An investigation of serum C–reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia

A Thesis Submitted

To

Sikkim University

In Partial Fulfilment of the Requirement For the

Degree of Doctor of Philosophy

By

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September 2021

Dedicated
to
My family

DECLARATION

I, Jiwan Gurung declare that the Ph.D. thesis entitled "**An investigation of serum C–reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia**" submitted by me for the award of the degree of Doctor of Philosophy in Zoology of Sikkim University under the supervision of Dr. Bisu Singh, Assistant Professor, Department of Zoology and co-supervisor Dr. Nirmal Kumar Bera, Professor, Department of Psychiatry, North Bengal Medical College and Hospital is original research work carried out by me in Department of Zoology, Sikkim University, Gangtok. This work has not been submitted for any other degree to this or any other University. All the sources of previous works of other used for the research have been properly cited.

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CERTIFICATE

This is to certify that the Ph.D. thesis entitled "**An investigation of serum C-reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia**" submitted to Sikkim University for the degree of Doctor of Philosophy in Zoology embodies the research work carried out by Mr. Jiwan Gurung from 2015 to 2021 at the Department of Zoology, School of Life Sciences, Sikkim University under our supervision. The work described here is original and no part of this thesis has been submitted elsewhere for the award of any Degree, Diploma, Associateship, Fellowship at this or any other University or Institution of higher learning. Mr Gurung is conversant with techniques and literature cited in the thesis and has fulfilled the requirements of the degree of Doctor of Philosophy in Science (Zoology) of Sikkim University. In character and demeanour, Mr. Jiwan Gurung is fit to submit the thesis for Ph.D. degree.

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"An investigation of serum C–reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia"

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CONTENTS

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ABBREVIATION

AIC	-	**Akaike's information criterion**
BBB	-	**Blood–Brain Barrier**
BMI	-	**Body Mass Index**
CI	-	**Confidence Interval**
CRP	-	**C-reactive protein**
PAMP	-	**Pathogen-associated molecular patterns**
DAMPs	-	**Damage-associated molecular patterns**
DNA	-	**Deoxyribonucleic acid**
DSM	-	**Diagnostic and Statistical Manual of Mental Disorders**
MyD88	-	**Differentiation factor 88**
ELISA	-	**Enzyme-Linked Immunosorbent Assay**
IL	-	**Interleukin**
IFN-γ	-	**Interferon-γ**
LD	-	**Linkage Disequilibrium**
MAPK	-	**Mitogen-activated protein kinase**
MAL	-	**Myeloid adapter-like protein**
NBMCH	-	**North Bengal Medical College and Hospital**
NF-κB	-	**Nuclear factor kappa B**
OD	-	**Odds ratio**
OPD	-	**Outpatient Department**
PPRs	-	**Pattern recognition receptors**
PCR	-	**Polymerase chain reaction**
PANSS	-	**Positive and Negative Syndrome Scale**
RBANS	-	**Repeatable Battery for the Assessment of Neuropsychological Status**
RE	-	**Restriction enzyme**
RFLP	-	**Restriction Fragment Length Polymorphism**
SD	-	**Standard Deviation**
SCID	-	**Structured Clinical Interview for DSM-5**
Th1	-	**T helper 1**
Th2	-	**T helper 2**
CNS	-	**Central Nervous System**
TRIF	-	**TIR-domain containing adaptor inducing protein inducing IFN-β**
TLR	-	**Toll Like Receptor**
TRAM	-	**TRIF-related adapter molecule**
TNF-α	-	**Tumor necrosis factor–α**
UV	-	**Ultraviolet**

PUBLICATIONS

1. **Jiwan Gurung**, Dependra Chamlagai, Nirmal Kumar Bera, Tapas Kumar Chaudhuri, and Bisu Singh. "Elevated levels of C-reactive protein and IL-6 among the antipsychotic medicating schizophrenia patients of Siliguri, West Bengal, India." *Nordic journal of psychiatry* 72, no. 4 (2018): 311-317.

2. Reshma Chettri, **Jiwan Gurung**, and Bisu Singh. "A 10-year retrospective study of suicide in Sikkim, India: Sociodemographic profile and risk assessment." *Indian journal of psychiatry* 58, no. 4 (2016): 448.

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6. Bisu Singh, **Jiwan Gurung**, and Aparajita Chakraborty. "Study of the mosquito larval diversity in the artificial breeding habitat of Gangtok, Sikkim." International Journal of Mosquito Research 6, no. 2 (2019): 27-31.

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8. Bisu Singh, Qubra, Khadijatul, Dependra Chamlagai, and **Jiwan Gurung**. "Study of In vitro Anti-inflammatory Property of Dendrocnide sinuata (Blume) Chew and Chenopodium ambrosioides (L.): Ethnomedicinal Plants from Assam." International Journal of Pharmaceutical Sciences Review and Research 54, no. 2, (2019): 20-24.

Manuscript communicated for Publication

1. **Jiwan Gurung**, Nirmal Kumar Bera, Manoj Lama, Bisu Singh. Association of TLR-4 rs4986790, rs4986791 and TLR-9 rs352140 gene polymorphism with schizophrenia.

Summary

Schizophrenia is a chronic and debilitating disorder that affects approximately 1% of the population. The underlying etiology of schizophrenia is largely controversial. It is suggested that chronic low-grade inflammation triggered by viral agents may play an important role in the etiopathology of schizophrenia. One of the key molecules of the innate immune system which can recognize pathogens such as viruses are Toll-like receptors (TLR). They also play an important role in inflammation by modulating the secretion of cytokines. C-reactive protein is an acute-phase protein and is a well-known marker for inflammation. It is synthesized during the inflammation by the hepatocytes under the influence of cytokine interleukin-6 (IL-6).

Various investigations have been undertaken with reference to TLR and CRP to better understand the role of inflammatory processes in schizophrenia. However, the results are largely inconsistent. With reference to Indian research, to date, only a few studies have been undertaken to shed the light on the role of inflammation in schizophrenia. Nonetheless, these studies are not comprehensive enough for arriving at a conclusion. Additionally, to date, no studies have been carried out to understand the role of TLR gene polymorphism in the etiopathology of schizophrenia among Indian patients. Therefore, the present study was conducted among the Indian Bengalee schizophrenia patients of West Bengal to ascertain the role of inflammation in the pathophysiology of schizophrenia with the objectives; (i) to study the prevalence of schizophrenia among the denizens of Siliguri to understand the disease burden, (ii) to study the serum levels of inflammatory marker C-reactive protein and its modulating cytokine interleukin-6 among the schizophrenia patients and compare with that of the controls, (iii) to study single nucleotide polymorphisms (SNPs) present in TLR-2, TLR-3, TLR-4, TLR-7, TLR-8, and TLR-9 genes which are previously observed to be associated with the viral diseases,

Quantitative estimation of serum CRP and IL-6 were performed by the ELISA method among the two groups of patients. The first group consisted of patients under antipsychotic medication (n=67) and the second group included psychotropic medication-free patients (n=28). The results were compared with age, sex, and ethnicity matched controls (n=72). Further, single nucleotide polymorphisms (SNPs) present in TLR genes were studied by the PCR-RFLP method among 120 schizophrenia patients and 145 controls. The prevalence rate of schizophrenia was studied based on a hospital study for one year i.e., from 31st January 2019 to 30th January 2020.

Based on our study, the prevalence of schizophrenia in the Siliguri sub-division of West Bengal was found to be 0.08 per 1,000 individuals. Further, a significantly increased level of CRP and IL-6 was observed among those patients who were under antipsychotic medication. Even though CRP levels were found to be higher in patients who are psychotropic medication-free, it was not found to be statistically significant. Conversely, psychotropic medication-free patients had significantly increased levels of IL-6. Correlation analysis showed that the level of CRP was significantly associated with the levels of IL-6. Genetic polymorphism study of TLR genes revealed that AG genotype and G allele of TLR-4 rs4986790, CT genotype and T allele of TLR4 rs4986791 were positively associated with schizophrenia. Even though the frequency of C/T genotypes and T allele of TLR-9 rs352140 were found to be higher in patients than the controls, only the T allele of TLR-9 rs352140 was found to be significantly associated with schizophrenia. Evaluation of association of TLR based on the genetic models revealed statistically significant association of TLR-4 rs4986790 in co-dominant, dominant and over-dominant models with schizophrenia. However, based on the lowest Akaike's information criterion (AIC) value, an over-

dominant model was observed to be the best-fit model. On the other hand, TLR-9 rs352140 in the co-dominant, dominant, recessive and over-dominant models were found to be significantly associated with schizophrenia. However, as per the AIC value dominant model was the best fit model. Haplotype analysis revealed a significantly increased frequency of G-C and A-T haplotypes of TLR-4 (rs4986790 v/s rs4986791), and T-T haplotype of TLR-9 (rs5743836 v/s rs352140) among the schizophrenia patients. Based on a logistic regression analysis of TLR genotype and demographic data, only the patient's age was found to be substantially linked with the TLR4 rs4986790 SNP.

The present study adds to the growing body of evidence that inflammatory processes may play an important role in the pathophysiology of schizophrenia. Future studies are warranted in other Indian populations to confirm our findings. In addition, investigations **with a more comprehensive study design involving virus, cytokines, TLR and other parameters of the immune system will help to better understand the underlying mechanism of the inflammatory process in schizophrenia.**

Key words: - Schizophrenia, Inflammatory processes, C-reactive protein, Interleukin-6,

Toll like receptors, West Bengal, Siliguri, India.

CHAPTER-01

Introduction

1. INTRODUCTION

Schizophrenia is a complex and multi-factorial psychiatric disorder and is perhaps the most mysterious and devastating amongst all psychiatric disorders. The term schizophrenia is derived from the Greek word schizo (split) and phren (mind) which means fragmented thinking. Historically the symptoms of schizophrenia are described among the ancient Egyptians. However, in the 19th century, the symptoms of schizophrenia were first described by Emil Kraepelin in 1896 and he termed the disorder as dementia praecox. Later, Paul Eugen Bleuler continued Kraepelin's work and coined the term schizophrenia in 1911. The symptoms of schizophrenia are considered as a syndrome comprising of positive, negative and cognitive abnormality (Strauss et al., 1974; Melssner, 1981). Commonly the symptoms of schizophrenia manifest at the onset of puberty which is characterized by impaired social functioning, neglect of self-care and disturbance of other mental processes. A significant dichotomy has been observed in the pathophysiology of schizophrenia among the males and females with males showing an early age of onset i.e., late teens to early 20s and while females showing late age of onset i.e., late 20s to 30s. However, both the sexes have been observed to be affected equally (Tochigi et al., 2004). An early sign of schizophrenia symptoms is difficult to understand or identify and is easily confused with typical adolescent behaviour. This early sign may include social withdrawal, loss of interests, unusual behaviour, or decrease functioning (such as in school, work or social relationships). Schizophrenia is found to occur equally among all the race, ethnicities, gender and socio-economic group. Presently the diagnosis of schizophrenia is performed by the psychiatrist through a complete medical interview with the patients and their relatives according to the guidelines of the Diagnostic and Statistical Manual of Mental Disorders V (Edition, 2013). Schizophrenia makes it

difficult for an individual to understand the reality around them, due to which they cannot behave normally in social situations. It causes severe impairments in functional capacity causing the problem in day-to-day activity. Due to impairment in functional capacity, people with schizophrenia generally experience social discrimination, financial difficulties, and family break-down. It is one of the leading causes of disability among young adults. Besides, people with schizophrenia have low rates of marriage (MacCabe et al., 2009), low fertility (Bundy et al., 2011), and unemployment. Most schizophrenia patients tend to have a poor diet, they have a smoking habit and are alcoholic. It is reported that schizophrenia patients have a 3.7 times higher risk of early death than the general population (Olfson et al., 2015). Besides, there is a 26.8% high risk of suicide in schizophrenia patients as compared to the general population. Despite decades of tireless research, to date, the etiology of schizophrenia is largely unknown. Currently, there is no cure for schizophrenia, and presently available treatment methods are focused on reducing the suffering of the patients by reducing the intensity and frequency of the symptoms, and improve the quality of life of the patients.

1.1. Symptoms of schizophrenia

To date, there are no biological diagnostic tests or biomarkers available for the diagnosis of schizophrenia. Hence, diagnosis is based on the analysis of the fairly complex symptoms. Therefore, the diagnosis is mainly based on the history and examination of the mental state and the symptom which must last for at least six months and have at least one-month active-phase symptoms. However, the symptoms of schizophrenia can vary between individuals. Besides, the variation of symptoms can occur in the same patient as the disease progresses (Shenton et al., 2010). Based

on its cumulative characteristic the symptoms of schizophrenia have been classified into positive, negative and cognitive symptoms.

1.1.1. Positive symptom

Positive symptoms generally mean psychotic syndrome such as thoughts, perceptions, and behaviors which are present in schizophrenia patients and absent in normal individuals. The common positive symptoms are described below: -

1.1.1.1. Hallucination

A hallucination is a perception of something that one can see, hear, feel, smell, or taste that is not there. Hallucinations are often divided into five categories: visual, auditory, olfactory, gustatory, and tactile. The most common type of hallucination reported among individuals with schizophrenia is an auditory hallucination (Rector and Beck, 2002).

1.1.1.2. Delusions

Delusion is a set of distorted thinking that includes false perceptions and beliefs in the patients, such as someone is plotting to harm him, patient's thoughts are being broadcasted in the air or controlled by some outside force.

1.1.1.3. Disorganized speech

Disorganized speech is difficulty in organizing a meaningful speech which manifested in the way the patients speak. The patient will be unable to stick to the subject and leap from one topic to another.

1.1.2. Negative symptoms

Negative symptoms are those symptoms that are present in a normal individual but absent in schizophrenia patients. Negative symptoms include an absence of memory, thoughts and a decrease in normal fundamental function. Negative symptoms are characterized by flowing symptoms.

1.1.2.1. Affective flattening

Affective flattening consists of a limited range of emotional expressions i.e., the facial expression is unresponsive and are not able to make eye contact with other people.

1.1.2.2. Alogia

Alogia is described as a lack of fluency in speech or logical or productive speech. The individual with alogia may speak few words and may give a short or empty answer to the question.

1.1.2.3. Avolition

Avolition refers to a lack of motivation or engagement in goal-directed behavior. The primary symptoms of avolition include sitting still for a long time without showing much interest in their surroundings. The symptoms also include not displaying any interest in work or social activities with others.

1.1.2.4. Anhedonia

Anhedonia is the inability to experience pleasure from activities one used to find enjoyable. For example, the person may not enjoy watching a sunset, going to the movies, or having a close relationship with other people.

1.1.3. Cognitive symptoms

Cognition refers to mental processes that allow us to perform day-to-day functions, such as the ability to pay attention, remember, and solve problems. Cognitive impairments are considered a core feature of schizophrenia and contribute to difficulties in work, social relationships, and independent living. Some examples of cognitive symptoms in schizophrenia include trouble in concentration or paying attention, poor memory, slow thinking, and poor executive functioning. Executive functions include the ability to plan, solve problems, and grasp abstract concepts.

1.2. Epidemiology

Epidemiology deals with the study of the determination and distribution of disease in a defined population in terms of incidence and prevalence (MacMahon, 1970). Epidemiology comprises prevalence and incidence studies of disease. Incidence is a measure of the probability of occurrence of new cases in a population over a given period. The study of incidence is useful for identifying disease risk factors, while the study of prevalence illustrates the burden of disease in society (Goldner et al., 2002). Schizophrenia is comparatively common across all the populations of the world (Jablensky, 2000). However, the study of incidence and prevalence of schizophrenia showed prominent variation between the studied populations in the different parts of the world (McGrath et al., 2008; Laursen et al., 2014). Variability in estimation in schizophrenia in epidemiological studies may be due to the differences in the methodological aspects of the studies (Perälä et al., 2007; Moreno-Küstner et al., 2014). In addition, diagnostic criteria for schizophrenia have changed over time which may be the reason for the variability in schizophrenia epidemiology. The Diagnostic and Statistical Manual of Mental Disorders (DSM-V) diagnose schizophrenia along a

continuum of severity, from the less severe delusional disorder to the more severe schizoaffective disorder (Bhati, 2013). Study setting such as institutionalized, incarcerated, homeless subjects may also contribute to variation (Simeone et al., 2015).

The annual incidence of schizophrenia was found to range from 16-40 per 100,000 a year using the broad criteria and 7-14 per 100,000 a year using narrow criteria for diagnosis of schizophrenia (Tandon et al., 2008). Recently, a meta-analysis reported that schizophrenia incidence had increased from 0.71/1000 in the year 2011 to 0.98/1000 in the year 2015 and reported the annual average rate of 0.79/1000 (Wu et al., 2018). According to one study, males have a greater incidence of schizophrenia than females, with a median ratio of 1.4 for males to females (McGrath et al., 2008). However, migrants were found to have a greater rate of schizophrenia than native-born people with the native-born/migrant median ratio of 4.6 (1.0 to 12.8) (McGrath et al., 2004). Similarly, the incidence rate in urban settings was significantly higher in comparison to mixed urban-rural, and the estimated median was 19 vs. 13.3 per 100,000, respectively (McGrath et al., 2008).

Prevalence quantifies the proportion of individuals in a population who have a disease during a specific time. The prevalence is categorized into three groups; point prevalence, period prevalence and lifetime prevalence. The point prevalence is the proportion of individuals who are manifesting a disease at a given time (e.g., one day or one week). The point prevalence of schizophrenia was found to range from 1.4 to 4.6 per 1000 people (Jablensky, 2000). The meta-analysis estimated the median point prevalence of schizophrenia to be 4.6 per 1,000 individuals (Saha et al., 2005). Recently, the median point of the prevalence of schizophrenia was found to be 3.89 per 1000 individuals (Moreno-Küstner et al., 2018). Periodic prevalence is described

as the proportion of the individuals who manifest the disorder during a specific time (e.g., one year). In the case of periodic prevalence, there is a disparity in the study between Saha et al. (2005) and Moreno-Küstner et al. (2018) in which Moreno-Küstner et al. (2018) reported a prevalence of 4.03 per 1,000 persons which is higher than Saha et al. (2005) who reported 3.3 per 1,000 person's prevalence. Lifetime prevalence is the proportion of the individuals who ever manifested a disorder, alive on a given day in the population. The lifetime prevalence reported by Moreno-Küstner et al. (2018) is 7.49 per 1,000 while Saha et al. (2005) reported a lifetime prevalence of 4.0 per 1,000 individuals. Prevalence of schizophrenia in migrant and native-born individuals suggested a higher prevalence among the migrants as compared to native-born with a median value of 1.8. Similarly, the prevalence of schizophrenia in developed countries was found to be higher than in developing countries in which the estimated median was 3.3 and 2.6 per 1,000 respectively (McGrath et al., 2004). The latitude-wise prevalence of schizophrenia was found to be increased in higher latitudes as compared to middle and low latitudes (McGrath et al., 2004).

Schizophrenia causes a global burden across human societies and cultures, and it may affect anyone regardless of sex, age, race and location. Understanding the epidemiology of schizophrenia could aid in identifying risk factors and predicting the risk associated with the etiology of the disease. The complete insight into the epidemiology of schizophrenia requires more intensive descriptive studies.

1.3. Eitology of schizophrenia

Despite decades of research, the etiology of schizophrenia is poorly understood. At present, the consensus is that genetic, as well as environmental factors, are equally

important in the genesis of schizophrenia. Recently, immune system dysfunction has also been associated with schizophrenia. Besides, various risk factors are proposed which may contribute to the development of the disorder.

1.3.1. Biological risk factors of schizophrenia

1.3.1.1. Genes and schizophrenia

A series of studies over the past decades have put forward the evidence that genetic factors may play an important role in the causation of schizophrenia. It has been suggested that the heredity of schizophrenia can be as high as 80% (Hosak, 2013). The family-based studies reported that the first-degree relatives of schizophrenia patients had a 10% risk for schizophrenia (Gottesman et al., 1987). Another study found that the risk of schizophrenia in offspring is 27.3% if both the parents have schizophrenia (Gottesman et al., 1991). It has been reported that the concordance rate of schizophrenia is about 41-56% for monozygotic twins and 28% for dizygotic twins (Cardno and Gottesman, 2000). These observational studies indicate the involvement of genetic factors in the development of schizophrenia. However, the underlying mechanisms of genetic transmission of schizophrenia are still uncertain. Linkage and association genetic studies have identified several putative susceptible genes such as human leukocyte antigen (HLA), catechol-O-methyltransferase (COMT), disrupted-in-schizophrenia 1 (DISC1), neuregulin 1 (NRG1) and dystrobrevin-binding protein 1 (DTNBP1) for schizophrenia (Sun et al 2002; Tosato et al., 2005; Singh et al., 2011; Li et al., 2012; Bakanidze et al., 2016). Recently, genome-wide association (GWAS) studies identified several susceptible genes for schizophrenia such as methylenetetrahydrofolate reductase (MTHFR), Latrophilin 2 (LPHN2), lactotransferrin (LFT), Ral GEF With PH Domain And SH3 Binding Motif 1

(RALGPS1) and transmembrane protein 74 (TMEM74) genes (Guo et al., 2021; Gennarelli et al., 2021). The GWAS approach has been effective in identifying genes associated with schizophrenia which is speculated to have multiple genes involved in its etiology. However, underlying etiopathological mechanisms for the associations of genes in schizophrenia are largely unknown to date. Therefore, studies have proposed that the development of schizophrenia could be driven by complex interactions between genes, neurotransmitters, neurodevelopment process, immune system as well as multiple biopsychosocial factors.

