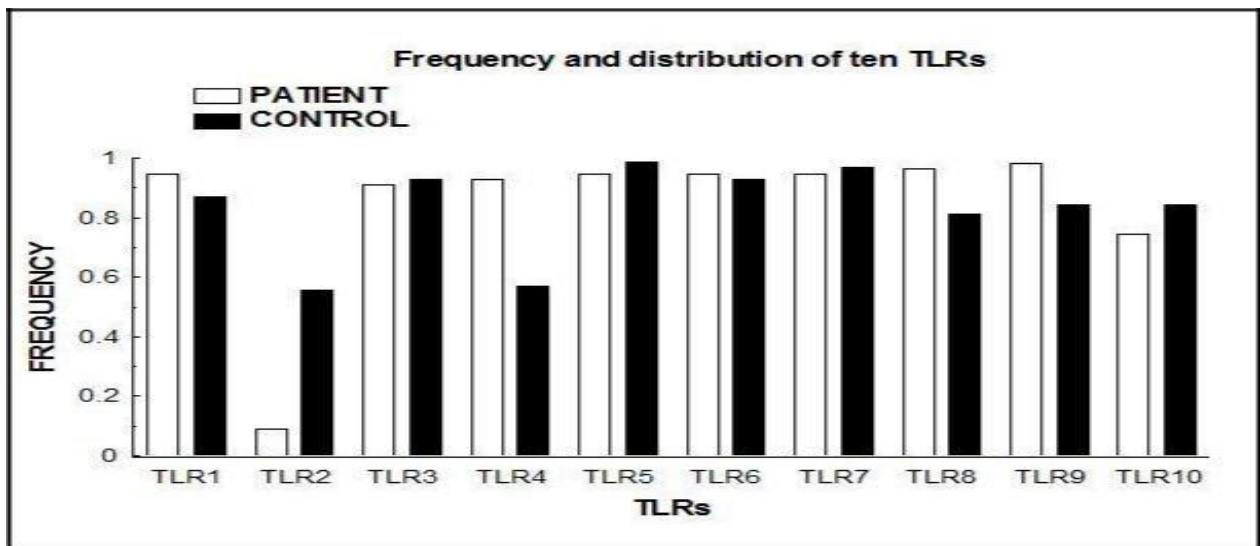


Fig 15 Frequency graph of ten TLR genes was of HIV positive patients.

4.5.2 Relative risk calculation

The relative risks for different TLRs were calculated among HIV+ patients (**Table 17**). The relative risks for TLR4 (RR- **1.62**, p- 0.41), TLR8 (RR- **1.18**, p- **0.007**) and TLR9 (RR- **1.16**, p- **0.005**) are very high, whereas door line association is found in case of TLR1 (1.08, p- 0.14) and TLR6 (1.01, p- 0.69). On the other hand low association is observed in case of TLR2 (0.16, p- < 0.0001) and TLR10 (0.88, p- 0.19).

ANOVA was performed for patient and control group and the p-value is observed 0.04 (< 0.05). Two-tailed t-test for p-value is assumed to be 0.93 for patient and control group.

Table 17: Gene frequencies of the 10 Human TLR genes in the control and patients and measurement of relative risk.

	Patients	Controls	χ^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

4.5.3 Odd ratio/ Risk ratio calculation

Fischer's exact test for probability showed significant association for TLR4 (p= 0.00001, >0.001), TLR8 (p= 0.01, >0.05) and TLR9 (p= 0.01, >0.05). The odd ratio and 95% confidence interval for ten different TLRs in HIV+ patients documented that in case of TLR4 the odd is 9.56 and CI is 3.11-29.37 for TLR8 odd is 6.04 and CI is 1.30-28.05 for TLR9 odd ratio is 10.06 and CI- 1 is 25-80.60 indicate high associations, whereas TLR2 (odd- 0.07, CI- 0.02- 0.22), TLR5 (odd- 0.25, CI- 0.02-2.48) and TLR7 (odd- 0.50, CI- 0.08-3.16) shows lower association among the patients and control samples. The risk ratio for TLR4 is 1.6227, TLR8 is 1.1834, and for TLR9 value is 1.1649 shows slightly higher association for the disease (**Table 19**).

Table 19: 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

4.5.4 Sensitivity and Specificity

The prevalence of the disease in the patients was estimated by the diagnostic test based on Bayer's theorem. The sensitivity is found very high in case of TLR8 (96.36) and TLR9 (98.18) (**Table 19**). A low sensitivity is observed in case of TLR2 (9.09) and TLR10 (74.55) which signify the low prevalence of the disease among the patients.

Table 20: Diagnostic test values for HIV patients based on baye'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

Table 18: CD4+marker test result of HIV patients

SAMPLE NO.	CD4+ RESULT
1	190
2	330
3	232
4	310
5	156
6	410
7	340
8	202
9	756
10	285
11	612
12	292
13	432
14	190

15	300
16	390
17	465
18	580
19	212
20	200
21	191
22	187
23	580
24	211
25	602
26	209
27	222
28	245
29	280
30	310
31	300
32	340
33	187
34	192
35	190
36	198
37	214
38	210
39	210
40	222
41	210
42	250
43	261
44	238

45	274
46	303
47	300
48	390
49	401
50	300
51	357
52	339
53	192
54	207
55	219