1.3.1.2. Neurological development and schizophrenia

The evidence for the abnormal neurological development as the risk for schizophrenia have come from the birth cohort studies where an impaired neuromotor and neurocognitive function in childhood was found to be associated with schizophrenia in later life (Rosso et al., 2000; Reichenberg et al., 2002). Studies have been observed that schizophrenia patients have abnormal craniofacial areas that suggest a uterine developmental disruption (Rakic and Swaab, 1988; O'connell et al., 1997). Furthermore, studies found that schizophrenia patient's brains are approximately 4% smaller and 5-8% lighter than healthy controls (O'connell et al., 1997). Recently, magnetic resonance imaging (MRI) studies provided further evidence of morphological alteration in the brain of schizophrenia patients in which decreased hippocampal and thalamic volumes and enlarged ventricles along with smaller total brain volume were observed (Olabi et al., 2011; Haukvik et al., 2013; Patrik and Filip, 2021). However, the MRI studies on schizophrenia brain are not yet conclusive with some of the studies reporting abnormalities in the brain whereas others reporting no observed abnormalities in the brain (Portas et al., 1998; Hazlett et al., 1999; Shenton

et al., 2014; Jiang et al., 2020). Besides, the MRI studies have not been able to throw light on the underlying pathophysiological mechanism of schizophrenia.

1.3.1.3. Neurotransmitters and schizophrenia

The postmortem studies of central nervous system tissue have highlighted the role of neurotransmitter receptors in the pathology of schizophrenia (Dean, 2002). Serotonin, dopamine, glutamate and gamma-aminobutyric acid are common neurotransmitters reported to be associated with schizophrenia. Grace et al. (2012) reported that the over activation of the dopamine system in the hippocampus in patients with schizophrenia (Grace et al., 2012). Further, another study reported that altered 5-HTT expression in certain brain regions in patients with schizophrenia (Hernandez and Sokolov, 1997). A postmortem brain study among schizophrenia patients reported that low levels of glutamate receptors in patients with schizophrenia (Konradi and Heckers, 2003). Further, postmortem studies have reported that GABAergic abnormalities in patients with schizophrenia (Benes, 2010; Nakazawa et al., 2012). Even though evidence from neurotransmitter studies supports the role of neurotransmitters in the etiology of schizophrenia, but it is not clear how these neurotransmitter abnormalities cause the manifestation of symptoms for schizophrenia.

1.3.1.4. Autoimmune disease and schizophrenia

Epidemiological studies provide evidence of an association between autoimmunity and schizophrenia (Jeppesen et al., 2019). According to a Danish study, 6% schizophrenia patients have an increased risk of autoimmune disorders (Benros et al., 2014). Further, another study from Taiwan reported that 3.4% of individuals with

autoimmune diseases also had schizophrenia (Chen et al., 2012). A Danish study based on the national patient register reported that the presence of autoimmune disease increases the risk for schizophrenia by 45% (Eaton et al., 2006). The relationship between autoimmune diseases and schizophrenia got further support when Wang et al. (2018) observed the association between schizophrenia and autoimmune disorders viz., systemic lupus erythematosus, rheumatoid arthritis and autoimmune vasculitis (Wang et al., 2018). Although most studies have reported the connection between schizophrenia and autoimmune diseases, it is still not clear whether autoimmune diseases cause schizophrenia or vice versa.

1.3.1.5. Prenatal age

Evidence suggests that prenatal age is a risk factor for schizophrenia. A study from Israel–investigated the risk of schizophrenia in advancing age of parents in a population-based birth cohort. The results revealed that in comparison to men aged less than 25, the relative risk for developing schizophrenia increases by 2.02 to 2.96 in offspring of the men whose age is 45-49 or more, however, advanced maternal age was not found to be associated with schizophrenia (Malaspina et al., 2001). A population-based cohort study reported that patients with schizophrenia without a family history show a significant advanced paternal age-related increase risk of schizophrenia (Sipos et al., 2004). Additionally, Frans et al. (2011) conducted a three-generation study and showed that there is an increase in association between grand paternal age and the risk of schizophrenia in offspring. Although, the actual mechanism behind the association between advanced paternal age and schizophrenia is poorly understood. However, it has been postulated that the association between parental advanced age and schizophrenia may be due to de novo mutation occurring

in the male spermatogonia. Every 16 days spermatogonia of man undergo cell division which results in approximately 200 divisions by age 20 years and 660 divisions by age 40 years (Drake et al., 1998). The replication of the genome introduces the possibility of copy error mutations at each time of cell division, which might result in point mutations or larger copy number variants (e.g., deletions or amplification). A significant number of the study reported that the sperm of the older man had more mutations (Crow, 2000; Bosch et al., 2003; Glaser et al., 2003).

1.3.2. Environmental risk factors of schizophrenia

1.3.2.1. Season of birth

In the year 1929, the first study was conducted by Tramer (1929) on the seasonality of birth in schizophrenia. He observed excess of winter birth (December-March) among the 2100 schizophrenia cases (Tramer, 1929). Subsequently, several studies reported the individuals with schizophrenia are more expected to be born in winter/spring (Torrey et al., 1997; Davies et al., 2003; Cheng et al., 2013). However, most of the studies investigating seasonality in schizophrenia have been conducted in the Northern and Southern hemispheres. Nonetheless, studies from the equatorial region studies reported no seasonal effects (Parker et al., 2000). Worldwide schizophrenia patients have been observed to have a 5-8% excess of winter-spring birth compared to the general population (Torrey et al., 1997). The explanation put forward for the seasonality of birth in schizophrenia is that during the second trimester of pregnancy if the foetus is exposed to flu season, the infections during that period may raise the risk of schizophrenia in the offspring (Messias et al., 2007). Moreover, the studies have also suggested that genetic vulnerability may increase the risk of seasonal effects in schizophrenia (Carrion-Baralt et al., 2004).

1.3.2.2. Pregnancy and birth complication

Complication during pregnancy and child delivery is found to be associated with susceptibility for schizophrenia (Sacker et al., 1996; Bennedsen et al., 2001). Preeclampsia, malformation and vacuum extraction are the main birth complication which is found to be associated with the development of schizophrenia. Most of the studies report that schizophrenia patients are more likely to have experienced hypoxia during the time of birth (Geddes et al., 1995; Zornberg et al., 2000; Dalman et al., 2001). Many studies have reported that women who suffer from preeclampsia during pregnancy have a greater chance of developing schizophrenia in their offspring later in life (Kendel, 1996). Further, it was estimated that the relative risk of schizophrenia is about 2.5 among the persons exposed to preeclampsia (Dalman et al., 1999). Studies have observed an association of low birth weight with schizophrenia. In addition, it has been suggested fetal hypoxia causes neurotoxic effects and early onset of schizophrenia due to premature cortical synaptic pruning (Rosso et al., 2000). Besides, an association between fetal hypoxia, ventricular enlargement and the reduced grey matter was found in schizophrenia (Cannon et al., 2002).

1.3.2.3. Prenatal exposure to infection

The seasonality of birth in schizophrenia further stimulated researchers to propose that prenatal infection during a specific season may play a very important role in the etiology of schizophrenia. A study conducted using archived maternal serum biomarkers drawn during pregnancy reported that prenatal infection increases the risk of schizophrenia in later life (Susser et al., 2000). Many other studies reported that infection during the period of pregnancy by various infectious agents such as Influenza (Mednick et al., 1988; Brown et al., 2004), Cytomegalovirus (Torrey et al.,

2006; Kim et al., 2007), and *Toxoplasma gondii* (Mortensen et al., 2007; Torrey and Yolken, 2007; Niebuhr et al., 2008), may lead to schizophrenia. These infectious agents act as teratogens to the fetal brain resulting in the disruption in the programmed maturation of various parts of the brain which in turn may increase the vulnerability to schizophrenia during late adolescence and early adulthood (Brown and Susser, 1996; Brown, 1999). The evidence for the prenatal infection in schizophrenia is supported by the finding by Bogerts et al. (1990) who demonstrated abnormalities of the brain such as enlarged cerebral ventricles and diminished hippocampal volume in the patient with schizophrenia during their first episode of schizophrenia.

1.3.2.4. Substance abuse

Mounting evidence suggests that continuous use of cannabis may lead to the genesis of psychotic illness including schizophrenia (Van et al., 2002; D'Souza, 2007; Minozzi et al., 2010). Besides, it is observed that psychotic patients use more cannabis than the general population. A study reported that individuals who have ever used cannabis had a two-fold increased risk of developing schizophrenia (Zammit et al., 2002). Various studies reported the dose-effect relationship between schizophrenia risk and cannabis use (Manrique-Garcia et al., 2012; Monteleone et al., 2014). It has been observed that a substantial amount of consumption of cannabis increases the likelihood of the development of schizophrenia by six times (Murray et al., 2016). This study got further strength when another study reported that cannabis abused by 18 years of age increase the risk of later developing schizophrenia symptoms (Zammit et al., 2002). The use of cannabis can induce the manifestation of psychosis up to 2.7 years earlier than those who developed the psychosis without a history of usage of

cannabis (Donoghue et al., 2014). It is well established that the use of cannabis is associated with a high risk of schizophrenia. However, all the individuals who are cannabis users do not develop psychosis, which suggests the association between schizophrenia and cannabis is due to the involvement of genetic factors (Henquet et al., 2005; Power et al., 2014; Giordano et al., 2015).

1.3.2.5. Urbanicity

Urban upbringing and urban residence have been suggested to be risk factors for schizophrenia and have been discussed for more than a decade. However, the number of possible factors such as age during urban upbringing, the dose-response relationship might enhance the association between schizophrenia and urbanicity. Danish population-based cohort study reported the risk of schizophrenia to be proportional to the degree of urbanization and larger town (Montensen et al., 1999; Predersen et al., 2001). Besides, the evidence suggests that the risk of schizophrenia may increase when the residence in childhood changes from rural area to urban area (Predersen et al., 2001).

1.3.2.6. Migration

Migration is suggested to be one of the risk factors for schizophrenia. A higher occurrence of schizophrenia has been observed in immigrants with 2.9 more likely to develop schizophrenia compared to the native-born population (Cantor-Graae, 2005). Further, meta-analysis studies show that the immigrants and their descendants are 2.5 times more likely to develop psychiatric disorder (Rai et al., 2013).

1.4. Immune system and schizophrenia

The immune system is a complex organization of various cells and mediators which protects the organisms from a variety of invading life-threatening foreign agents. The immune system is divided into two components i.e., the innate immune system (first line of immune surveillance) and the adaptive immune system (second line of immune surveillance). Further, each arm of the immune system is subsequently divided into cellular and humoral components. The innate immune system is a non-specific first line of defense consisting of effector cells viz. monocytes/macrophages, granulocytes and natural killer (NK) cells, etc. The humoral component of the innate immune systems encompasses the acute-phase proteins and the complement system. The adaptive immune system is more sophisticated and works on the ability to memorize the antigens. The cellular components of the adaptive immune system are B-lymphocytes, T lymphocytes, macrophages, neutrophils, etc. The humoral component of the immune system consists of antibodies produced by B-lymphocytes. The T lymphocytes are divided into the T helper-1 (Th-1) and the T helper-2 (Th-2) based on the synthesis of cytokines. The cellular components of the adaptive immune systems are activated by T helper–1 (Th-1) cells that produce the pro-inflammatory cytokines such as IL-2, IFN- γ, TNF-α, etc. While the humoral components are activated by T helper 2 (Th-2) cells and support the synthesis of antibody-mediated immune responses as well as the production of anti-inflammatory cytokines.

1.4.1. Cytokine

Cytokines are low molecular weight polypeptides with a molecular mass of 6-70 kDa. It is indispensable for communications between the cells of the immune system (Kumar and Clark, 2012). Cytokines are named in a varied way as per their synthesis

and functions. The cytokines synthesized by monocytes are called monokine, those cytokines synthesized by lymphocytes are referred to as lymphokine, cytokines having chemotactic activities are called chemokine, and the cytokines synthesized by one leukocyte and acting on other leukocytes are called interleukin. The cytokines may act in autocrine (acting on same cells that secrete cytokine), paracrine (acting on nearby cells) and endocrine pattern (acting on distant cells). Cytokines are pleiotropy in nature i.e., a single cytokine act on several different cell types producing a different response. Besides, cytokines can be redundant too i.e., many cytokines exert the same function. Cytokines control the responses of the innate immune system and the adaptive system. Based on the functions, the cytokine has been divided into pro-inflammatory cytokine and anti-inflammatory cytokines. The cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interferon-γ (IFN-γ), interleukin-6 (IL-6) etc. are pro-inflammatory cytokines which augment the inflammation by promoting leukocyte recruitment to inflammatory sites and/or activating inflammatory cells. On the other hand, interleukin-1 receptor antagonist (IL-1ra), interleukin-4 (IL-4) and interleukin-10 (IL-10) etc. are considered to be anti-inflammatory cytokines that counteract the hyperalgesic effects of the pro-inflammatory response.

1.4.2. Inflammation

Inflammation is a defense response of the immune system in response to infection or injury. However, persistent activation of inflammatory processes is the hallmark of the dysregulation of the immune system and can have a negative effect on an individual. Inflammation is of two types, acute and chronic inflammation. Acute inflammation is a nonspecific response lasting for few days, characterized by redness, swelling, heat, pain, and loss of tissue function, which results from local immune,

vascular and inflammatory cell responses to infection or injury (Takeuchi et al., 2010). During acute inflammation, leukocytes migrate to the area of injury, the blood supply to the area increases, and blood vessels become more permeable, allowing cells and molecules to enter the injured tissue. However, acute inflammation may turn into chronic, contributing to a variety of chronic inflammatory diseases (Zhou et al., 2016). Chronic inflammation is usually a lower grade response lasting for a prolonged period of several months to a year. Chronic inflammation is systemic inflammation rather than localized inflammation.

1.4.3. Cytokine, inflammation and schizophrenia

Accumulating evidence suggests that inflammatory processes may play an important role in schizophrenia which is evident by the presence of the imbalance between pro-inflammatory (Goldsmith et al., 2016; Frydecka, et al., 2018) and anti-inflammatory cytokines (Drexhage et al., 2011). Several studies have reported a reduced level of Th-1 cytokine such as interleukin-2 among schizophrenia patients (Kim et al., 1998), while Th-2 cytokines such as interleukin-10 were observed to be high in schizophrenia patients (Kim et al., 2002). Thus, Schwarz et al. (2001) suggested a shift of immune response toward Th-2 in schizophrenia. Contrastingly, studies have reported an increased level of pro-inflammatory cytokines viz. IFN-γ, IL-1β, IL-6 and IL-12, TNF-α in schizophrenia patients (Kozora, 2005; Miller et al., 2011). Thus, there is a lack of agreement in the Th-2 immune response shift in schizophrenia (Potvin et al., 2008). To date, the evidence put forward for the altered level of inflammatory cytokine in schizophrenia is not convincing due to a lack of consensus studies. However, it can be mentioned that inflammatory processes could indicate a vulnerability factor in some cases of schizophrenia.

The term ‘mild localized chronic encephalitis’ was proposed to describe the inflammatory processes in schizophrenia (Bechter et al., 2001). Activation of the inflammatory arm of the immune system and increased levels of the inflammatory marker has been found in many individuals with schizophrenia leading to the concept that schizophrenia is associated with a low degree of inflammation. Immune activation in the periphery can influence the shape and function of the central nervous system (CNS) and is associated with an increased risk of developing neuropsychiatric and neurodegenerative diseases (Dantzer et al., 2008; Czirr and Wyss-Coray, 2012). Further, evidence suggests that peripheral inflammation may increase the permeability of the blood-brain barrier and allow the recruitment of peripheral leukocytes to the central nervous system, thus regulating CNS immune surveillance (Engelhardt and Sorokin, 2009; Khandaker et al., 2015; Peralta-Ramos et al., 2019). Microglia and astrocytes are the major immunocompetent cells that control both the induction as well as limitation of inflammatory processes in the brain (Aravena et al., 2011). IL-6 is a major cytokine involved in microglial activities which can exert a neurotoxic or neuroprotective effect (Eskes et al., 2002; Krady et al., 2008). Aravena et al. (2011) observed that overexpression of IL-6 inhibits hippocampal neurogenesis in the CNS. Increased levels of TNF-α were also found to inhibit hippocampal neurogenesis (Monje et al., 2003; Cacci et al., 2005). Further, the expression of IL-1β in the CNS inhibits the formation of the hippocampus-mediated memory in an inverted U-shaped pattern (Goshen et al., 2007).

Evidence also suggests that neuroinflammation of the CNS involves the kynurenine pathway of tryptophan metabolism. In support of this Erhardt and Engberg (2002), reported that the increased levels of the kynurenic acid stimulate hyperactivity of the mesocorticolimbic dopamine system in schizophrenia patients (Erhardt and Engberg,

2002). Inhibition of COX-2 by celecoxib decreases levels of kynurenic acid, while inhibition of COX-1 increases levels of kynurenic acid (Muller and Schwarz, 2010; Schwieler et al., 2015). Inflammatory cytokines such as IL-1β and tumor necrosis factor TNF-α have been shown to enhance the expression of COX-2 mRNA (Muller and Schwarz, 2010; Sommer et al., 2012). Further, it has been reported that IL-6 can activate the COX-2 signaling pathway (Akarasereenont et al., 1999; Chun and Surh, 2004). These evidences suggest the role of inflammatory processes in the development and progression of schizophrenia.

1.5. NEED FOR THE PRESENT STUDY

Traditionally the research on schizophrenia has focused mostly on neurodevelopmental and neurotransmitter systems. Recently, researchers have focused their attention to understand the role of inflammatory processes in schizophrenia. Thus, various studies have been carried out using the biological markers to understand the role of inflammatory processes in schizophrenia (Dickerson et al., 2007; Miller et al., 2011; Müller et al., 2015). However, there is a lack of consensus among the studies (Na et al., 2007; Potvin et al., 2008; Reale et al., 2011; Zhu et al., 2018). In addition, the administration of anti-inflammatory drugs has also been found to improve the psychotic symptoms in patients with schizophrenia (Sommer et al., 2014; Cho et al., 2019). Hence, there is a need for further studies to throw light on the role of inflammation in the pathophysiology of schizophrenia. Even though some of the studies have been conducted by the Indian researchers to understand the role of inflammatory process in schizophrenia, still this domain of the study has been insufficiently explored. Therefore, the present study was conducted among the Indian Bengalee patients to understand the role of the inflammatory

process in schizophrenia using a common inflammatory marker C-reactive protein (CRP), interleukin-6 (a modulating cytokine of CRP), and a very important component of the innate immune system toll-like receptor (TLR). The study may shed the light on the role of the inflammatory process in Indian schizophrenia patients which may provide valuable input in future treatment strategies.

1.6. OBJECTIVES

i. To investigate the prevalence of schizophrenia among the population of North Bengal on the basis of hospital study.

ii. To study the serum level of CRP in schizophrenic patients and compare it with that of the controls.

iii. To evaluate whether selected polymorphism of TLR-2 (rs5743708, rs211917864), TLR-3 (rs3775296, rs3775290), TLR-4 (rs4986790, rs4986791), TLR-7 (rs179009), TLR-8 (rs3764880), TLR-9 (rs352140, rs5743836) influence the incidence and clinical picture of schizophrenia among the Indian schizophrenia patients.

iv. To investigate the role of specific haplotype of TLR in susceptibility to schizophrenia in Indian population.

v. To study the association of functionally-relevant TLR genetic variations and reported demographic characteristics of the patients.

CHAPTER-02

Literature and Review

2. LITERATURE REVIEW

The present investigation had three components viz. the study of prevalence of schizophrenia among the population of North Bengal, the study of CRP (a state marker for inflammation) and its modulating cytokine IL-6 among the schizophrenia patients, and genetic polymorphism study of selected TLR genes among the schizophrenia patients. Hence, in this section, the past ten years of literature have been reviewed under separate headings.

2.1. Prevalence of schizophrenia

A prevalence study measures the proportion of individuals in a population who have a disease at any given time. The prevalence is categorized into three types; point prevalence, period prevalence and lifetime prevalence. The point prevalence is defined as a proportion of individuals who are manifesting a disease at a specific time (e.g., a day or a week). Period prevalence (12-months) is defined as the proportion of a population who are manifesting a disease between 1 month and 12 months. The percentage of people in a population who have ever manifested a disorder at any point in their lives is known as lifetime prevalence. Schizophrenia is one of the top 15 causes of disability in the world (Vos et al., 2016). However, as per the literature, the studies on the prevalence of schizophrenia are very limited. Prevalence studies may provide valuable information about the disease burden in society. Therefore, it is essential to measure the proportion of the disease to understand the burden of schizophrenia.

2.1.1. Studies on the prevalence of schizophrenia: International status

The studies on the prevalence of schizophrenia are summarized in table 1. It has been reported that the prevalence of schizophrenia may vary by the range of 2-3/1,000 population all over the world (Moreno-Küstner et al., 2018). However, there is variation in the prevalence rate as reported by various studies. In a study from Canada, the 12-months prevalence of schizophrenia was reported to be 1.2 per 1,000 population (Nimgaonkar et al., 2000). In another study from Canada, an investigation was conducted to estimate the 12-months prevalence for three conjugative years and the prevalence rate was observed as 0.45 cases per 100 population for the 1996–1997 year, 0.45 cases per 100 population for 1997–1998 and 0.42 cases per 100 population for 1998–1999 (Goldner et al., 2003). Further, a study from Netherland reported the point prevalence rate of 2.1 per 1,000 population for schizophrenia (Schrier et al., 2001). On the other hand, in studies among Asian countries such as China the lifetime prevalence rate was observed to be 4.13 per 1,000 population, and males were found to have an earlier mean age of onset than females (Ran et al., 2003). In agreement with these results, a study from Ethiopia also observed a similar lifetime prevalence rate (4.7 per 1,000 population) for schizophrenia, but contrary to the study from China, females had an earlier mean age of onset than males (Kebede et al., 2003). In yet another Chinese study, a higher point prevalence (55%; relative risk-1.77) of schizophrenia was observed among the women belonging to urban areas (Phillips et al., 2004). Contrastingly, a study from Bangladesh reported a higher 12-month prevalence (57%) of schizophrenia among men than women (Mahmud et al., 2015). In a population survey study, the point prevalence of schizophrenia was reported to be 4.2 per 1,000 population among the Indonesian population (Kurihara et al., 2005). A study from Scotland reported the point prevalence rate of 3.59 per1000 population (Shivashankar et al., 2013). In a study among

the USA population, Wu et al. (2006) observed a prevalence of 5.1 per 1000 population (Wu et al., 2006). A comparative study was conducted among the population of Finland by Peraela et al. (2007) in which the highest rate of lifetime prevalence was observed for schizophrenia (0.87%), followed by schizoaffective disorder (0.32%), schizophreniform disorder (0.07%), delusional disorder (0.18%), bipolar I disorder (0.24%) and major depressive disorder (0.35%) (Peraela et al., 2007). In a study from Sweden 12-months prevalence rate of schizophrenia was found to be 0.37% (Fors et al., 2007). Another Swedish study based on a 12-months investigation reported a prevalence rate of 3.7 per 1,000 population for schizophrenia (Jorgensen et al., 2013). In a similar approach of study, the prevalence was found to be 0.97% for schizophrenia among the Australian population (Kake et al., 2008). In a cohort of Chinese schizophrenia patients with a history of suicide attempts, the lifetime prevalence of schizophrenia was found to be 0.49% and 9.7% (Xiang et al., 2008). In the same study, urban residency, lower monthly income, unmarried status and family history of schizophrenia were found to be associated with an increased risk of schizophrenia among the Chinese population (Xiang et al., 2008). Recently, a study observed a 23% lifetime prevalence of schizophrenia among the population from Singapore (Subramaniam et al., 2021).

Table 1. Studies on prevalence of schizophrenia

Name	Year	Country	Study	Prevalence type	Sample size	Major findings
Nimgaonkar et al.,	2000	Canada	Hospital based	12-month	8542	Annual prevalence of schizophrenia was 1.2 per 1,000 individuals.
Schrier et al.,	2001	Netherland	Hospital based	Point	337362	The point prevalence rate of schizophrenia was 2.1 per 1000 (males 2.6 per 1000; females 1.6 per 1000).
Ran et al.,	2003	China	General population	Lifetime	89512	The prevalence rate of schizophrenia was 4.13 per 1000 population. Males had an earlier mean age of onset (29.6 years) than females (32.3 years).
Goldner et al.,	2003	Canada	Hospital based	12-month	11516	One-year prevalence rate for schizophrenia was significantly correlated with low income.
Kebede et al.,	2003	Ethiopia	General population	Lifetime	68378	Estimated lifetime prevalence of schizophrenia was 4.7 per 1,000 population. Females had an earlier mean age of onset (21.0 years) than males (23.8 years).
Phillips et al.,	2004	China	General population	Point	19223	Prevalence of schizophrenia was higher in women than men and most of the patients belong to urban than rural areas.
Kurihara et al.,	2005	Indonesia	General population	Point	8546	The point prevalence of schizophrenia was 4.2 per 1000 population.
Wu et al.,	2006	USA	Hospital based	12-month	6800000	The 12-month prevalence of schizophrenia was estimated at 5.1 per 1000 population.
Fors et al.,	2007	Sweden	Hospital based	12-month	68041	The prevalence of schizophrenia was 0.37% and the mortality rate schizophrenia patients was higher than normal individuals.
Peraela et al.,	2007	Finland	General population	Lifetime	8028	Lifetime prevalence was 0.87% for schizophrenia, 0.32% for schizoaffective disorder, 0.07% for schizophreniform disorder, 0.18% for delusional disorder, 0.24% for bipolar I disorder, 0.35% for major depressive disorder.
KaKe et al.,	2008	Australia	Hospital based	12-month	3736269	The estimated 12-month prevalence of schizophrenia was 0.97%.

Table 1. Continue

Author	Year	Country	Study	Prevalence type	Sample size	Major findings
Xiang et al.,	2008	China	General population	Lifetime	5926	The lifetime prevalence of schizophrenia was 0.49% and 9.7% of the subjects with lifetime schizophrenia reported a history of suicide attempts.
Jorgensen et al.,	2013	Sweden	Hospital based	12-month	946381	The 12-month prevalence of schizophrenia was 3.7 per 1000 population.
Shivashankar et al.,	2013	Scotland	General population	Point	205	Point prevalence was found to be 3.59 per 1000 general population.
Mahmud et al.,	2015	Bangladesh	Hospital based	12-month	83	Higher prevalence of schizophrenia among the male than the female.
Subramaniam et al.,	2021	Singapore	General population	Lifetime	6,126	Lifetime prevalence of rate was found to be 23%.

2.1.2. Studies on prevalence of schizophrenia: National status

It has been reported that the burden of psychiatric disorders has double in India since 1990 and after the depressive and anxiety disorders, schizophrenia is the highest disease burden in India among the mental disorders (Sagar et al., 2020). However, the available literature regarding the Indian studies on the prevalence of schizophrenia is meagre (Table 2). A study was conducted in the urban area of Ahmedabad among 461 families consisting of 2712 individuals to understand the prevalence of schizophrenia. The results showed a prevalence of 1.5 per 1,000 individuals (Shah et al., 1980). Further, a survey was conducted among 1,00,000 individuals from Madras and reported the prevalence rate of 2.49 per 1,000 population for schizophrenia (Padmavathi et al., 1987). In a retrospective investigation of fifteen epidemiological studies, the prevalence of schizophrenia was reported to be 2.5 per 1,000 individuals (Ganguli et al., 2000). Prevalence studies demonstrate the disease burden in society, and understanding the burden of disease in populations could help to identify the risk factors as well as predict the risk related to schizophrenia.

Table 2. Indian studies on prevalence of schizophrenia.

Author	Year	Country	Study	Prevalence type	Population	Major findings
Shah et al.,	1980	India	General population	Not mentioned	128	Prevalence rate of schizophrenia was 1.5/1000 population
Padmavathi et al.,	1987	India	Attended	Not mentioned	252	Prevalence rate of schizophrenia was 2.49/1000 population
Ganguli et al.,	2000	India	Retrospective	Pooled	--	Prevalence of schizophrenia was 2.5 /1000 population

2.2. C-reactive protein: Genetics, synthesis, structure and functions

C-reactive protein (CRP) is a well-established biomarker of systemic inflammation which increases in response to infectious and non-infectious exposures. CRP was

discovered by Tillett and Francis in 1930. The name was given as a CRP because of its capacity to precipitate the somatic C- (capsular) polysaccharide of pneumococcus. CRP was first identified in the serum of patients with acute inflammation and it was thought to be a product of pathogenic secretion (Tillett and Francis, 1930). Later it was established that the level of CRP may increase in a variety of illnesses including cancer (Pepys and Hirschfield, 2003). Nevertheless, now it has been well established that CRP level increases in a variety of infections and inflammatory conditions such as myocarditis and rheumatic fever (Kaneko et al., 2000; Gölbasi et al., 2002; Dhingra et al., 2007). In addition, an elevated level of CRP in the blood is considered to be the risk factor for cardiovascular diseases, diabetes and other metabolic dysfunction (Bassuk et al., 2004; Pfutzner and Forst, 2006).

CRP is synthesized by the hepatocyte cells of the liver in response to the cytokines namely IL-6, IL-1, and TNF-α (Matthew et al., 2007; Yap et al., 1991; Zhang et al., 1996). The gene encoding for CRP is located in chromosome 1q21–23 region (Walsh et al., 1996). It is composed of 2301 bases with two exons and 280 base pair introns (Lei et al., 1985, Woo et al., 1985). CRP is a non-glycosylated, 224-residue circulating protein composed of five identical subunits with a monomer molar mass of 25,039 Da (Figure 1) (Pepys et al., 1978; Volanakis and Wirtz, 1979; Baltz et al., 1982; De-Beer et al., 1982; Kushner et al., 2006). It is arranged in pentameric symmetry and has a characteristic calcium-dependent binding to specific ligands, including binding to low-density lipoprotein cholesterol (Volanakis and Kaplan, 1971; Pepys et al., 1978; Volanakis and Wirtz, 1979; Baltz et al., 1982; De Beer et al., 1982; Pepys et al., 1985). It has been observed that the specific genetic polymorphisms in the CRP gene are associated with higher basal CRP levels (Kluft and de Maat, 2003). Moreover, the levels of CRP vary with age, gender and ethnicity (Ford et al., 2003; Rifai and Ridker, 2003; Imhof et al.,

2003; Woloshin and Schwartz, 2005). Further, slight elevation of CRP was observed to be found in people with low socio-economic status which may be due to the high prevalence of infection and diseases among them (Mackenbach and Howden-Chapman, 2003). Further, it was observed that the food habits and poor dietary supply of nutrients also modulate the CRP level (Kushner et al., 2006).

During acute inflammation, the concentration of CRP abruptly increases to 1000-fold in blood within 24-28 hours (Du Clos and Mold, 2004). Later the concentration quickly returns to basal values as the half-life of CRP is about 19 hours (Pepys and Hirschfield, 2003). During inflammation, the monomeric CRP is synthesized and is assembled into a pentamer which binds to the endoplasmic reticulum (ER) with the help of two carboxylesterases i.e., gp60a and gp50b (Macintyre et al., 1994). Later, CRP slowly dissociates from ER during the resting (non-inflammatory) state. Studies suggest that monomeric and pentameric CRP have distinct functions. Pentameric CRP possesses both pro-inflammatory and anti-inflammatory properties in a context-dependent manner whereas, monomeric CRP exerts potent pro-inflammatory actions on endothelial cells, endothelial progenitor cells, leukocytes, platelets and may amplify the inflammatory response (Wu et al., 2015).

A study by Kingsley and Jones, (2008) showed that CRP levels can be used as a marker for infection, rather than distinguishing different types of infection. A recent study suggested that CRP is not only a marker of infection and inflammation but it also has a protective role against bacterial infections, principally through the activation of complement and subsequent opsonization of pathogens (Sproston et al., 2018). A study hypothesized that CRP is involved in pattern recognition, host defense, and enhancement of the innate immune response (Volanakis, 1982). This hypothesis is supported by the evidence from both evolutionary and phylogenetic conservation of CRP (Baltz et al.,

1982). Moreover, CRP is also suggested to be associated with inhibition of fibrinolysis, promotion of tissue factor, reduction of endothelial nitric oxide, increase in cellular adhesion, induction of gluconeogenesis and leptin resistance (Pasceri et al., 2001; Venugopal et al., 2002; Devaraj et al., 2003; Bisoendial et al., 2005; Chen et al., 2006; Birjmohun et al., 2007). Besides, CRP is also believed to play an important role in the innate immune system (Du Clos and Mold, 2004). Since there are different conditions in which elevation of CRP may take place, the increase CRP levels do not diagnose a specific disease or infection, rather it can provide us information about the presence of inflammation in patients (Whicher et al., 1985; Masi et al., 2001). In addition, studies have revealed that CRP can be a predictor of mortality (Caixeta et al., 2011; Gradel et al., 2011; Morrow et al., 1998; Seligman et al., 2006; Chundadze et al., 2010). However, according to a meta-analysis, early increases in CRP levels are not a strong predictor of survival in critically ill patients, whereas late increases in CRP levels are a good marker of death risk (Zhang and Ni, 2011). The higher late elevated CRP concentrations in the non-survivors were suggested to be due to the persistent inflammatory load and may predict the poor clinical outcome (Zhang and Ni, 2011).

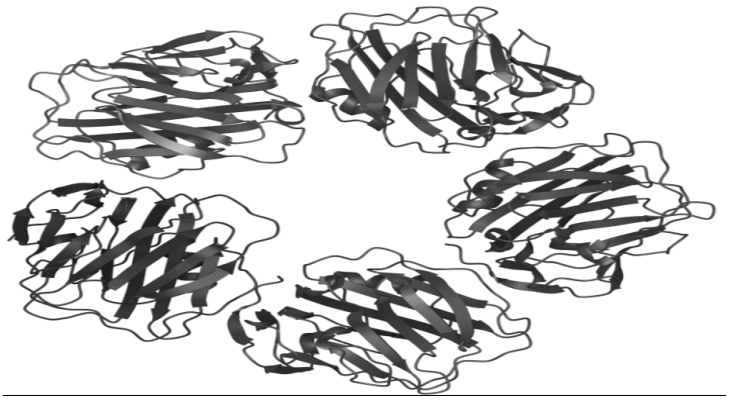

Figure 1. Pentameric structure of C-reactive protein

Retrieved from Protein data bank. (https://www.rcsb.org/structure/1GNH)

2.2.1. Study of C-reactive protein in schizophrenia: International status

C-reactive protein is studied by several researchers to understand the role of inflammatory processes in schizophrenia. In this section retrospective studies of CRP in schizophrenia for the past ten years have been reviewed (Table 3). Most of the studies conducted in the last decade tried to correlate the levels of CRP with a psychiatric parameter or else with the antipsychotic treatment. In one such study, a higher CRP level was observed among the antipsychotic free male Egyptian schizophrenia patients. Further, in the same study, the levels of CRP were found to be correlated with the severity of psychopathology as per PANSS (Fawzi et al., 2011). In another study from Finland, levels of CRP were reported to be significantly high in schizophrenia patients than in the controls. Further, in the same study medication of antipsychotic drugs were found to be significantly associated with elevated levels of CRP (Suvisaari et al., 2011). In accordance with the above studies, further investigations have also observed a significantly high level of CRP in schizophrenia patients (Dickerson et al., 2013; Lin et al., 2013; Frydecka et.al., 2015; Joseph et.al., 2015; Aas et.al., 2017; Ayari et al., 2020). Investigations involving a comparison of CRP levels in deficit and non-deficit schizophrenia patients revealed that deficit schizophrenia patients had higher levels of CRP than non-deficit schizophrenia patients (Garcia-Rizo et al., 2012). Further, studies have observed the association of higher levels of CRP with worse cognitive performance in patients with deficit schizophrenia (Pan et al., 2020). Besides, a study reported that elevated levels of CRP and exposure to Herpes simplex Virus -1 were associated with the severity of cognitive impairment in schizophrenia (Dickerson et al., 2012). It has been observed that the level of CRP in schizophrenia patients is modulated by several factors like physiological condition, substance abuse, waist circumference and diastolic blood pressure (Vuksan-Cusa et al., 2013). The elevated CRP level in schizophrenia

patients was also observed to be correlated with metabolic syndrome and it was suggested that elevated CRP levels are associated with increased risk for the development of metabolic syndrome in schizophrenia patients (Popovic et al., 2015). In addition, high levels of CRP are associated with obesity in a patient with schizophrenia (Klemettila et al., 2014). Studies have also shown the gender specificity in the level of CRP in schizophrenia. Thus, in a study among Czechoslovakian schizophrenia patients, an increased level of CRP was observed only among the female patients (Wyzokinski et al., 2015). In concordance to this study, a cross-sectional study from Spain also observed the sex-specific association of CRP with schizophrenia and reported the association between high levels of CRP and visual learning performance only among the female schizophrenia patients but not with the male patients (Dal-Santo et al., 2020). Another cross-sectional study looking at the link between the quality of life, CRP, and schizophrenia found that physical and psychological well-being, as well as sentimental life, are linked to CRP levels in schizophrenia (Faugere et al., 2015). A study conducted on family matched control to understand the role of CRP in Chinese schizophrenia patients showed that the levels of CRP were significantly higher in the patient than the family matched control (Zhu et al., 2015). In a study, Bulzacka et al. (2016) reported an association of CRP with poor abstract reasoning, general intellectual aptitude, the decline of all components of working memory and a wide range of other impaired cognitive functions. Studies have also reported the association of elevated levels of CRP with aggressive behavior in patients with schizophrenia (Barzilay et al., 2016). Besides, higher levels of CRP were found to be associated with an increase in disease severity in schizophrenia patients (Christiano et al., 2017). Further, higher CRP levels were found to be correlated with positive and negative symptoms, and Brief Psychiatric Rating Scale (BPRS) score in schizophrenia (Boozalis et al., 2018; Bolu et al., 2019 and Zhu et al.,

2019). A recent study also observed an association of CRP with thought and language dysfunction and the score of the Positive and Negative Syndrome Scale (PANSS) in schizophrenia (Chang et al., 2019). Nevertheless, in the longitudinal studies decreased CRP levels have been found to be correlated with improvement in positive symptoms of schizophrenia (Steiner et al., 2020). The abnormal CRP levels have also been found to be associated with antidepressant medicating schizophrenia patients (Fond et al., 2016). Recently, it was reported that abnormal CRP levels were significantly associated with increased rates of non-remission in schizophrenia patients who were under antidepressants (Fond et al., 2020). Contrastingly, studies have also reported no significant association between CRP levels and antidepressant consumption (Faugere et al., 2018). Even though numerous investigations have been undertaken to understand the effect of antipsychotics on the CRP levels, the findings are largely inclusive. Moreover, it is still not understood whether increase CRP levels results in the disease manifestation or else schizophrenia itself causes the elevated levels of CRP.

Table 3. Study of C-reactive protein in schizophrenia

Author	Year	Country	Study sample	Patients	Controls	Major findings
Fawzi et al.,	2011	Eygpt	Serum	200	200	Higher CRP level was found in male Egyptian antipsychotic-free patients, and is positively correlated with the severity of the psychopathology.
Suvisaari et al.,	2011	Finland	Serum	45	45	Patients with schizophrenia have significantly higher CRP levels which were modulated by both antipsychotic medication and nonaffective psychosis.
Garcia-Rizo et al.,	2012	Spanish	Blood	62	--	CRP levels were significantly higher in the deficit patients.
Dickerson et al.,	2012	Maryland	Serum	588	--	Elevated levels of CRP and exposure to herpes simplex virus -1 are found to be associated with severity of cognitive impairment in schizophrenia
Vuksan-Cusa et al.,	2013	Zagreb	Serum	181	--	CRP was correlated with waist circumference and diastolic blood pressure in schizophrenia patients.
Sicras-Mainar et al.,	2013	Spain	Serum	705	--	CRP was associated with cardiovascular disease in patients with schizophrenia.
Dickerson et al.,	2013	Maryland	Blood	295	228	The levels of CRP in the schizophrenia patients were significantly increased compared to controls adjusting for age, gender, race, maternal education, smoking status, and body mass index.
Lin et al.,	2013	Taiwan	Serum	36	36	Significant increase of hsCRP levels in schizophrenia than controls.
Klemettila et al.,	2014	Finland	Serum	190	--	The levels hs-CRP was associated with obesity in patients with schizophrenia.

Table 3. Continue.

Author	Year	Country	Study sample	Patients	Controls	Major findings
Joseph et.al.,	2015	Norwegians	Plasma	88	71	hs-CRP levels were higher in individuals with schizophrenia than controls.
Zhu et al.,	2015	China	Plasma	93	93	Mean levels of CRP were higher in patients compared to controls.
Popovic et al.,	2015	Serbia	Plasma	93	--	Elevated levels of CRP were identified as a predictor of metabolic syndrome independently of diabetes mellitus in family history in schizophrenia patients.
Wyzokinski	2015	Czechoslovakia	Serum	485	--	Increased CRP levels were associated with age and female gender in patients with schizophrenia
Micoulaud-Franchi et al.,	2015	France	Blood	55		Abnormal CRP levels in schizophrenia patients were associated with higher rate of sensory gating deficit.
Faugere et al.,	2015	France	Serum	256	--	Psychological well-being, physical well-being and sentimental life is associated with CRP in schizophrenia
Bulzacka et al.,	2016	France	Plasma	369	--	Abnormal CRP levels in schizophrenia patients were associated with impaired general intellectual ability, abstract reasoning, decline of all components of working memory and a wide range of other impaired cognitive functions.
Barzilay et al.,	2016	Israel	Serum	213	--	Elevated CRP associated with aggressive behavior in schizophrenia patients.
Fond et al.,	2016	France	Serum	219	--	Abnormal CRP levels are associated with schizophrenia patients who were under antidepressant treatment
Aas et al.,	2017	Norway	Plasma	148	212	Schizophrenia patients had increased levels of hs-CRP than the controls
Christiano et al.,	2017	Brazil	Serum	35	--	CRP levels were higher in schizophrenia patients with greater disease severity.

Table 3. Continue.

Author	Year	Country	Study sample	Patients	Controls	Major findings
Boozalis et al.,	2018	Texas	Plasma	39	--	Positive correlation was observed between CRP and PANSS negative symptoms in schizophrenia
Faugere et al.,	2018	France	Serum	307	--	No association between CRP levels and antidepressants consumption
Bolu et.al.,	2019	Turkey	Serum	74	54	Positive correlation between hsCRP levels and severity of positive symptoms with schizophrenia
Zhu et al.,	2019	China	Serum	58	31	Serum hsCRP levels and BPRS scores were significantly higher in schizophrenia patients than controls
Chang et al.,	2019	China	Plasma	60	--	CRP levels were significantly associated with thought and language disorder (TALD) score and positive and negative syndrome scale (PANSS)
Dal-Santo et al.,	2020	Spain	Plasma	132	--	High levels of CRP and visual learning performance was associated only among the female schizophrenia patients.
Pan et al.,	2020	China	Serum	91	--	Higher serum CRP level was associated with lower cognitive performance in the deficit schizophrenia patients
Fond et al.,	2020	France	Serum	272	--	Abnormal CRP levels were significantly strongly associated with increased rates of non-remission under antidepressants in schizophrenia patients
Steiner et al.,	2020	Germany	Serum	253	204	Level of CRP correlated with PANSS-P at baseline in schizophrenia and declining CRP level correlated with improvement of positive symptoms after treatment.
Ayari et al.,	2020	Tunisia	Serum	128	63	hs-CRP levels were significantly higher in schizophrenia patients than controls.

2.2.2. Studies of CRP in schizophrenia: National status

Very few studies have been carried out to understand the role of CRP in schizophrenia among Indian patients (Table 4). In one study cardiovascular disease risk was estimated using CRP among schizophrenia patients from Aurangabad. The results showed that the levels of CRP were higher in schizophrenia patients than in the controls (Joshi et al., 2013). In concordance to the previous investigation, another study among the South Indian population of Puducherry reported higher levels of CRP in schizophrenia patients than the controls (Devenarayan et al., 2016). Even though the studies of CRP so far have yielded inconsistent results, the mounting evidence suggests that immune dysfunction and inflammatory process may play a very important role in the etiopathology of schizophrenia.

Table 4. Study of C-reactive protein among the Indian schizophrenia patients.

Author	Year	Population	Study sample plasma/serum	Patients	Controls	Major findings
Joshi et al.,	2014	India	Serum	45	41	Increased levels of hs-CRP in schizophrenia patients.
Devaranayanan et al.,	2016	Indian	Serum	40	40	Increased levels of CRP in schizophrenia patients than the controls

2.3. Interleukin-6: Genetics, structure, synthesis and function

The cytokine interleukin-6 was originally identified as a soluble protein produced by T cells that activate the differentiation of B cells into antibody-producing cells. Thus, it was initially known as B cell stimulatory factor 2 (BSF-2) (Kishimoto and Ishizaka, 1976). In 1986, IFN-β2 and a 26-kDa protein were identified in fibroblasts which were observed to be identical to BSF-2 (Haegeman et al., 1986; Zilberstein et al., 1986). Simultaneously, the cDNA of the human BSF-2 gene was successfully cloned

(Hirano et al., 1986). Later the hepatocyte-stimulating factor which was initially designated as plasmacytoma growth factor was found to be IL-6, highlighting the protein's diverse biological activities (Gauldie et al., 1987). The name IL-6 was first designated in 1988 at a conference entitled "Regulation of the Acute Phase and Immune Responses: A New Cytokine" (Sehgal et al., 1989).

IL-6 is a pleiotropic cytokine that regulates antigen-specific immune responses and inflammatory reactions. In addition to its role in the acute phase response, IL-6 has diverse roles in autoimmunity, endothelial cell dysfunction, fibrogenesis and organ development. The gene for human IL-6 is located on chromosome 7p21 (Sehgal et al., 1986) and consists of four introns and five exons within the protein-coding region of the gene. The positions of exon/intron boundaries, exon lengths, and location of cysteine residues within exons are conserved across the species. However, differences occur at the 5' boundary of exon 1 and the 3' boundary of exon 5, which lie outside the coding region. Human IL-6 is a single-chain glycoprotein with a molecular mass ranging from 21kDa to 30kDa (Figure 2) depending on the cellular source and method of synthesis (Hirano et al., 1985; Cayphas et al., 1987; van Damme et al., 1987). Under strongly reducing and denaturing conditions, the modified forms of natural IL-6 tend to cluster around two molecular weights. The 23-25kDa forms are exclusively O-glycosylated, while the 28-30kDa forms are both N- and O-glycosylated (May et al., 1988). Of the two potential N-glycosylation sequons (Asn-Xaa-Ser/Thr) in human IL-6 (Asn 45 and Asn 144), only one (not yet identified) is utilized (Gross et al., 1989). In addition, most forms of natural IL-6 are phosphorylated at multiple serine residues, although the extent of phosphorylation is very tissue-specific (May et al., 1988). The four-helical cytokine IL-6 on cells binds to a membrane-bound IL-6

receptor (IL-6R) which dimerizes and initiates intracellular signaling via the JAK-STAT pathway.

IL-6 is a pro-inflammatory cytokine that is produced by a variety of cell types such as lymphocytes, monocytes and granulocytes in response to various stimuli (Akira et al., 1993; Li and He, 2006; Zimmermann et al., 2015). A number of transcription factors have been shown to regulate the IL-6 gene transcription. Nuclear factor IL-6 (NF-IL6) (also known as CCAAT/enhancer-binding protein β), NF-κB, specificity protein 1 (SP1), and interferon regulatory factor 1 are the binding site for the functional *cis*-regulatory elements in gene's 5′ flanking vicinity of IL-6. (Libermann and Baltimore, 1990; Kishimoto et al., 1992; Matsusaka et al., 1993). Activation of *cis*-regulatory elements by stimulation with TNF, IL-1, forskolin and TLR-mediated signal results in activation of the promoter of IL-6 and synthesis of IL-6 (Tanaka et al., 2014). IL-6 transmits the signal through mIL-6R (classical signaling pathway) or through sIL-6R (trans-signaling pathway). In both cases, IL-6 first binds to the receptor IL-6R and then to gp130 via cellulose-binding domains, but elicits different biological effects depending upon the receptor form (Bousoik et al., 2018). Classic signaling is manifested mainly in leukocytes and liver cells, which express both mIL-6Rα and gp130, and promote anti-inflammatory responses. In contrast, trans-signaling can be manifested in all gp130-expressing cells and leads to pro-inflammatory responses (Masjedi et al., 2018). These pro- and anti-inflammatory responses are elicited by a hexameric complex of IL-6 with IL-6R and gp130. This hexameric complex activates Janus kinase (JAK) (Li et al., 2014) that, in turn, activates three possible signaling routes. In route 1, JAK induces tyrosine phosphorylation of itself and subsequently activates dimerization of signal transducer and transcription-3 (STAT3) (Heinrich et al., 2003). In route 2, JAK activates Ras/Raf pathway, which subsequently causes

hyperphosphorylation of mitogen-activated protein kinases (MAPK) and an increase in its serine/threonine kinase activity (Bousoik et al., 2018). Route 3 involves the activation of phosphoinositol-3 kinase (PI3K) – protein kinase B (PKB)/Akt pathway, wherein JAK phosphorylates and activates PI3K, which then phosphorylates certain phosphatidylinositides to phosphatidylinositol-4,5-bisphosphate (PIP2) and phosphatidylinositol-3,4,5-trisphosphate (PIP3). PIP3, in turn, phosphorylates and activates PKB/Akt, which is recruited to the plasma membrane. Under homeostatic conditions, IL-6 levels in the circulation are as low as 1–5 pg/ml, but during inflammatory states, these levels can rise more than 1,000-fold (Waage et al., 1989). IL-6 is produced by myeloid cells upon Toll-like receptor stimulation together with the cytokines IL-1β and TNFα, which via a feed-forward loop, lead to an immense amplification of IL-6 production during inflammatory conditions (Tanaka et al., 2014). The increased level of IL-6 then stimulates liver to synthesize acute-phase proteins viz. CRP, haptoglobin, serum amyloid A, α1-antichymotrypsin, and fibrinogen (Heinrich et al. 1990). Furthermore, IL-6 promotes specific differentiation of naive CD4+ T cells, thus performing an important function in the linking of innate to acquired immune response. It is demonstrated that IL-6, in combination with transforming growth factor-β (TGF-β), is indispensable for Th17 differentiation from naive CD4+ T cells (Korn et al., 2009). However, IL-6 also inhibits TGF-β-induced T-reg differentiation (Bettelli et al., 2006). Up-regulation of the Th17/Treg balance is considered to be responsible for the disruption of immunological tolerance and is thus pathologically involved in the development of autoimmune and chronic inflammatory diseases (Kimura and Kishimoto, 2010). Further, IL-6 is known to promote T-follicular helper-cell differentiation as well as the production of IL-21 (Ma et al., 2012), which regulates immunoglobulin (Ig) synthesis and more specifically IgG4.

Besides, IL-6 also play an important role in the maturation of naïve CD8+ T cells to cytotoxic T cells (Okada et al., 1988). In addition, IL-6 induces the differentiation of activated B cells into antibody-producing plasma cells.

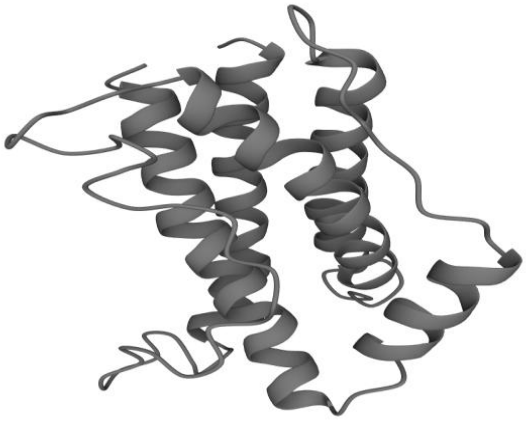

Figure 2. Structure of Interleukin-6.
Retrieved from Protein data bank. (https://www.rcsb.org/structure/1IL6).

2.3.1. Study of IL-6 in schizophrenia: International status

Functionally interleukin-6 is a pleiotropic cytokine and owing to its important role in the inflammatory process it has been investigated intensively to understand its role in schizophrenia. In this section, retrogressive ten-year studies of IL-6 in schizophrenia have been reviewed (Table 5). Various studies observed increased levels of IL-6 in schizophrenia patients (García-Miss et al., 2010; Zakharyan et al., 2012; Frydecka et al., 2015). Besides, increase levels of IL-6 were observed at the early and late stages of schizophrenia (Pedrini et al., 2012). In addition to its high level in serum, significantly high levels of IL-6 were also found in cerebrospinal fluid (CSF) of schizophrenia patients (Sasayama et al., 2013). On the other hand, studies reported that levels of IL-6 may reduce following medical treatment (Sobi´s et al., 2014). However, other studies reported antipsychotic treatment does not have a modulatory effect on the levels of IL-6 (Lin et al., 2011). Studies have further revealed that higher

levels of IL-6 in schizophrenia patients have a significant association with the severity of clinical symptoms as measured by the Positive and Negative Syndrome Scale (PANSS) (Dahan et al., 2018). More specifically, IL-6 was found to be positively correlated with the negative score of PANSS (Hatziagelaki et al., 2019). The levels of IL-6 were also found to be associated with the cortical thickness in the left pars opercularis, right pars triangularis, left superior temporal gyrus, and right middle temporal gyrus (Wu et al., 2019). Recently, it has been suggested that the higher levels of IL-6 are a marker for reduced improvement of depressive symptoms in schizophrenia (He et al., 2020).

Table 5. Study of Interleukin-6 in schizophrenia.

Author	Year	Country	Study sample plasma/serum	Patients	Controls	Major findings
García-Miss et al.,	2010	Mexico	Serum	70	70	The high levels of IL-6 are associated with schizophrenia
Lin et al.,	2010	China	Serum	34	30	Significantly higher levels of IL-6 in schizophrenia patients. There were no significant changes in levels of IL-6 even after one month treatment
Zakharyan et al.,	2012	America	Plasma	103	62	IL-6 levels in schizophrenia were 1.5-fold higher than controls
Pedrini et al.,	2012	Brazil	Serum	61	57	IL-6 levels were significantly high in patients with schizophrenia at early and late stages than in controls.
Sasayama et al.,	2013	Japan	Serum	32	35	Patients with schizophrenia had significantly higher IL-6 levels in both CSF and serum than controls and IL-6 levels were significantly higher in the CSF than in the serum
Sobi´s et al.,	2014	Poland	Serum	17	NIL	A significant reduction in levels of IL-6 after the treatment with antipsychotic drugs for 28 days
Frydecka et al.,	2015	Poland	Serum	151	194	Serum IL-6 levels were significantly higher in schizophrenia patients in comparison with healthy controls
Dahan et al.,	2018	Israel	Serum	41	25	Elevated concentrations of IL-6 were associated with severe clinical symptoms as measured in PANSS scale
Hatziagelaki et al.,	2019	Greece	Serum	14	0	IL-6 was found to be positively correlated with the negative score of PANSS
Wu et al.,	2019	China	Serum	44	44	IL-6 level was significantly associated with the cortical thickness in the left pars opercularis, right pars triangularis, left superior temporal gyrus, and right middle temporal gyrus
He et al.,	2020	China	Serum	35	36	Higher levels of IL-6 are a marker for reduced improvement of depressive symptoms in schizophrenia.

2.3.2. Study of IL-6 in schizophrenia: National status

As far as the study of IL-6 among Indian schizophrenia patients is concerned, only four studies have been conducted to date (Table 6) to understand its role in schizophrenia. In one of the earlier studies among the Indian Bengalee patients from Siliguri, West Bengal, the decreased level of IL-6 was observed in both psychotropic medications free and antipsychotic medicating patients (Singh et al., 2009). In another study conducted at the National Institute of Mental Health & Neurosciences, Bangalore, plasma levels of IL-6 were found to be significantly high in schizophrenia patients than the controls, and the IL-6 level was found to have a significant negative correlation with left hippocampal volume in schizophrenia patients (Kalmady et al., 2014). This finding was again replicated among the antipsychotic free and antipsychotic naïve patients where significantly high levels of IL-6 were observed among the schizophrenia patients than the controls (Kalmady et al., 2018). In a recent longitudinal study, at the National Institute of Mental Health & Neurosciences, Bangalore, plasma levels of IL-6 were reported to be significantly reduced after three months of treatment with antipsychotic medication, which suggest that antipsychotic medication has an immunomodulatory effect in schizophrenia patients (Subbanna et al., 2020). The cytokine IL-6 is a plausible inflammatory biomarker as its production is rapidly increased in course of acute inflammatory reactions along with other cytokines. The present literature provides the further potential relationship between immunological dysfunction and the consequent inflammatory processes mediated through cytokines in schizophrenia patients.

Table 6. Study of Interleukin-6 among the Indian schizophrenia patients.

Author	Year	Country	Study sample plasma/ serum	Patients	Controls	Major findings
Singh et al.,	2009	India	Serum	50	30	IL-6 significantly decreased in both antipsychotic medicating and psychotropic medication free patients were than the controls.
Kalmady et al.,	2014	India	Plasma	28	37	IL-6 levels were significantly higher in schizophrenia patients than controls.
Kalmady et al.,	2018	India	Plasma	75	102	Schizophrenia patients showed significantly higher levels of IL-6 in comparison to healthy controls.
Subbanna et al.,	2020	India	Plasma	27	--	Treatment with antipsychotic medication for 3 months results in significant reduction in plasma levels of IL-6.

2.4. Genetics, structure and function of Toll like receptor

The Toll gene was first discovered in *Drosophila melanogaster* by Christiane Nüsslein-Volhard and co-workers and this gene was found to control dorsoventral patterning in the flies during development (Anderson et al., 1985). Later Jules Hoffman and colleagues showed that the Toll gene is important for the flies' resistance to fungal infection (Lemaitre et al., 1996). The first human homolog of the Toll gene was described by Nomura and colleagues in 1994 (Nomura et al., 1994). Charles Janeway and Ruslan Medzhitov suggested that the human homolog hToll [now known as Toll-like receptor-4 (TLR-4)] induced activation of NF-κB,

proinflammatory cytokines and costimulatory molecules (Medzhitov et al., 1997). Subsequently, Bruce Beutler showed that C3H/HeJ mice were unresponsive to lipopolysaccharide (LPS) due to the proline to histidine point-mutation at position 712 in the TIR domain of TLR-4, identifying TLR-4 as a key receptor for LPS, which suggests that TLRs are indispensable for the recognition of microbial surface molecules (Poltorak et al., 1998). Akira (2006) and colleagues later described the functions of other TLRs through the generation of an extensive collection of mice with targeted deletions of TLRs and TLR signaling proteins (Akira et al., 2006).

To date, ten functional TLR (TLR-1-TLR-10) genes have been identified in the human genome and they are found to be located in different chromosomes. However, the protein product of the TLR gene has been classified into two subfamilies such as cell surface TLRs and intracellular TLRs. The TLRs which are located on the plasma membrane of the cells include TLR-1, -2, -4, -5, -6, and -10. The intracellular TLRs which are located in the endosomal compartment are TLR-3, -7, -8 and -9 (Kawai and Akira, 2010; Celhar et al., 2012). Thus, TLRs are localized as transmembrane proteins in the plasma membrane or intracellular vacuolar membranes. The cytoplasmic domain of TLRs is referred to as the Toll/IL-1 receptor (TIR) domain because it is similar to the interleukin IL-1 receptor family (Rock, 2006; Takeda and Akira, 2003). However, the extracellular region of TLRs and IL-1R are markedly different. The IL-1R possesses an Ig-like domain, TLRs contain leucine-rich repeats (LRR) (Bowie and O'Neill, 2000). The LRR domains are composed of 19–25 tandem leucine-rich repeat motifs, each of which is 24–29 amino acids in length, containing the motif XLXXLXLXX (L-leucine: X- amino acid) as well as other conserved amino acid residues (XΦXXΦXXXXFXXLX; Φ = hydrophobic residue) (Akira et al., 2006). Each LRR consists of α-helix connected and β-strand. The ligand binds with the

concave surface of the LRR domain of TLRs and forms a horseshoe structure. The LRR domain binds ligands, while the cytoplasmic Toll-IL-1 receptor (TIR) domain initiates intracellular signaling pathways through a homotypic protein-protein interaction with TIR-adapter molecules (O'Neill and Bowie, 2007). TLRs are expressed in a variety of mammalian immunologically important cells viz. B cells (Gerondakis et al., 2007), mast cells, natural killer cells (Eriksson et al., 2006), regulatory T cells (Sutmuller et al., 2007), macrophages, monocytes, dendritic cells (Kaisho and Akira, 2006), neutrophils (Sabroe and Whyte, 2007) and basophils. In addition, non-immune cells such as epithelial (Yoshimoto and Nakanish, 2006) and endothelial cells (Gibson et al., 2008) can also express TLRs. They are also present in the brain cells such as microglia (Olson and Miller, 2004), astrocytes (Bowman et al., 2003) and oligodendrocytes (Aravalli et al., 2007). However, neurons, as well as neuronal progenitor cells may also express TLRs (Tang et al., 2007).

2.4.1. TLR-1, TLR-2 and TLR-6

The genes for TLR-1 and TLR-6 are mapped to chromosome 4p14 and the coding region consists of one exon. On the other hand, the TLR-2 gene is located in chromosome 4q32 and consists of two exons. The TLR-2 recognizes the microbial ligands by dimerizing with either TLR-1 and TLR-6 (Figure 3 [A] and [B]) which in turn dictates the specificity of the ligand recognition (Ozinsky et al., 2000; Takeuchi et al., 2001). TLR-2 recognizes the broad range of microbial products including Gram-positive bacteria, mycobacteria, fungi, spirochaetes (*Treponema maltophilum*) and protozoa (*Trypanosoma cruzi*) (Takeda et al., 2003). Moreover, TLR-2 also recognizes lipopolysaccharide preparations from non-enterobacteria such as

Helicobacter pyroli and *Leptospira interrogans* (Hirschfeld et al., 2001; Werts et al., 2001).

2.4.2. TLR-3

TLR-3 maps to chromosome 4q35 and has five exons, however, the protein is encoded by exons 2 to 5 (Figure 3 [C]). Studies have shown that TLR-3 functions as a cell-surface receptor for double-stranded RNA (dsRNA) (Alexopoulou et al., 2001). dsRNA is a molecular pattern produced by most viruses at some point in their infection cycle. It has long been known to have immunostimulatory activity, partly because of its ability to activate the dsRNA-dependent protein kinase R (PKR) (Williams et al., 1999). However, PKR-deficient cells are still able to respond to both dsRNA and its synthetic analogue, polyinosine-polycytosine (polyIC) (Chu et al., 1999), indicating the existence of another receptor for dsRNA. This receptor seems to be TLR-3, as cells deficient for TLR-3 have a profound defect in their responsiveness to polyIC, as well as to viral dsRNA (Alexopoulou et al., 2001).

2.4.3. TLR-4

The TLR-4 gene has four exons which are mapped to chromosome 9q32–33 (Figure 3 [D]). TLR-4 is an important receptor for lipopolysaccharide recognition (Poltorak et al., 1998; Hoshino et al., 1999). Lipopolysaccharide is known to be a very strong immuno-activator and a very small quantity of it can activate the TLR-4 (Gao et al., 2003). In addition, TLR-4 has been reported to be involved in the endogenous ligand recognition, such as the heparn sulfate, oligosaccharides of hyaluronic acid, extra domain A of fibronectins, heat shock proteins and fibrinogen (HSP60 and HSP70). In addition, it is observed that contamination of the Hsp70 with LPS confers the ability

to activate TLR-4 (Gao et al., 2003). Nevertheless, viral glycoproteins are also found to activate TLR-4 causing increase expression of cytokines such as IFN-β (Rassa et al., 2002).

2.4.4. TLR-5

Gene for TLR-5 is mapped to chromosome 1q33.3 and has five exons (Figure 3 [E]). TLR-5 responses to a monomeric constituent of bacterial flagella (Hayashi et al., 2001). They are expressed on the intestinal endothelial cells of the subepithelial compartment, hence they can recognize microbial antigens on the mucosal surface of the intestine (Gewirtz et al., 2001; Maaser et al., 2004; Hawn et al., 2003). It has been observed that polymorphism in the peptide-binding region of TLR-5 may provide vulnerability to *Legionella pneumophila,* a causative agent of pneumonia (Hawn et al., 2003).

2.4.5. TLR-7 and TLR-8

TLR-7 and TLR-8 genes are found in tandem on chromosome Xp22 and have two exons each (Figure 3 [F] and [G]). They can recognize the same ligand in some cases. In humans both TLR-7 and TLR-8 recognize imidazoquinoline compounds (Jurk et al., 2002). Both TLR-7 and TLR-8 are receptors that can identify guanosine- or uridine-rich single-stranded RNA (ssRNA) from viruses viz. human immunodeficiency virus, influenza virus and vesicular stomatitis virus. (Heil et al., 2004; Diebold et al., 2004; Lund et al., 2004). Due to its expression only in the endosome TLR-7 or TLR-8 are not able to detect ssRNA derived from the host as it is not delivered to the endosome (Takeda and Akira, 2005).

2.4.6. TLR-9

The TLR-9 gene is located at chromosome number 3p21.3 and consists of two exons (Figure 3 [H]). However, the second exon is the major coding region. TLR-9 is known to recognize the bacterial CpG DNA. In addition, TLR-9 has also been shown to recognize viral-derived CpG DNA (Lund et al., 2003; Krug et al., 2004). The mutation in the TLR-9 gene has been shown to confer susceptibility to mouse cytomegalovirus infection (Tabeta et al., 2004).

2.4.7. TLR-10

TLR-10 gene is located in chromosome 4p14 and is structurally similar to TLR-1 and TLR-6 (Figure 3 [I]). The TLR-10 can bind to tripalmitoyl-*S*-glyceryl-cysteine (Pam3Cys) lipopeptides present in Gram-negative bacteria. It has been observed that TLR10 and TLR-2 share common ligands and both of them can bind to PAMPs of *Borrelia burgdorferi* (Oosting et al., 2014). Besides, studies have observed that heterodimerized TLR-2/TLR-10 can bind to various PAMPs similar to TLR-1/TLR-2 heterodimer (Verma et al., 2014). In addition, TLR-2/TLR-10 heterodimer can also recognize the lipopolysaccharides present in *Helicobacter pylori*. It is interesting to note that lipopolysaccharides are traditionally recognized by TLR-4 but *H. pylori* do not induce TLR-4 response (Nagashima et al., 2015)

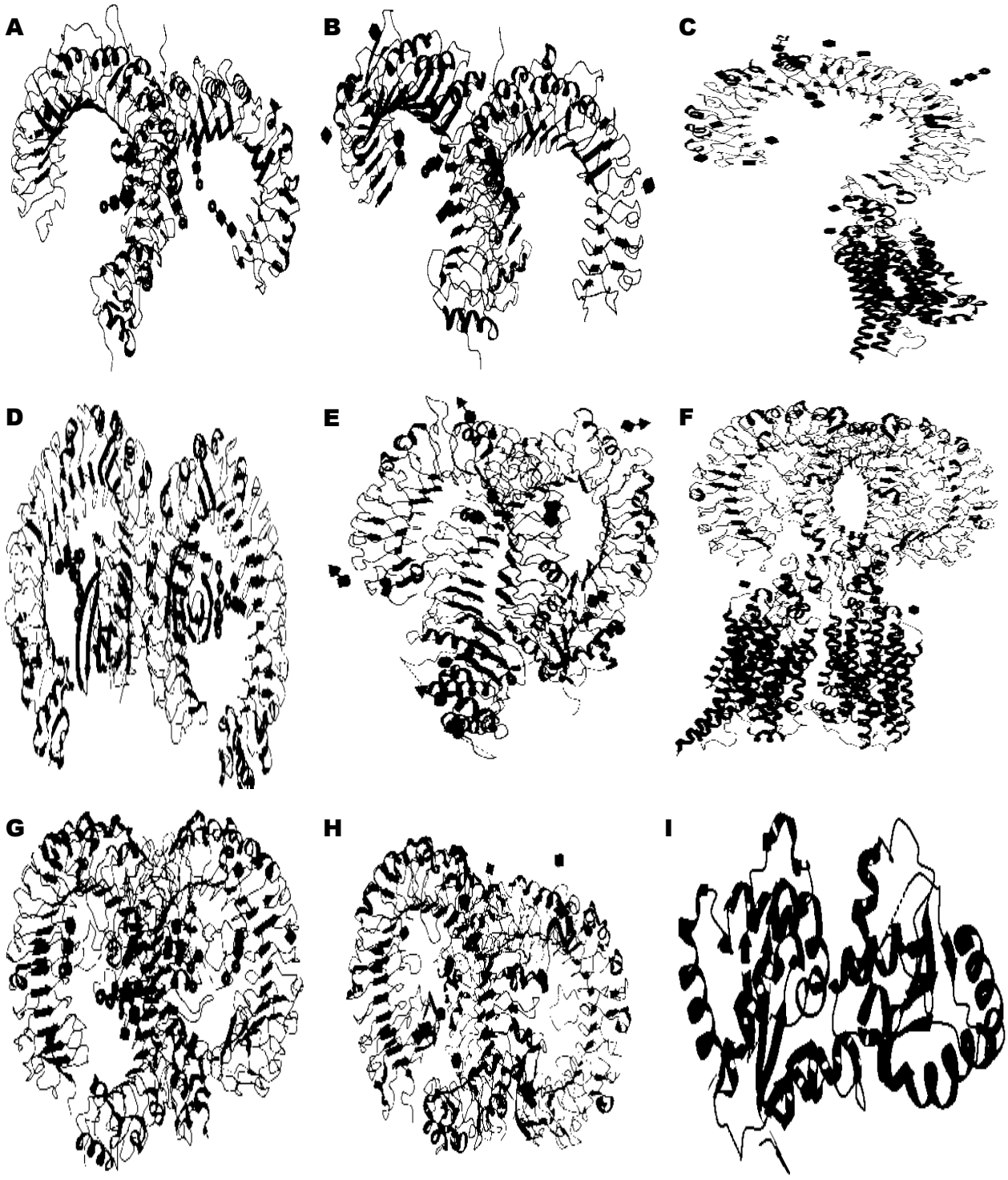

Figure 3. Structure of TLRs: (A) TLR-2/TLR-1 heterodimer, (B) TLR-2/TLR-1 heterodimer, (C) TLR-3, (D) TLR-4, (E) TLR-5, (F) TLR-7, (G) TLR-8, (H) TLR-9, (I) TLR-10.

Retrieved from the protein data bank, (https://www.rcsb.org/).

2.4.8. Mechanism of TLR action

The specific microbial structures (PAMPs) are recognized by a specified TLR which relies on receptor dimerization to achieve specificity in agonist recognition. The signaling pathways activated by TLRs are divided into myeloid differentiation factor 88 (MyD88) dependent pathways and TIR-domain containing adaptor inducing protein inducing IFN-β (TRIF) dependent pathway. MyD88 is the universal adapter protein recruited by all TLRs except for TLR-3, which utilizes TIR domain-containing adapter-inducing interferon-β (TRIF) to mediate signaling. TLR-4 utilizes both MyD88-dependent and TRIF-dependent signaling pathways (Kawai and Akira, 2007).

MyD88-dependent signaling pathway involves N-terminal death domain and C-terminal TIR domain, which are associated with the toll/interleukin-1 receptor domain of TLRs. MyD88 can activate IL-1 receptor-associated kinase (IRAK)-4 via the interaction with death domains of TLRs and facilitate IRAK-4-mediated phosphorylation of IRAK-1. The phosphorylation of IRAK-1 leads to its activation which then associates with TRAF6 and leading to the activation of two different signaling pathways. One of the pathways of TLR activation is MAPK (mitogen-activated protein kinase), which mediates activation of Activator Protein-1(AP-1) transcription factors, which regulate the inflammatory cytokine gene expression. Another pathway is TAK1/TAB complex activation, which enhances IkB kinase (IKK) complex activity. These pathways finally lead to the nuclear translocation of transcription factor NF-kB and the production of inflammatory cytokines. In addition, the second TIR domain containing molecule is Mal (MyD88-adaptor-like)/TIRAP (TIR domain-containing adaptor protein) which is structurally related to MyD88, also leads to the production of inflammatory cytokines. Similar to MyD88, TIRAP/Mal

also leads to the production of the inflammatory cytokine. TIRAP/Mal-deficient cells respond to ligands of TLR-3, TLR-5, TLR-7 and TLR-9 but do not respond to ligands of TLR-2 and TLR-4. TLR-3 and TLR-4 utilize the MyD88-independent/TRIF-dependent component to induce NF-kB. TRIF activates the NF-kB through two different regions. TRAF6-binding motifs of the N-terminal region assist the binding of TRIF with TRAF6 which in turn activates TAK1. The C-terminal region of TRIF contains receptor-interacting protein homotypic interaction motif (RHIM), which provides the domain for interaction with RIP1 (receptor-interacting protein-1). The ubiquitination of RIP1 causes the activation TRAF6 and TAK1 resulting in NF-kB activation. In addition, MyD88-independent/TRIF-dependent pathway is induced through the IKK-related kinases viz. IKKi and TANK which play an important role in expression of type I IFN through phosphorylation and activation of interferon regulatory factor-3 (IRF3) and interferon regulatory factor-7 (IRF7) which are transcription factors. The stimulation of cells causes the IKKi and TBK1-mediated phosphorylation of the IRF3 at the C-terminal regions, which enables the formation of a homodimer and translocation into the nucleus. The N-terminal region of TRIF associates with the TBK1 and IKKi and also associate with the TRAF6 thus regulating the NF-kB activation through the N-terminal portion of TRIF. In addition, IKKi and TBK1 can also phosphorylate and activate IRF7 and lead to activation of NF-kB (Figure 4).

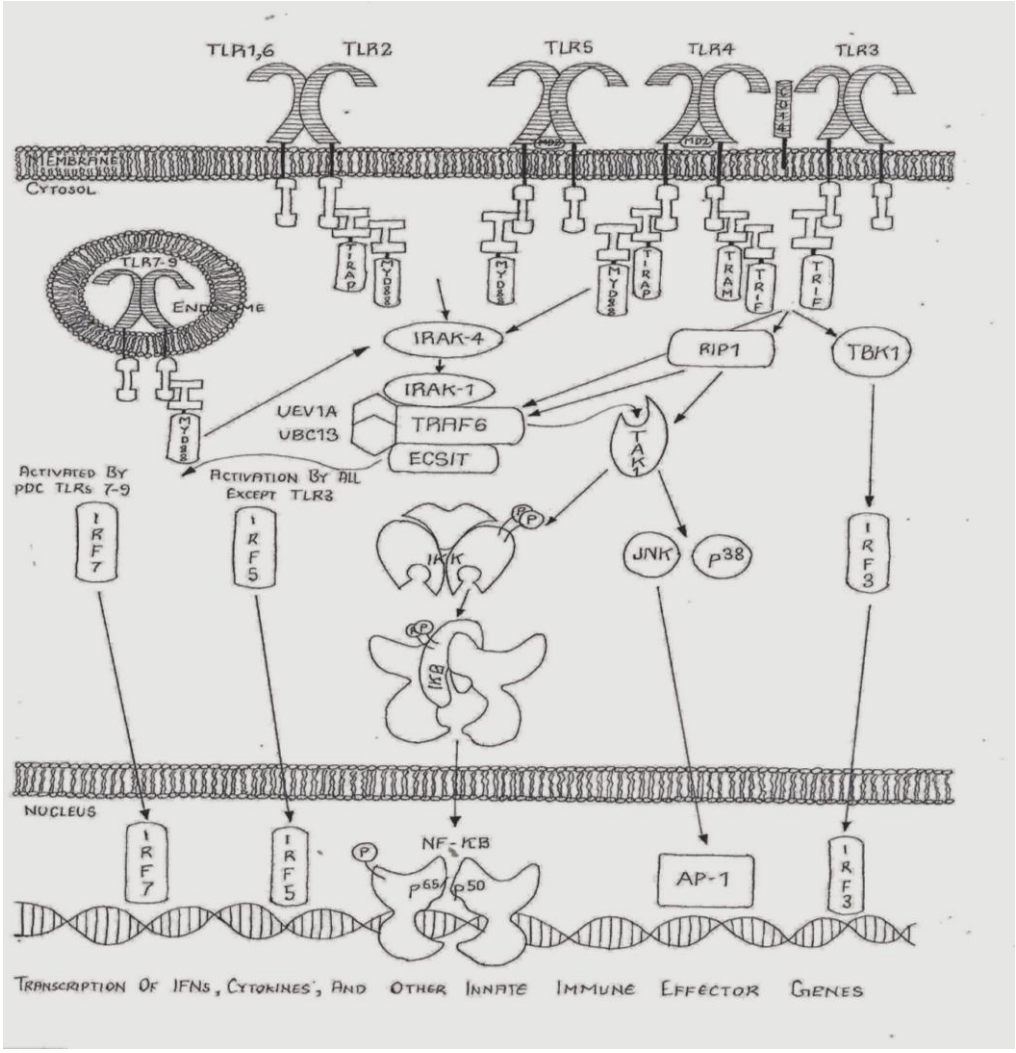

Figure 4. Overview of Toll like receptor signaling pathways. Adopted from West et al. (2006)

2.4.9. Function of TLR

TLRs are a member of pattern recognition receptors (PRRs) which are crucial for immune recognition of many PAMPs and Damage-associated molecular patterns (DAMPs). TLR activation results in the induction of pro-inflammatory cytokines (i.e., chemokines and type I IFNs) and upregulation of co-stimulatory molecules. They are the important mediators of both innate immunity and adaptive immune response. TLRs are not solely dedicated to eliciting pathogen-related immune responses but also bear physiological as well as pathological roles unrelated to infection. TLRs are

classically studied in relation to immunity, however recent evidence implicates TLRs as mediators of central nervous system (CNS) plasticity. Studies have shown that pathogen-derived TLR activation in the brain adversely affects cognition. A study demonstrated a significant alteration of cognitive functions in TLR-2-deficient amyloid precursor protein/presenelin 1 mouse (Richard et al., 2008). In another study, transgenic Alzheimer's disease mice deficient in TLR-4 exhibit reduced cognitive function (Song et al., 2011).

In CNS, microglia express various types TLR, which on activation by PAMPs stimulate the release of pro-inflammatory cytokines including TNF-α and IL-6 (Lehnardt, 2010; Suh et al., 2009). Microglia can be activated during systemic infections without the blood-brain barrier being compromised, suggesting that PAMPs can cross the blood-brain barrier and/or activate macrophages and microglia in circumventricular organs. Alternatively, macrophages and/or circulating cytokines can pass the blood-brain barrier and intercept invading pathogens in the brain and/or activated microglial cells. There is evidence that localized activation of TLR-4 in blood-brain barrier-associated macrophages/microglia can trigger a 'wave' of microglial activation that spreads within the brain parenchyma. This transcellular wave of innate immune cell activation is believed to be propagated by TNF- α (Baker et al., 2010). Activation of TLRs can result in different outcomes in different types of CNS cells. When TLR-3 is activated in astrocytes, a comprehensive neuroprotective response was suggested to occur, in contrast to the pro-inflammatory reaction of microglial cells (Bsibsi et al., 2006). However, the majority of studies support the view that TLR-3 activation in human astrocytes contributes to a pro-inflammatory phenotype of astrocytes (Kim et al., 2008). The family of the innate immune receptors is now being recognized as modulators of CNS plasticity. TLRs influence neural

progenitor cells proliferation, differentiation, neurite outgrowth and behavioral plasticity. Although evidence from mice deficient in TLRs strongly implicates the role of these receptors in neuroplasticity, the distinction between developmental and functional effects of life-long deficiency of a TLR, as well as specific roles for TLRs in neuroplasticity following infection and injury remains unclear.

2.4.10. Studies of TLRs in schizophrenia: International status

Studies have put forward the hypothesis that dysfunction of the immune system shall cause hindrance in the elimination of pathogens from the body which in turn may lead to low-grade inflammation in patients with schizophrenia (Muller et al., 2012). Since TLR is an important molecule for the recognition of PAMPs, so any abnormality in the TLR may lead to abnormality in pathogen recognition and clearance, leading to inflammation. Even though the proposed low-grade inflammatory process in schizophrenia has been regarded as one of the etiological factors for the disorder the available literature regarding the study of TLR in schizophrenia is meagre (Table 7). Moreover, very few studies have been conducted to understand the role of single nucleotide polymorphism (SNP) within TLR genes in schizophrenia. In one of the studies, the C allele of TLR-2 rs3804099 and TLR-2 rs3804100 SNPs was found to be associated with a poor concentration in schizophrenia. However, no direct association was observed for TLR-2 gene polymorphism in susceptibility to schizophrenia (Kang et al., 2013). In concordance with this study, García-Bueno et al. (2016) also reported no direct association of TLR-4 SNPs rs4986790, rs4986791 and rs11536889 with schizophrenia (Garcia-Bueno et al., 2016). In another approach of study, the expression of TLR in the monocyte cells of schizophrenia patients was found to be significantly associated with the disease condition (Chang et al., 2011). In a

comparative study among bipolar disorder and schizophrenia patients an increased expression of IL-1β, IL-6 and TNF-α were observed following treatment with the TLR-2 and TLR-4 agonist in both disorders as compared to controls. However, in the same study TLR-9 agonist was found to induce an increase in IL-8 in schizophrenia. The findings suggest that specific alterations in TLR agonist-mediated cytokine production may contribute to immune surveillance malfunction in psychotic illnesses (McKernan et al., 2011). In a pilot study, peripheral blood mononuclear cells were analyzed for the expression of 84 genes of the TLR pathway after the stimulation with LPS. The results indicated a diminished capacity for the response after LPS stimulation for factors that activate the TLR pathway. However, there was an increased expression of inhibiting factors of the TLR pathway compared to healthy controls (Weidinger, 2013). In another study, pregnant mice were exposed to, a synthetic polyinosinic-polycytidylic acid (Poly I·C), double-stranded RNA molecular mimic of replicating virus. It was observed that Poly (I·C) inhibits embryonic neuronal stem cell replication and population of the superficial layers of the neocortex by neurons. Poly (I·C) also led to impaired neonatal locomotor development and abnormal sensorimotor gating responses in adult offspring. Using TLR-3 deficient mice, it was established that these effects were dependent on TLR-3. Inhibition of stem cell proliferation was also abrogated by pre-treatment with the nonsteroidal anti-inflammatory drug (NSAID) carprofen, a cyclooxygenase (COX) inhibitor. The findings provide insights into mechanisms by which maternal infection can induce subtle neuropathology and behavioral dysfunction, and it may open new strategies to reduce the risk of neuropsychiatric disorders subsequent to prenatal exposures to pathogens and other triggers of innate immunity (De Miranda et al., 2010). In the classic study, the expressions of Toll-like receptors (TLRs) in monocytes were

examined among schizophrenia patients by using RT-PCR. The results showed significant reductions of TLR-3 and TLR-5 mRNA in schizophrenia patients. These findings demonstrated the connection between TLR and schizophrenia (Chang et al., 2011). In a study among the cultured neurons and *in vivo* mouse brain it was observed that Toll-like receptor 3 (TLR-3) acts through MYD88 pathway to downregulate the expression of Disrupted in schizophrenia 1 (Disc1) gene, resulting in impairment of neuronal development. In addition, TLR-3 activation at the neonatal stage was found to increase dendritic spine density but found to narrow spine heads suggesting a long-lasting effect of TLR-3 activation on spinogenesis. This study reveals the role of TLR-3 in the regulation of dendritic morphology and explains how environmental factors may influence mental health (Chen et al., 2016). Recently, a study was conducted to investigate the effect of TLR-2 gene on neurobehavioral functions in TLR-2 knockout mice. The results showed decreased cognitive function and locomotor activity, as well as increased anxiety in TLR-2 knockout mice. Further, regional cerebral blood flow (rCBF), inhibited long-term potentiation (LTP), and increased blood-brain barrier (BBB) permeability was found to be significantly reduced in TLR-2 knockout mice (Hu et al., 2020). Another study was conducted to understand the relationship between peripheral TLR expressing cells and regional brain volumes in humans. Results revealed a significant negative correlation between the percentage of TLR-4+ monocytes and brain volume in the frontal and anterior cingulated region. The study revealed an association of abnormal TLR-activation with decreased brain volumes in schizophrenia patients (Li et al., 2020). In a longitudinal study by Keri et al. (2017a), drug-naïve schizophrenia patients were found to have a higher proportion of TLR-4+, TLR-5+ and TLR-5+ Treg/Tact cells. However, following the treatment an increase in the number of Treg/Tact cells and TLR-2+

monocytes were observed. On the other hand, no effect was observed in the number of TLR-4+ monocytes. These results indicate that abnormal expression of TLRs may have a role in the etiopathology of schizophrenia, and antipsychotics may modulate their expression (Keri et al., 2017a). Another study, Keri et al. (2017b) observed an increased expression of TLR-4 and TLR-5 in schizophrenia patients than the controls. Further, TLR-4 stimulation with bacterial lipopolysaccharides was found to increase IL-1β, IL-6 and TNF-α in schizophrenia patients, however, the level of IL-10 was not found to be changed. However, in the case of TLR-5, the levels of IL-1β, IL-6, TNF-α and IL-10 cytokine were found to have no significant differences between patients and controls after stimulation with flagellin. Recently, a European study observed the down-regulation in the expression of TLR-1, TLR-2, TLR-4, TLR-6, and TLR-9 and upregulation of TLR-3 and TLR-7 in schizophrenia patients (Kozłowska et al., 2019).

Table 7. Study of Toll like receptors in schizophrenia

Gene	SNP ID	Author	year	Model	Country	Patients	Controls	Method	Major findings
TLR-3	--	De Miranda et al.,	2010	Mouse	USA	--	--	Flow cytometry	Exposure to polyinosinic-polycytidylic acid to TLR-3 wild type mice during gestation increases the risk of neuropsychiatric disorders in offspring
TLR-1 to TLR-5	--	Chang et al.,	2011	Human	Taiwan	46	22	RT-PCR	mRNA levels of TLR-3 and TLR-5 were significantly reduced in schizophrenia patients.
TLR-1, TLR-3, TLR-5, TLR-6, TLR-7	--	McKernan et al.,	2011	Human	Irish	40	40	ELISA	Specific alterations in TLR agonist-mediated cytokine release contribute to schizophrenia.
TLR-2	rs3804099 rs3804100	Kang et al.,	2013	Human	Korean	286	305	Direct Sequencing	C allele of TLR-2 SNPs rs3804099 and rs3804100 was association with poor concentration in schizophrenia
TLR-4	rs4986790 rs4986791	García-Bueno et al.,	2016	Human	Spain	214	216	Direct Sequencing	No association with schizophrenia

Table 7. Continue.

Gene	SNP ID	Author	year	Model	Country	Patients	Controls	Method	Major findings
TLR-2 TLR-4 TLR-5	--	Kéri et al.,	2017	Human	Hungary	35	30	Flow cytometry	Drug-naïve patients with schizophrenia exhibited an increased percentage of TLR-4+ and TLR-5+ monocytes and TLR-5+ Treg/Tact cells.
TLR-4 TLR-5	--	Kéri et al.,	2017	Human	Hungary	42	42	Flow cytometry	Increased expression of TLR-4 and TLR-5 in schizophrenia patients.
TLR-1 to TLR-9	--	Kozłowska et al.,	2019	Human	Europe	27	29	qRT-PCR	TLR-1, 2, 4, 6, and 9 expression were down-regulated in schizophrenia.
TLR-2	--	Hu et al.,	2020	Mouse	China	--	--	Western blot	TLR-2 knockout mice showed decreased cognitive function, locomotor activity and increased anxiety
TLR-1, TLR-4	--	Li et al.,	2020	Mouse	Hungary	--	--	Flow cytometry	Negative association between the percentage of TLR-4+ monocytes and brain volume in frontal and anterior cingulated region

2.4.11. Studies of TLRs in schizophrenia: National status

To date, only one study has been performed to understand the role of TLR in schizophrenia among Indian patients (Table 8). Balaji et al. (2020) investigated the expression profile of TLR-3 and TLR-4 genes among the 31 schizophrenia patients and 30 controls. The results showed the gene expression levels of TLR-4 were significantly upregulated in patients with schizophrenia compared to controls. However, no significant difference was observed for the TLR-3 gene. The results of the study provided evidence for the possible contribution of TLR-4 in the immunopathogenetic pathway in schizophrenia (Balaji et al., 2020). In another study, it was hypothesized that in some cases of schizophrenia where immune-inflammatory and oxidative and nitrosative stress responses are evident, the prenatal infection might lead to neuro progressive changes in a TLR-3/TLR-4-dependent pathway. It was referred to as "The TRIPS (Toll-like receptors in immuno-inflammatory pathogenesis) Hypothesis" (Venkatasubramanian and Debnath, 2013). From the above literature review, it is evident that TLR may have an important role in the etiopathology of schizophrenia.

Table 8. Study of Toll like receptors among the Indian schizophrenia patients.

Gene	SNP ID	Author	year	Model	Country	Patients	Controls	Method	Major findings
TLR-3, TLR-4	--	Balaji et al.,	2020	Human	India	31	30	qRT-PCR	TLR-4 gene was significantly up-regulated in drug-naïve patients.

CHAPTER-03

Materials And Methods

3. MATERIALS AND METHODS

3.1. Study Design

The present investigation was designed as a cross-sectional case-control study to explore the role of inflammation in the etiopathology of schizophrenia. The process of inflammation (if any) in schizophrenia was investigated by using the marks such as CRP, IL-6 and polymorphism in TLR genes. In addition, the prevalence of schizophrenia was also investigated in this region based on the hospital study. The present investigation was approved by the Institutional Ethics Committee of University of Sikkim, reference no: SU/IEC/2016/07 (Annexure-I) and was carried out according to the Declaration of Helsinki principles (1964).

3.2. Study of prevalence of schizophrenia

The prevalence of schizophrenia in the North Bengal region was studied for one year i.e., from 31st January 2019 to 30th January 2020 at the Psychiatry Department of North Bengal Medical College and Hospital (NBMCH), West Bengal, India. The study was carried out at NBMCH as the patients from all parts of North Bengal visit here for the treatment. The psychiatric patients who visited the OPD were considered for the study. After the diagnosis of the patients by the psychiatrist, the details of the patients such as diagnosis of the psychiatric disorder, age, ethnicity, gender etc. were recorded. Duplicate documentation of the same patients was cross-checked and one of the entries was excluded. Participation in the study was voluntary and confidentiality of data was maintained. The prevalence was calculated by using the formulae (Noordzij et al., 2010):-

$$\text{Prevalence} = \frac{\text{Number of subjects having the disease at a time point}}{\text{Total population of Siliguri}}$$

The total population of Siliguri, North Bengal was calculated using an online population growth calculator for 2021. The conditions for calculating population growth were initial population = 513264, rate of growth (%) = 40 and of year (t) = 10.

3.3. Study population

All the participants included in the present study were India-born Bengalee individuals from Siliguri, West Bengal, India. A total of 265 participants were included in the study out of which 120 were schizophrenia patients and 145 were healthy controls. Further, the number of patients and controls included in the study for CRP, IL-6 and TLRs varied between each other.

3.4. Characteristics of the patients

Schizophrenia patients who visited the Outpatient Department (OPD) of the Department of Psychiatry, NBMCH, Siliguri, West Bengal were included in the present study. The patients were diagnosed by the psychiatrist based on Structured Clinical Interview (First et al., 1996) and Diagnostic and Statistical Manual of Mental Disorders (DSM-5) criteria (American Psychiatric Association). The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS), Positive and Negative Syndrome Scale (PANSS), and Brief Psychiatric Rating Scale (BPRS) were also used to evaluate psychiatric parameters (Overall et al., 1962). The study protocol was explained to the patients and their relatives and those who were willing to participate in the study voluntarily were requested to give written consent for the collection of blood samples after the study procedure was explained (Annexure-II).

The patients were made to answer a questionnaire to collect the history and demographic data (Annexure-III).

The following criteria were followed for the selection of the schizophrenia patients; (i) diagnosis of schizophrenia as per the DSM V criteria (American Psychiatric Association, 2013), (ii) unrelatedness of the individuals with one another.

The exclusion criteria were; (i) history of substance abuse and personality disorder, (iii) history of mental retardation and dementia in the patients, (iv) the recent history of allergies and recurrent infections, (v) personal or family history of autoimmune disorder.

3.5. Control's characteristics

Healthy volunteers from the Siliguri sub-division of the Darjeeling District, West Bengal, were considered as a potential control for the study. At first, the study protocol was explained to the potential participants, and those who fulfil the inclusion criteria and were willing to participate voluntarily in the study were included in the study and written consent was obtained. The controls were assessed by the General Health Questionnaire-60 criteria to rule out psychiatric morbidity (Annexure-IV).

For the inclusion of the controls following criteria were followed; (i) healthy individuals who matched in age, gender and ethnicity with the patients, (ii) absence of personal and family history of psychiatric disorders, mental retardation and autoimmune disorder (iii) unrelatedness with one another. To confirm the unrelatedness of the individuals three-generation pedigree charts was prepared. The exclusion criteria for the controls were as follows: (i) recent history of recurrent infections and allergies. (ii) habit or the history of substance abuse.

3.6. Collection of blood samples and sample preparation

Before the collection of the blood samples, the patient and controls were made to rest for half an hour. Approximately 5 ml of blood samples were drawn from the cubital vein using a hypodermic syringe. From the collected blood samples, 3ml were allowed to clot at room temperature for 2 to 3 hours. From the clotted blood serum was collected and centrifuged to separate the contamination. The supernatant serum was collected and aliquoted in an eppendorf tube and stored at -20°C refrigerator until further analysis. The remaining 2ml blood samples were stored at -20°C for DNA extraction.

3.7. Quantitative estimation of CRP and IL-6

For the estimation of CRP and IL-6 in the serum, patients were divided into psychotropic medication free and antipsychotic medicating groups. The psychotropic medication free group included 28 schizophrenia patients who were not under antipsychotic medications for six weeks. On the other hand, the antipsychotic medicating group consisted of 67 patients who were under long-term antipsychotic medication. Seventy-two healthy volunteers who were matched in age, sex and ethnicity with the patients were considered as the controls. The levels of CRP and IL-6 in the blood serum were determined using an ELISA kit (RayBio). The sensitivity of the assay for CRP was 34 pg/ml, and for IL-6 it was 3 pg/ml. The inter-and intra assay coefficient for CRP was <10%, and for IL-6 it was <12%. The assay was carried out as per the instruction manual provided with the kit.

3.8. TLR polymorphism study

3.8.1. DNA Extraction

Genomic DNA was extracted from the blood of 120 schizophrenia patients using a commercially available DNA extraction kit (Qiagen, Germany). The assay was carried out according to the manufacturer's instructions.

3.8.2. SNP genotyping of TLR

SNPs in TLR-2 (rs5743708, rs121917864), TLR-3 (rs3775290, rs3775296), TLR-4 (rs4986790, rs4986791), TLR-7 (rs179009), TLR-8 (rs3764880), and TLR-9 (rs352140, rs5743836) were genotyped using PCR RFLP method. The primers used for the study, restriction enzymes, amplicon size and the digested product sizes are summarized in table 11. The PCR reactions were–set at 25 μL total volume which consisted of 100 ng genomic DNA, 1xTaq buffer (New England Biolabs), 1.5mM $MgCl_2$ (New England Biolabs), 200 mM of deoxynucleotide triphosphates, 20 pmol of each primer (Eurofins), 0.5 units of Taq DNA polymerase (New England Biolabs) (Table 9). The amplification was carried out in a thermal cycler (ProFlex PCR System, Thermo Fisher) consisting of initial denaturation at 95°C for 8 min. It was followed by 35 cycles each consisting of 94 °C for 30s, annealing at respective temperatures (Table 10), 72°C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were run in 2% agarose gel to confirm the amplification. Further, 10 µL amplified products were mixed with 1X restriction buffer and 1µL restriction enzyme (New England Bio Labs) and were incubated overnight at 37°C. The digested products were separated using 3% agarose gel and documented in the gel doc system (Figure 5 [A] to [J]). In case of any ambiguity in the results, the assay was repeated.

Table 9. Details of polymerase chain reaction mixture.

Chemicals	Reaction Concentration	Reaction volume (25 ul)
dH_2O	--	18.6 ul
Taq buffer	1X	2.5 ul
Deoxynucleotide triphosphates(dNTPs)	200 mM	1 ul
$MgCl_2$	1.5mM	0.5 ul
Forward primer	20 pmol	0.5 ul
Reverse primer	20 pmol	0.5 ul
Taq DNA polymerase	0.5 units	0.4 ul
Genomic DNA	100 ng	1 ul

Table 10. Details of polymerase chain reaction condition.

Conditions	Temperature	Time	No. of cycles
Initial denaturation	95 °C	8 Minutes	1 cycle
Denaturation	95 ° C	1 Minutes	35 cycles
Annealing	55 ° C (TLR-3 rs3775290 and (TLR-9 rs5743836) 53 ° C (TLR-8rs3764880) 54 ° C (TLR-7rs179009) 56 ° C (TLR-3rs3775296) 60 ° C (TLR-2 rs121917864) 61° C (TLR-4 rs4986790 and (TLR-4 rs4986791) 65° C (TLR-9 rs352140) 66° C (TLR-2 rs5743708)	1 Minutes	
Extension	72 °C	1 Minutes	
Final extension	72 °C	10 Minutes	1 cycle

Table 11. Primer sequences, restriction enzymes, and fragment sizes for TLR SNPs.

SNP Polymorphism	Primer sequence (5′–3′)	Restriction enzyme	Fragments size (bp)	References
TLR-2 Arg753Gln (rs5743708)	CATTCCCCAGCGCTTCTGCAAGCTCC GGAACCTAGGACTTTATCGCAGCTC	AciI	GG: 104,25 bp GA: 125,104,25 bp AA: 129 bp	Folwaczny et al., 2004
TLR-2 Arg677Trp (rs121917864)	GCCTACTGGGTGGAGAAGCTT CCAGTTCATACTTGCACCACT C	MspI	CC: 124, 75 bp CT: 199, 124, 75 bp TT: 199 bp	Habibzadeh et al., 2018
TLR-3 _7C/A (rs3775296)	GCATTTGAAAGCCATCTGCT AAGTTGGCGGCTGGTAATCT	MboII	AA: 257, 17 bp AC: 279, 257, 17 bp CC: 279 bp	Habibabadi et al., 2020
TLR-3 c.1377C/T (rs3775290)	CCAGGCATAAAAAGCAATATG GGACCAAGGCAAAGGAGTTC	TaqI	CC: 274, 63 bp CT: 337, 274, 63 bp TT: 337 bp	Zayed etal., 2017
TLR-4 gene Asp299Gly (rs4986790)	GATTAGCATACTTAGACTACTACCTCCATG GATCAACTTCTGAAAAAGCATTCCCAC	NcoI	AA: 249 bp AG: 249, 223 bp GG: 223 bp	Chen et al., 2012
TLR-4 Thr399Ile (rs4986791)	GGTTGCTGTTCTCAAAGTGATTTTGGGAGAA ACCTGAAGACTGGAGAGTGAGTTAAATGCT	HinfI	CC: 406 bp CT: 406,377 bp TT: 337 bp	Chen et al., 2012

Table 11. Continue.

SNP Polymorphism	Primer sequence (5′–3′)	Restriction enzyme	Fragments size (bp)	References
TLR-7 Gln11Leu (rs179009)	TAACAACGAATAGGAAAATGC GTTTTAGGAAACCATCTAGCC	NlaIII	AA: 122, 247 bp AG: 122, 247, 369 bp GG: 369 bp	Singh et al., 2020
TLR-8 Met1Val (rs3764880)	GTGTGTGTCTGATTTGGGTTG TTCTAGGCTCACACCATTTG	NlaIII	AA: 156, 137, 97 bp AG: 256,156,137,97 bp GG: 256, 137 bp	Engin et al., 2010
TLR-9 Pro545Pro (rs352140)	GCCAGGTAATTGTCACGGAG GATGTTTGCCCAGCTCTCG	BstU I	CC: 362, 166 bp CT:528, 362, 166 bp TT: 528 bp	Loganathan et al., 2017
TLR-9 1237T/C (rs5743836)	ATGGGAGCAGAGACATAATGGA CTGCTTGCAGTTGACTGTGT	BstNI	TT: 108, 27 bp TC:108, 60, 48, 27 bp CC: 60, 48, 27 bp	Zayed etal., 2017

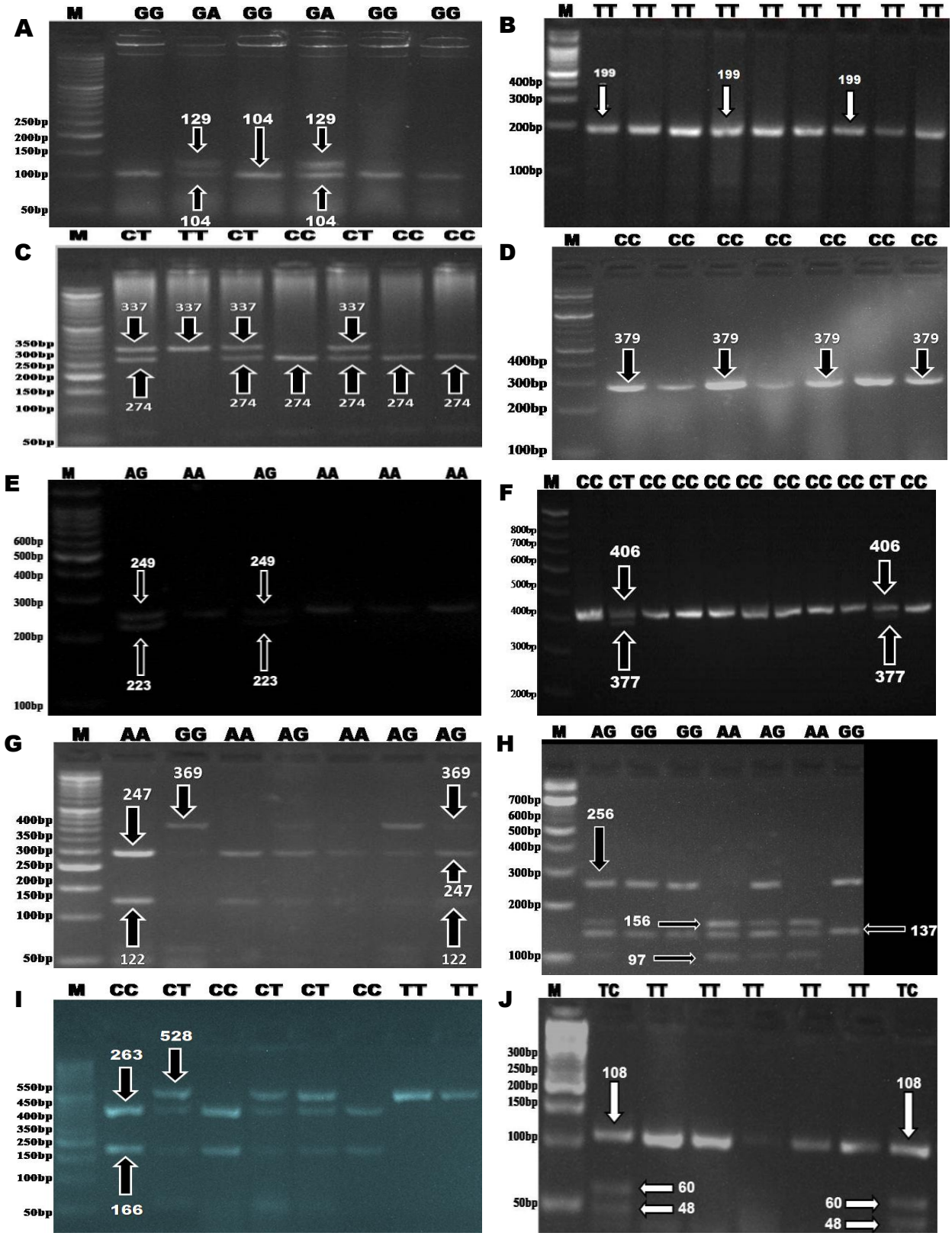

Figure 5. Agarose gel electrophoresis results showing PCR-RFLP products; (A) TLR-2 (rs5743708), (B) TLR-2 (rs121917864), (C) TLR-3 (rs3775290), (D) TLR-3 (rs3775296), (E) TLR-4 (rs3775290), (F) TLR-4 (rs4986791), (G) TLR-7 (rs179009), (H) TLR-8 (rs3764880), (I) TLR-9 (rs352140), (J) TLR-9 (rs5743836).

3.9. Statistical analysis

All the statistical analysis was performed using SPSS software, version 23, IBM Crop, and R studio, version (3.6.1). Socio-demographic continuous variables were described as mean and standard deviation (SD) while categorical variables were represented as percentages**.**

3.9.1. Statistical analysis for prevalence study

Chi-squared ($\chi 2$) test was performed to analyze the difference in the prevalence of schizophrenia between males and females. Further, the student's *t*- test was performed to study the age of onset of symptoms between the male and female.

3.9.2. Statistical analysis for CRP and IL-6

The kurtosis and skewness analysis were first performed to check the data for its distribution. The results showed that the data were skewed. Hence, non-parametric tests viz., Mann–Whitney U test, and Spearman correlation test was performed. The p values of <0.05 were considered statistically significant.

3.9.3. Statistical analysis for TLR polymorphism

Genotype frequencies were tested for deviation from Hardy-Hardy-Weinberg Equilibrium (HWE) using a goodness of fit Chi-squared ($\chi 2$) test. Chi-square test and Fisher's exact test was performed to check whether allele frequency and genotype distributions in cases were significantly different from that of the controls. Association between the TLR SNPs and schizophrenia susceptibility under five different inheritance models (codominant, dominant, recessive, overdominant, and additive model) were analyzed as odds ratio (OR) and 95% confidence interval (CI)

using an online SNPStats analysis program (https ://www.snpst ats.net/start.htm) (Qi et al., 2015). Akaike's information criterion (AIC) was applied to estimate the optimal genetic model of each SNP. Haplotype construction and Pairwise linkage disequilibrium were performed using the online SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) (Shi and He, 2005).

CHAPTER-04

Analyis and Interpretation of Data

4. ANALYSIS AND INTERPRETATION OF DATA

4.1. Results of prevalence study

During one year of study, 10212 number of psychiatric patients visited OPD of Dept. of Psychiatry, North Bengal Medical College, Siliguri. Among them, the total number of schizophrenia patients was 1128, consisting of 708 males and 420 females (Table 12). Thus, the overall prevalence rate of schizophrenia was found to be 0.08 per 1000 populations. In addition, the prevalence of schizophrenia was found to be higher in males (0.09) than the females (0.06) (χ^2=71.78, df=1, p=0.00). The total number of patients from the rural area was 732 (64.89%), and 396 (35.11%) patients were from urban areas. The age of onset of symptoms was found to be earlier in males (29.70±13.69 years) than the females (31.16 ±12.78 years) though it was not statistically different.

Table 12. Prevalence of schizophrenia among the patients who visited Department of Psychiatry, North Bengal Medical College, Siliguri.

Variable	**Schizophrenia (N=1128)**	**Test**
Gender Male Female	 708 (62.77%) 420 (37.23%)	χ^2=71.78, df=1, p=0.00
Mean age Male Female	36.94±11.99 35.43 ±12.95 39.48±9.82	t=1.60, df=92, p=0.11
Age of onset Male Female	 29.70±13.69 31.16±12.78	t=0.49, df=92, p=0.62
Urban Rural	396 (35.11%) 732 (64.89%)	

4.2. Results of ELISA assay for CRP and IL-6

The demographic details of study subjects included in this study are presented in table 13. A total of 167 participants were recruited for the study, among them 67 were antipsychotic medicating schizophrenia patients, 28 psychotropic medication-free schizophrenia patients and 72 were age, sex, and ethnicity matched healthy controls. Among the antipsychotic medicating schizophrenia patients 51 (76.1%) were male and 16 (23.9%) were females and among psychotropic medication-free schizophrenia patients 17 (60.7%) were male and 11 (39.3%) were females. Among the controls 55 (76.4%) were male and 17 (23.6%) were females. The mean age of the antipsychotic medication patients (33.89±11.28) and psychotropic medication-free patients (35.21 ± 11.10) were not significantly different than the controls (34.23±9.2). Further, the mean body mass index (BMI) between psychotropic medication-free, antipsychotic medicating patients and the controls were not found to be significantly different from each other.

The comparison of the serum levels of CRP and IL-6 between the patients and controls are summarized in table 14. The serum levels of the CRP were found to be significantly higher in patients who were under the antipsychotic medication than the controls (U=2.76, p=0.01) (Figure 6). On the other hand, although serum CRP levels were found to be higher among the patients who were psychotropic medicating free than the controls, the value was not statistically significant (U=0.49, p=0.62). Besides, the serum levels of IL-6 were found to be significantly high among the patients who were under antipsychotic medication (U=3.68, p=0.00) and patients who were psychotropic medication-free (U=2.44, p= 0.02) than the controls (Figure 7).

Table 13. Demographic characteristics of schizophrenia patients and controls included in CRP and IL-6 study.

Group	**Antipsychotic medication patients N = 67 (a)**	**Psychotropic medication free patients N =28 (b)**	**Control N= 72 (c)**	**Test**
Male	51 (76.1%)	17 (60.7%)	55 (76.4%)	
Female	16 (23.9%)	11 (39.3%)	17 (23.6%)	
Age	Mean =33.89 SD =± 11.28	Mean = 35.21 SD =± 11.10	Mean = 34.23 SD =± 9.2	(a)vs(c): t = 0.13, df =137, p=0.90 (b) vs (c): t = 0.36, df= 93, p=0.72
Duration of illness	Mean = 5.78 SD = ± 5.41	Mean = 5.33 SD = ± 3.68		t = 0.39, df = 93, p=0.69
Age of onset	Mean = 27.85 SD= ±10.23	Mean = 29.50 SD =± 9.53		t = 0.72, df = 93, p=0.46
Body mass index (Kg/m^2)	Mean = 22.37, SD =±3.16	Mean = 21.76 SD = ± 4.31	Mean = 23.21 SD =±3.6	(a)vs(b) : t=0.76, df=93, p=0.44 (a) vs (c) : t=1.68, df=137, p=0.14 (b) vs (c) : t=68, df=98, p=0.09
Smoking status	0	0	0	
BPRS	Mean = 40, SD=1.5	Mean = 44, SD=1.5		t=14.20, df=93, p=0.01
PANSS	Mean=124, SD=±2.0	Mean=115, SD=1.2		t=27.50, df=93, p=0.01
RBANS	Mean= 105, SD=1.0	Mean= 95, SD=1.0		t=9.45, df=93, p=0.01
Antipsychotic dose				
Olazapine	10–20 mg/day			
Quetiapine	200–600 mg/day			
Clozapine	100–300 mg/day			
Risperidone	2–8 mg/day			

Table 14. Comparison of serum CRP and IL-6 between psychotropic medications free and antipsychotic medicating schizophrenia patients, and controls.

Variables	Antipsychotic medicating patients (n=67)		Controls (n=72)		p-Value	Mann-Whitney test
CRP (pg/ml)	Mean=471.0 SD= ±391,5	Median=421.0 IR=393.1-548.9	Mean=337.6 SD=±297.9	Median=212.3 IR=267.6-407.7	0.01	U=2.76
IL-6 (pg/ml)	Mean=105.2 SD= ±43.2	Median=89.2 IR=94.7-115.8	Mean=89.9 SD= ±72.6	Median=48.9 IR=72.9-107.0	0.00	U=3.68
Variables	Psychotropic medication free patients (n=67)		Controls (n=72)		p-Value	Mann-Whitney test
CRP (pg/ml)	Mean=349.1 SD=±296.6	Median=290.2 IR=234.1-464.1	Mean=337.4 SD=±297.9	Median=212.3 IR=267.6-407.7	0.62	U=0.49
IL-6 (pg/ml)	Mean=104.0 SD=±55.0	Median=80.0 IR=82.7-125.6	Mean=89.9 SD=±72.6	Median=48.9 IR=72.9-107.0	0.02	U=2.44

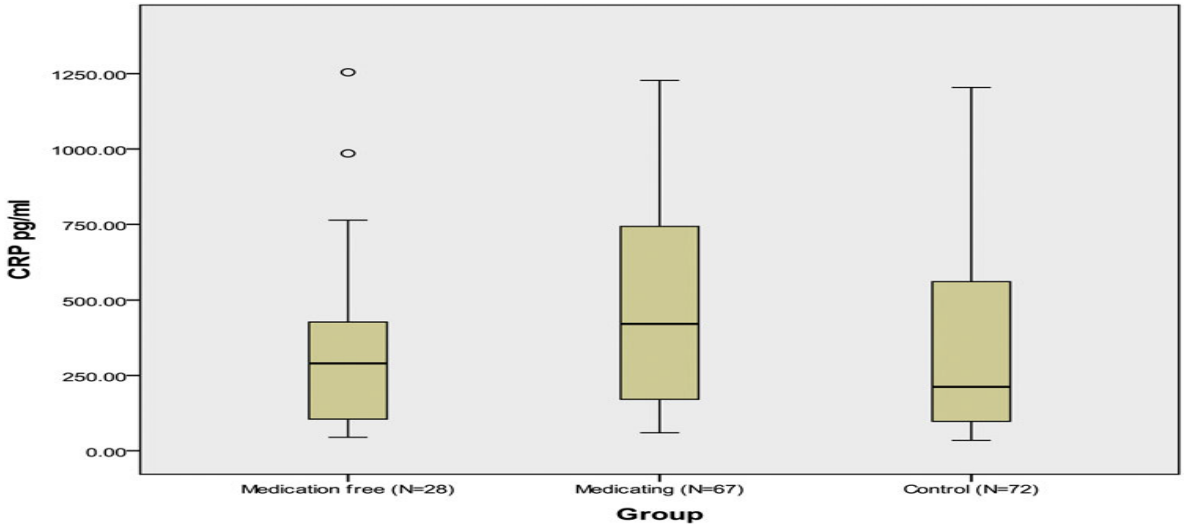

Figure 6. Box plot of serum CRP levels of psychotropic medication free, antipsychotic medicating schizophrenia patients and the controls. Median values are indicated by horizontal lines, outliers are indicated by o.

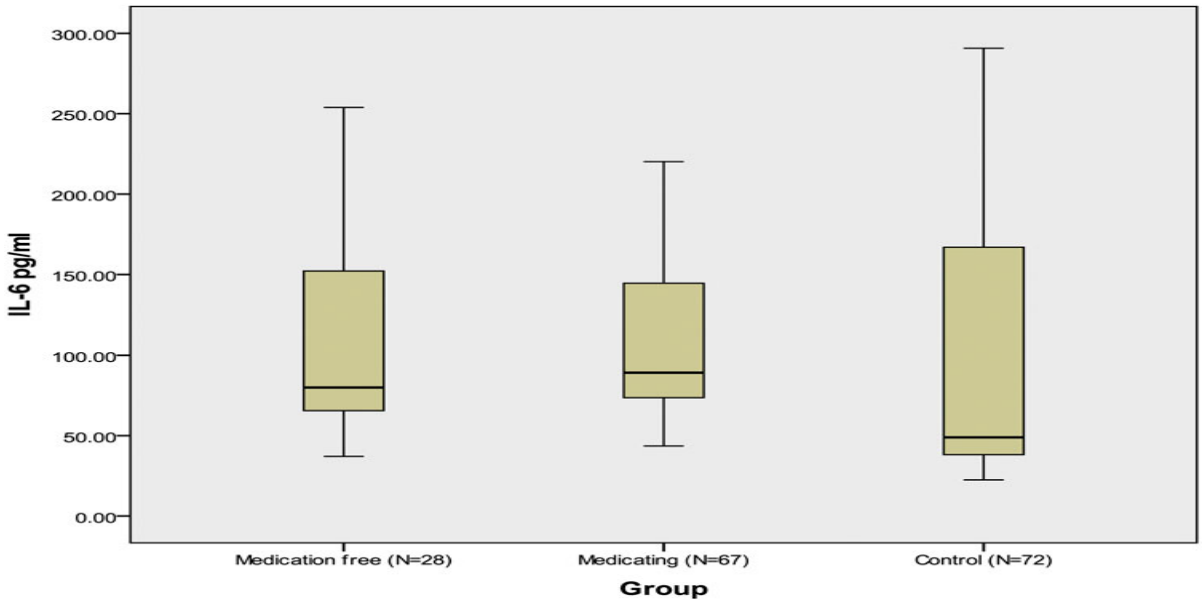

Figure 7. Box plot of serum IL-6 levels of psychotropic medication free, antipsychotic medicating schizophrenia patients and the controls. Median values are indicated by horizontal lines.

4.2.1. The correlation analysis between clinical characteristic and serum levels of CRP and IL-6

Spearman correlation analysis was performed between the serum CRP levels and IL-6 levels. The results revealed that levels of CRP were positively correlated with the

levels of IL-6 (r=0.89, p=0.00) (Figure 8). Further, spearman correlation analysis was performed to understand the correlation between demographic characteristics of the patients and serum levels of CRP and IL-6 (Table 15). The results showed no significant correlation of CRP and IL-6 with age (CRP: r=-0.01, p=0.90; IL-6: r=-0.015, p=0.88), duration of illness (CRP: r=0.05, p=0.57, IL-6: r=0.01, p=0.93) and age of onset (CRP: r=0.00, p=0.99; IL-6: r=0.06, p=0.59). Further, BMI of antipsychotic medicating patients (r=0.04, p=0.73), psychotropic medication-free patients (r=0.00, p=0.99) and controls (r=0.01, p=0.94) was not correlated with the serum levels of CRP. Similarly, BMI of antipsychotic medicating patients (r=-0.23. p=0.06) and the controls (r=0.14, p=0.23) was also not correlated with the levels of IL-6. However, the BMI of psychotropic medication-free patients (r=0.03, p=0.05) was significantly correlated with the levels of IL-6.

Table 15. Spearman's correlation test between demographic details and serum levels of CRP and IL-6 among psychotropic medication free, antipsychotic medicating schizophrenia patients and controls.

Variables	CRP	IL-6
Age	r=-0.01, p=0.90	r=0.015, p=0.88
Age of onset	r=0.00, p=0.99	r=0.06, p=0.59
Duration of illness	r=0.05, p=0.57	r=0.01, p=0. 93

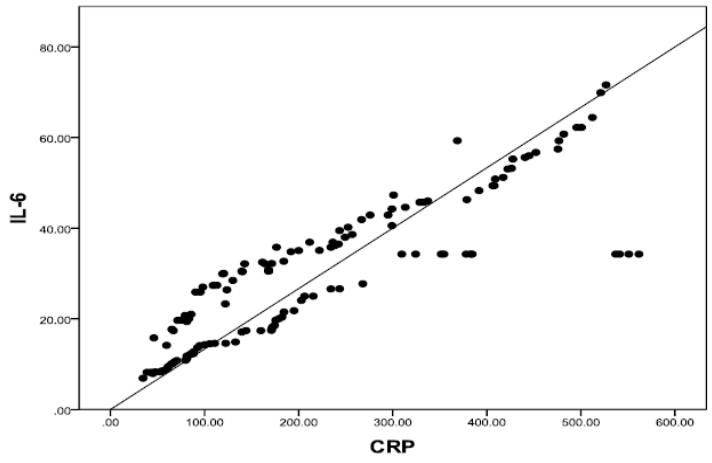

Figure 8. Correlation between CRP and IL-6.

4.3. Results of TLR polymorphism study

The demographic characteristics of patients and controls included in the TLR polymorphism study are presented in table 16. Among 120 recruited patients 67 (55.83%) were male and 53 (44.17%) were females. On the other hand, among 145 recruited controls 90 (62%) were males and 55 (38%) were females. The number of patients (males and females) and controls (males and females) was not significantly different (χ^2=2.36, df=1, p=0.14). The mean age of patients (32.80±11.72) and controls (31.94±12.13) were not significantly different (t=0.53 df=263, p=0.57). Among the patients, the mean age of onset of the symptoms was 27.35 ±12.96 years, and the duration of illness was 7.10 ±6.99 years. All the genotype distributions were in Hardy-Weinberg equilibrium (HWE) for patients and controls (P>0.05), except TLR-7 (rs179009), TLR-8 (rs3764880), TLR-9 (rs5743836) in both patients and controls group (*P*<0.05) (Table 17).

Table 16.Demographic characteristic of schizophrenia patients and control subjects.

Variables	Schizophrenia (n=120)	Control (n=145)	Test
Gender Male Female Total	 67 (55.83%) 53 (44.17%) 120	 90 (62%) 55 (38%) 145	 χ^2=3.37, df=1, p=0.06 χ^2=0.03, df=1, p=0.85 χ^2=2.36, df=1, p=0.14
Age (mean ± SD, years)	32.80±11.72	31.94±12.13	t=0.53 df=263, p=0.57
Duration of illness (mean ± SD, years)	Mean = 7.10 SD±6.99	--	--
Age of onset (mean ± SD, years)	Mean= 27.35 SD±12.96	--	--

Table 17. Hardy Weinberg equilibrium test for the TLR SNPs.

TLR genes	Patients (N=120)	Controls (N=145)
TLR-2 Arg753Gln (rs5743708)	HWE= 0.18, p=0.66	HWE=0.02, p=0.86
TLR-3 c.1377C/T (rs3775290)	HWE=3.13, p=0.07	HWE = 0.02, p=0.88
TLR-4 Asp299Gly(rs4986790)	HWE =0.01, p= 0.91	HWE = 3.24, p=0.07
TLR-4 Thr399Ile (rs4986791)	HWE =0.39, p= 0.59	HWE =0.02, p=0.86
TLR-7 IVS-151 (rs179009)	HWE = 94.2, p= 0.00*	HWE =58.76, p= 0.00*
TLR-8 Met1Val (rs3764880)	HWE = 58.74, p= 0.00*	HWE =76.03, p= 0.00*
TLR-9 Pro545Pro (rs352140)	HWE =0.61, p= 0.43	HWE 2.71, p=0.09
TLR-9 1237T/C (rs5743836)	HWE =108,48, p=0.00*	--

The genotype and allele frequency of TLR among the patients and controls is summarized in table 18. It was observed that the frequency of G/A heterozygous mutant genotype of TLR-2 rs5743708 was higher in patients than the controls, however, it was not found to be significant. Besides, A/A mutant homozygous recessive genotype was not detected in both the patients and controls (Figure 9). Although the A allele of TLR-2 rs5743708 was found to be higher in schizophrenia patients (OR=2.78, 95%CI=0.84-9.16, p=0.07) it was not significant. Further, in the case of TLR-2 rs121917864 SNP, no mutation was observed in both patients and controls. On the other hand, for TLR-3 rs3775290 T/T mutant homozygous recessive genotype was significantly higher in controls than the patients. Similarly, the frequency of T allele of TLR-3 rs3775290 was found to be significantly higher in controls than the patients (OR=0.63, 95%CI= 0.44-0.90, p=0.01). Moreover, no mutation was observed for TLR-3 rs3775296 in both the patients and controls. Further, the frequency of heterozygous mutant A/G genotype of TLR-4 rs4986790 was found to be significantly high in patients than the controls and the G allele was found to be significantly high in schizophrenia patients (OR=2.68; 95% CI =1.28-5.63, p=0.01). Similarly, the frequency of C/T heterozygous mutant genotypes of TLR-4 rs4986791 was found to be significantly high in patients than the controls. Contrastingly, the mutant homozygous recessive genotype TT was not observed the

studied subjects. Moreover, T allele was found to be significantly high in the patients (OR= 4.09; 95% CI=1.31-12.73, p=0.01). With reference to TLR-7 rs179009 SNP, the heterozygous mutant genotype A/G of was found to be significantly high in control than the patients. Even though the frequency of G allele (OR=0.84, 95%CI=0.49-0.42, p=0.51) was high in the controls it was not significant. Since TLR-7 gene is located in the X chromosome, it was further analyze the genotype frequency between males and females separately. The results showed that A/G heterozygous mutant genotype frequency was significantly high in female controls than the female patients. However, G/G or G/- mutant homozygous recessive genotypes were not significant in both the male and female subjects. Similarly, in the case of TLR-8 rs3764880, the frequency of A/G heterozygous mutant and G/G homozygous mutant recessive genotype was high in controls than the patients, but it was not significant. The frequency of G allele of TLR-8 was also not found to vary between patients and controls (OR=1.08, 95%CI=0.77-1.52, p=0.51). Since the gene for TLR-8 is also located in X chromosome, therefore, it was further analyze the male and female separately. However, the genotypes and allelic distribution of TLR-8 rs3764880 was not found to vary between male and female subjects (Table 19). On the other hand, though the frequency of C/T heterozygous mutant and TT homozygous mutant genotypes of TLR-9 rs352140 was found to be higher in patients than the controls, it was not significantly different. However, the frequency of T allele of TLR-9 rs352140 was found to be significantly high in patients than the control (OR=1.77, 95%CI=(1.37-2.83, P=0.00). Besides, the study of TLR-9 rs5743836 revealed the presence of only three C/T heterozygous mutant genotypes in patients whereas no mutation was observed in the controls.

Table 18. Genotype distribution and allele frequency of TLRs gene between patients and controls. N= number of participants.

TLR SNP	Genotype/Allele	Patients (N=120) frequency %	Control (N=145) frequency %	Odd ratio (95% CI)	P-value
TLR-2 Arg753Gln (rs5743708)	GG	111 (92.50%)	141(97.24)	--	0.06
	GA	9(7.50%)	4(2.76)	--	0.17
	AA	00 (00%)	00 (00%)	--	--
	G	231 (96.25%)	286 (98.62%)	1	--
	A	9 (3.75%)	4 (1.38%)	2.78 (0.84-9.16)	0.07
TLR-2 Arg677Trp (rs121917864)	TT	120 (100%)	145 (100%)	--	0.14
	TC	00 (00%)	00 (00%)	--	--
	CC	00 (00%)	00 (00%)	--	--
	T	240 (100%)	290 (100%)	--	--
	C	00 (00%)	00 (00%)	--	--
TLR-3 c.1377C/T (rs3775290)	CC	54(45.00%)	50(34.48%)	--	0.15
	CT	59(49.17%)	71(48.97%)	--	0.08
	TT	7(5.83%)	24(16.55%)	--	0.00*
	C	167(69.58%)	171(58.97%)	1	--
	T	73(30.42%)	119(41.03%)	0.63(0.44-0.90)	0.01*
TLR-3 _7 C/A (rs3775296)	CC	120 (100%)	145 (100%)	--	0.14
	AC	00 (00%)	00 (00%)	--	--
	AA	00 (00%)	00 (00%)	--	--
	C	240 (100%)	290 (100%)	--	--
	A	00 (00%)	00 (00%)	--	--
TLR-4 Asp299Gly (rs4986790)	AA	98 (81.67%)	135 (93.10%)	--	0.02*
	AG	21 (17.50%)	9 (6.21%)	--	0.04*
	GG	1 (0.83%)	1 (0.69%)	--	1
	A	217 (90.42%)	279 (96.21%)	1	--
	G	23 (9.58%)	11 (3.79%)	2.68 (1.28-5.63)	0.01*

Table 18. Continue.

TLR SNP	Genotype/Allele	Patients (N=120) frequency %	Control (N=145) frequency %	Odd ratio (95% CI)	P-value
TLR-4 Thr399Ile (rs4986791)	CC	107 (89.17%))	141 (97.24%)	--	0.04
	CT	13 (10.83%)	4 (2.76%)	--	0.05*
	TT	00 (00%)	00(00%)	--	--
	C	227(94.92%)	286 (98.62%)	1	--
	T	13(5.08%)	4(1.38%)	4.09 (1.31-12.73)	0.01*
TLR-7 IVS-151 (rs179009)	AA or A/-	105 (87.50%)	120 (82.76%)	--	0.32
	AG	3 (2.50%)	12 (8.28%)	--	0.02*
	GG or G/-	12 (10.00%)	13(8.96%)	--	0.84
	A	213 (88.75%)	252 (86.90%)	1	--
	G	27 (11.25%)	38 (13.10%)	0.84 (0.49-0.42)	0.51
TLR-8 Met1Val (rs3764880)	AA or A/-	49 (40.83%)	63(43.45%)	--	0.22
	AG	18 (15.00%)	20(13.79%)	--	0.10
	GG or G/-	53 (44.17%)	62 (42.76%)	--	0.70
	A	116 (48.33%)	146 (50.34%)	1	--
	G	124(51.67%)	144 (49.66%)	1.08 (0.77-1.52)	0.34
TLR-9 Pro545Pro (rs352140)	CC	37 (30.83%)	80 (55.18%)	--	0.00*
	CT	63 (52.50%)	50 (34.48%)	--	0.22
	TT	20 (16.67%)	15(10.34%)	--	0.39
	C	137 (57.08%)	210 (72.41%)	1	--
	T	103 (42.92%)	80 (27.59%)	1.77 (1.37-2.83)	0.00*
TLR-9 1237T/C (rs5743836)	TT	117 (97.5%)	145 (100%)	--	0.08
	TC	3 (2.5%)	00 (00%)	--	--
	CC	00 (00%)	00 (00%)	--	--
	C	237 (98.75%)	290 (100%)	--	--
	T	3 (1.25%)	00 (00%)	--	--

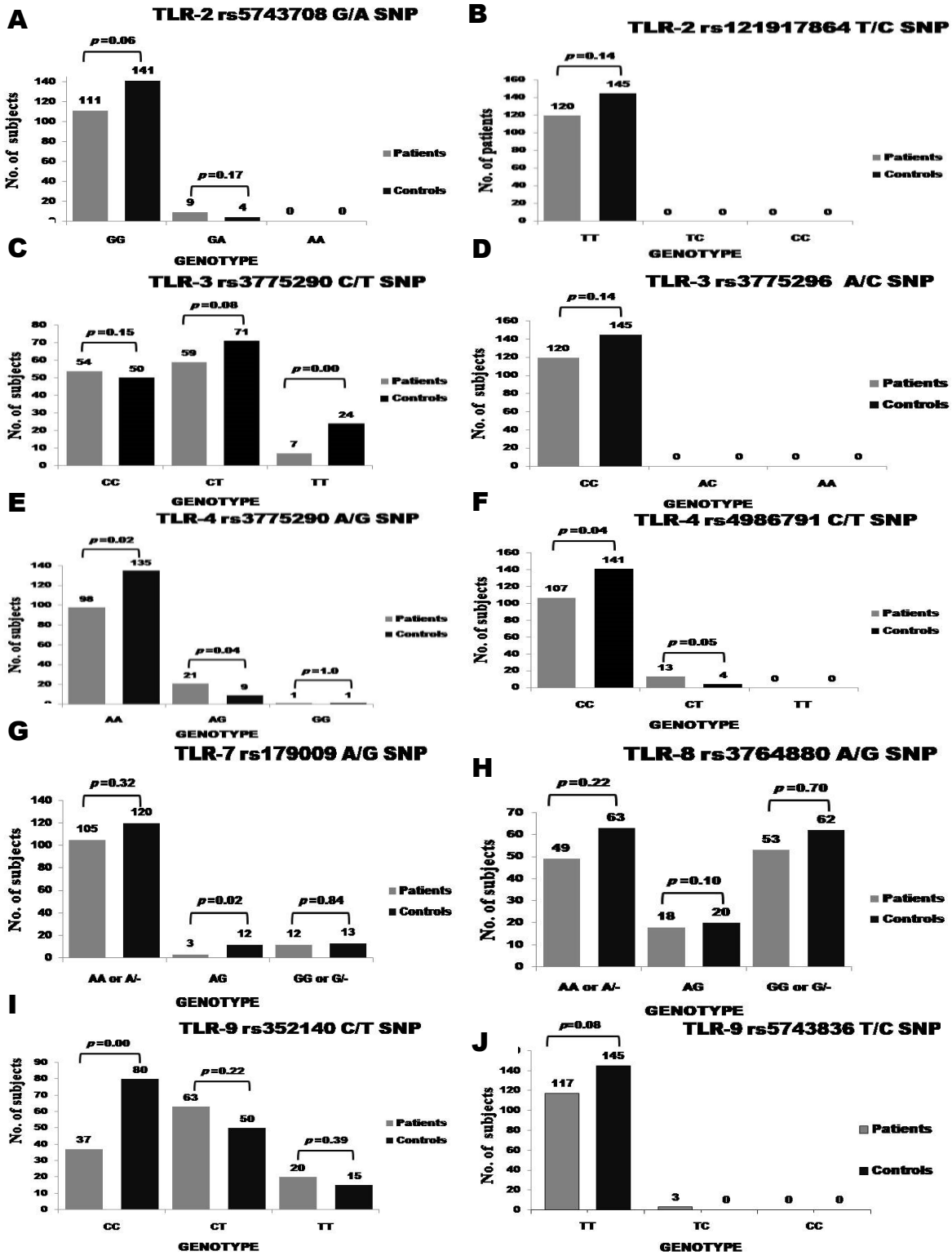

Figure 9. Bar diagram showing genotype frequencies of TLR genes: (A) TLR-2 rs5743708 G/A SNP, (B) TLR-2 rs121917864 T/C SNP, (C) TLR-3 rs3775290 C/T SNP, (D) TLR-3 rs3775296 A/C SNP, (E) TLR-4 rs3775290 A/G SNP, (F) TLR-4 rs4986791 C/T SNP, (G) TLR-7 rs179009 A/G SNP, (H) TLR-8 rs3764880 A/G SNP (I) TLR-9 rs352140 C/T SNP, (J) TLR-9 rs5743836 T/C SNP.

Table 19. Gender specific distribution of genotype and allele of TLR- 7 and TLR-8 among the patients and controls. * Significant.

TLR SNP	Genotype	Patients	Controls	P-value
TLR-7 Female	AA	48 (87.5%)	40 (72.73%)	0.39
	AG	3 (2.5%)	12 (21.82%)	0.02*
	GG	2 (10%)	3 (5.45%)	0.65
TLR-7 Male	A /-	57 (85.07%)	80 (88.89%)	0.49
	G /-	10 (14.93%)	10 (11.11%)	1
TLR-8 Female	AA	16 (30.19%)	17 (30.91%)	0.65
	AG	18 (33.96%)	20 (36.36%)	0.10
	GG	19 (35.85%)	18 (32.73%)	0.65
TLR-8 Male	A/-	33 (49.25%)	46 (51.11%)	0.14
	G/-	34 (50.75%)	44(48.89%)	0.25

4.3.1. Optimal genetic model study of TLR SNPs in schizophrenia

Optimal genetic model was used to analyze the association of SNPs on the basis of lower Akaike information criterion (AIC) value (Shimo-onoda et al., 2002) (Table 20). For TLR-3 rs3775290 genotype C/T (OR= 0.74, 95%CI=0.43-1.26, p=0.01), T/T (OR=0.20, 95%CI=0.08-0.55, p=0.01) in Co–dominant model, C/C, T/T in dominant model (OR=0.61, 95%CI= 0.36-1.02, p= 0.05) and T/T in recessive model (OR=0.25, 95%CI=0.01-0.62, p=0.01) was found to be negatively associated with schizophrenia. Based on ACI value recessive model was the optimal genetic model for TLR-3 rs3775290. Further, TLR-4 rs4986790 A/G genotypes (OR=3.48, 95%CI=1.51-8.00, p=0.01) and G/G genotype (OR=1.27, 95%CI=0.08-20.74, p=0.01) in co-dominant model, A/G and G/G in dominant model (OR=3.24, 95%CI=1.46-7.22, p=0.00), and A/G genotype (OR=1.23, 95%CI=0.55-2.77, p=0.02) in over-dominant model were found to be significantly associated with schizophrenia. Based on the ACI value, over-dominant model was the best-fit model. In case of TLR-7 rs179009 SNP A/G, G/G in co-dominant model (OR = 0.15, 95%CI=0.04-0.66, p=0.01) (OR=0.23, 95%CI=0.02-2.26, p=0.01), A/G, G/G in dominant model (OR=0.17, 95%CI=0.05-0.61, p=0.00) and A/G in over-dominant model (OR=0.16, 95%CI=0.04-0.70,

p=0.01) was found to be significantly associated with schizophrenia. Based on the AIC value dominant model was found to be the optimal genetic model. Contrastingly, in case of TLR-8 rs3764880 SNP none of the genotype was found to be significantly associated in any of the models. However, A/G and G/G genotype in the dominant model was the optimal genetic model. The genetic modelling study showed that in case of TLR-9 rs352140 SNP C/T (OR=2.83, 95%CI=1.64-4.89, p=0.00) and T/T (OR=3.06, 95%CI=1.40-6.73, p=0.00) genotypes in co-dominant model, C/T and T/T in dominant model (OR=2.88, 95%CI= 1.72-4.83, p=0.00), T/T in recessive model (OR=1.81, 95%CI= 0.87-3.74, p=0.04) and C/T of over-dominant (OR=2.14, 95%CI=1.30-3.53, p=0.00) was found to be significantly associated with schizophrenia. TLR-9 rs352140 in the dominant model was found to be the optimal model.

Table 20. Genetic model study of the association of TLR polymorphism with schizophrenia. * Significant

SNP ID	Model	Genotype	Patients (N=120)	Control (N=145)	OR (95% CI)	P-value AIC value
TLR-3c.1377C/T (rs3775290)	Co–dominant	C/C	54 (45.0%)	50 (34.5%)	1	0.01* 360
		C/T	59 (49.2%)	71 (49.0%)	0.74 (0.43-1.26)	
		T/T	7 (5.8%)	24 (16.6%)	0.20 (0.08-0.55)	
	Dominant	C/C	54 (45.0%)	50 (34.4%)	1	0.05* 366
		C/C-T/T	66 (55.0%)	95 (65.5%)	0.61 (0.36-1.02)	
	Recessive	C/C-C/T	113 (94.2%)	121 (83.5%)	1	0.01* 359.2
		T/T	7 (5.8%)	24 (16.6%)	0.25 (0.01-0.62)	
	Over -dominant	C/C-T/T	61 (50.8%)	74 (51.0%)	1	0.87 369.6
		C/T	59 (49.2%)	71 (49.0%)	1.04 (0.64-1.70)	
	Log-additive	--	--	--	0.55 (0.37-0.82)	0.00 360.7
TLR-4 Asp299Gly (rs4986790)	Co–dominant	A/A	98 (81.7%)	135 (93.1%)	1.00	0.01* 362.1
		A/G	21 (17.5%)	9 (6.2%)	3.48 (1.51-8.00)	
		G/G	1 (0.8%)	1 (0.7%)	1.27 (0.08-20.74)	
	Dominant	A/A	98 (81.7%)	135 (93.1%)	1	0.00* 360.6
		A/G-G/G	22 (18.3%)	10 (6.9%)	3.24 (1.46-7.22)	
	Recessive	A/A-A/G	119 (99.2%)	144 (99.3%)	1	0.94 360.6
		G/G	1 (0.8%)	1 (0.7%)	1.12(0.07-18.20)	
	Over -dominant	A/A-G/G	99 (82.5%)	136 (93.8%)	1	0.02* 360.2
		A/G	21 (17.5%)	9 (6.2%)	1.23 (0.55-2.77)	
	Log-additive	--	--	--	2.75 (1.30-5.82)	0.01* 361.9

Table 20. Continue.

SNP ID	Model	Genotype	Patients (N=120)	Control (N=145)	OR (95% CI)	P-value AIC value
TLR-7 IVS-151 (rs179009)	Co–dominant	A/A	48 (90.6%)	40 (72.7%)	1	0.01* 133.9
		A/G	3 (5.7%)	12 (21.8%)	0.15 (0.04-0.66)	
		G/G	2 (3.8%)	3 (5.5%)	0.23 (0.02-2.26)	
	Dominant	A/A	48 (90.6%)	40 (72.7%)	1	0.00* 132
		A/G-G/G	5 (9.4%)	15 (27.3%)	0.17 (0.05-0.61)	
	Recessive	A/A-A/G	51 (96.2%)	52 (94.5%)	1	0.3 139.8
		G/G	2 (3.8%)	3 (5.5%)	0.31 (0.03-3.34)	
	Over -dominant	A/A-G/G	50 (94.3%)	43 (78.2%)	1	0.01* 133.5
		A/G	3 (5.7%)	12 (21.8%)	0.16 (0.04-0.70)	
	Log-additive	--	--	--	0.27 (0.09-0.78)	0.01* 133.4
TLR-8 Met1Val (rs3764880)	Co–dominant	A/A	20 (37.7%)	18 (32.7%)	1	0.58 141.8
		A/G	17 (32.1%)	20 (36.4%)	0.62 (0.26-1.66)	
		G/G	16 (30.2%)	17 (30.9%)	0.64 (0.23-1.80)	
	Dominant	A/A	20 (37.7%)	18 (32.7%)	1	0.3 139.8
		A/G-G/G	33 (62.3%)	37 (67.3%)	0.63 (0.26-1.50)	
	Recessive	A/A-A/G	37 (69.8%)	38 (69.1%)	1	0.66 140.7
		G/G	16 (30.2%)	17 (30.9%)	0.83 (0.33-2.01)	
	Over -dominant	A/A-G/G	36 (67.9%)	35 (63.6%)	1	0.54 140.5
		A/G	17 (32.1%)	20 (36.4%)	0.76 (0.32-1.80)	
	Log-additive	--	--	--	0.79 (0.47-1.33)	0.38 140.2

Table 20. Continue.

SNP ID	Model	Genotype	Patients (N=120)	Control (N=145)	OR (95% CI)	P-value AIC value
TLR-9 Pro545Pro (rs352140)	Co–dominant	C/C	37 (30.8%)	80 (55.2%)	1	0.00* 353.9
		C/T	63 (52.5%)	50 (34.5%)	2.83 (1.64-4.89)	
		T/T	20 (16.7%)	15 (10.3%)	3.06 (1.40-6.73)	
	Dominant	C/C	37 (30.8%)	80 (55.2%)	1	0.00* 351.9
		C/T-T/T	83 (69.2%)	65 (44.8%)	2.88 (1.72-4.83)	
	Recessive	C/C-C/T	100 (83.3%)	130 (89.7%)	1	0.04* 366.3
		T/T	20 (16.7%)	15 (10.3%)	1.81 (0.87-3.74)	
	Over -dominant	C/C-T/T	57 (47.5%)	95 (65.5%)	1	0.00* 359.8
		C/T	63 (52.5%)	50 (34.5%)	2.14 (1.30-3.53)	
	Log-additive	--	--	--	1.99 (1.38-2.89)	0.00* 354.9

4.3.2. Results of TLR haplotype analysis

The results of the haplotype analysis showed that G-C (OR=2.6, 95%CI=1.20-5.70, p=0.02) and A-T haplotypes (OR=3.8, 95%CI=1.17-1.26, p=0.03) between TLR-4 rs4986790 and TLR-4 rs4986791 SNPs were significantly associated with schizophrenia. On the other hand, only T-T haplotype between the SNPs TLR-9 rs5743836 and TLR-9 rs352140 was found to be significantly associated with schizophrenia (OR=2.01, 95%CI=1.38-2.92, p=0.00). Further, none of the haplotypes of TLR-7 rs179009 and TLR-8 rs3764880 was found to be significantly associated with schizophrenia (Table 21). Linkage disequilibrium analysis (LD) showed a limited LD among the haplotypes.

Table 21. Haplotype construction of the TLR gene. * Significant

SNP ID	Haplotypes	Frequencies%		OR (95% CI)	P-	D'	r^2
		Patients	controls		value		
TLR-4 rs4986790 v/s TLR-4 rs4986791	A-C	0.855	0.95	1	--	0.07	0.03
	G-C	0.086	0.038	2.6 (1.20-5.70)	0.02*		
	A-T	0.045	0.012	3.8 (1.17-1.26)	0.03*		
	G-T	0.014	NA	--	--		
TLR-7 rs179009 v/s TLR-8 rs3764880	G-A	0.53	0.44	1	---	0.97	0.08
	A-A	0.41	0.4	0.79 (0.45-1.38)	0.41		
	A-G	0.05	0.09	0.29 (0.07-1.15)	0.08		
	G-G	0.01	0.07	0.18 (0.03-1.24)	0.08		
TLR-9 rs5743836 v/s TLR-9 rs352140	C-T	0.55	0.72	1	--	0.08	0.00
	T-T	0.43	0.28	2.01 (1.38 - 2.92)	0.00*		
	C-C	0.02	NA	--	--		
	T-C	0.00	NA	--	--		

Table 22. Multivariate logistic regression for TLR-4 rs4986790 SNP and TLR-9 rs352140 SNP. * Significant.

Variable	TLR-4 rs4986790	TLR-9 rs352140
Age	OR=0.94, CI=0.6-1.2, p=0.04*	OR=1.02, CI=0.7-1.4, p=0.49
Age of onset	OR=1, CI=0.9-1.1, p=0.19	OR=0.96, CI=0.8-1.2, p=0.12
Duration of illness	OR=1, CI=0.9-1.1, p=0.36	OR=1.1, CI=0.9-1.2, p=0.84

4.3.3. Multivariate logistic regression analysis

Multivariate logistic regression analysis was performed for TLR-4 rs4986790 SNP and TLR-9 rs352140 SNP to understand the association of schizophrenia with demographic characteristics of the patients viz. age, age of one set and duration of illness (Table 22). The results of TLR-4 showed that only age (OR=0.94, CI=0.6-1.2, p=0.04) was significantly associated with schizophrenia, while other variables such as the age of onset (OR=1, CI=0.9-1.1, p=0.19), and duration of illness (OR=1, CI=0.9-1.1, p=0.36) were not significantly associated with schizophrenia [Figure 10 (A) and (B)]. Further, in case of TLR-9, age (OR=1.02, CI=0.7-1.4, p=0.49), age of onset (OR=0.96, CI=0.8-1.2, p=0.12), and duration of illness (OR=1.1, CI=0.9-1.2, p=0.84) was not significantly associated with schizophrenia.

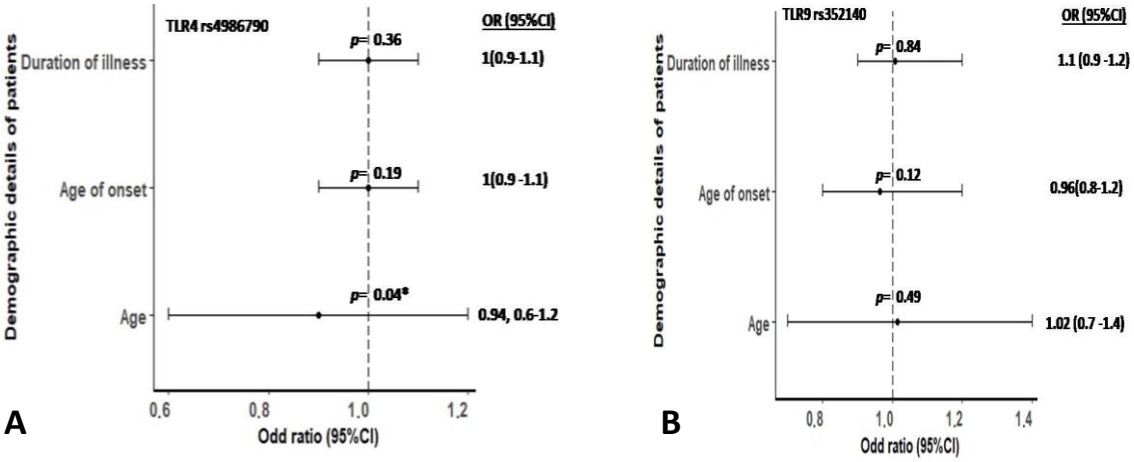

Figure 10. Multivariate logistic regression analysis for association of TLR-4 rs4986790 (A) and TLR-9 rs352140 (B) polymorphisms with demographic details of patients. Data are expressed as odds ratio (OR) at 95% confidence interval (CI) with the p-values < 0.05 considerded as significant.

CHAPTER-05

Discussion

5. DISCUSSION

Despite decades of research the pathophysiology of schizophrenia is still not clearly understood. Evidence from the various studies has advocated that multiple etiological factor involving genetic, neurodevelopment, neurotransmitters, immunological and inflammatory processes may play a very important role in schizophrenia. Even though, the possible role of inflammatory processes in the pathophysiology of schizophrenia was proposed forty years ago, the research in this aspect of schizophrenia had long been neglected (Torrey and Peterson, 1973; Horrobin, 1977). Recently, immunological and genetic studies have put forward a strong evidence for the involvement of an immunological process and inflammation in the etiopathology of schizophrenia. Although several studies were carried out to investigate the role of inflammatory processes in schizophrenia, the findings are largely inconsistent. The lack of consistency in the findings regarding inflammatory processes in schizophrenia may be due to the small sample size, nonconsideration of medication status and lack of cumulative approach in the study. The present study is the first of its kind among the schizophrenia patients of Siliguri, West Bengal to understand the role inflammatory process in the etiopathology of schizophrenia. I investigated the components of the immune system viz. C-reactive protein, interleukin-6, and Toll-like receptors, which play a very important role in the inflammatory process. In addition, the prevalence of schizophrenia was studied to investigate the disease burden among the denizens of North Bengal, India. In the following section, each component of the study is discussed separately.

5.1. Prevalence study

It is reported that after depressive and anxiety disorders, schizophrenia is the highest disease burden in India among all the mental disorders. Irrespective of it the studies of the prevalence of mental disorders in the various states of India are meagre (Sagar et al., 2020). In the present study, prevalence of schizophrenia was studied among the denizens of North Bengal based on single centric study at NBMCH, Siliguri. It was observed that the prevalence of schizophrenia among the denizens of North Bengal is 0.08 per 1000 population. Besides, schizophrenia was found to be common among the age group of 20-65 years. Our findings are not in concordance with national prevalence which is 2.3 per 1000 population (Rajan et al., 2012). The present finding is also not in agreement with the study conducted in Chennai where the prevalence of schizophrenia was found to be 3.87/1000 population (Indian Council of Medical Research, 1990). The non-agreement of the present prevalence rate with previous studies may be due to the study settings i.e., general population vs. hospital-based study, urban/rural vs. mixed population etc. It was observed that the prevalence of the mental disorder is high in Southern states than in the Northern states of India (Sagar et al., 2020). It was hypothesized that the higher levels of modernization and urbanization in Southern states could be a factor for the higher prevalence of mental disorders (Trivedi et al., 2008; Jiloha et al., 2009; Hidaka et al., 2012; Chandra et al., 2018). Further, the prevalence study of schizophrenia in other countries such as Taiwan reported a rate of 3.3 per 1000 population (Chen et al., 2004). Saha et al. (2005) conducted a meta-analysis by the inclusion of 188 articles published in the international journals and reported schizophrenia prevalence of 3.3 per 1000 (Saha et al., 2005). However, a recent meta-analysis of 73 studies has observed a prevalence of 4.03 per 1000 individuals (Moreno-Küstner et al., 2018). Thus, the prevalence of

schizophrenia appears to differ by a range of 2-3/ 1000 population all over the world (Moreno-Küstner et al., 2018). Besides, most of the studies involving gender-wise distribution of schizophrenia suggest a higher prevalence of schizophrenia among the male than the female (Tu et al., 2017; Aleman et al., 2003). Studies from Nepal and Bangladesh also reported that higher prevalence of schizophrenia among the male than the female (Banerjee et al., 2012; Mahmud et al., 2015). On the contrary, studies from China reported a higher prevalence of schizophrenia among the female than the male (Cooper et al., 1996; Ding et al., 2020). Many studies reported a late onset of schizophrenia among females (Castle et al., 2000; Borowska-Beszta et al., 2014). Thus, it has been speculated that a higher prevalence of schizophrenia in females is confounded by age bias (Aleman et al., 2003). Therefore, the reason behind the discrepancies between the present and previous studies may be due to the inclusion of older-aged patients. It has been estimated that the average age of onset of schizophrenia for males is 15–25 years and for females 25–35 years (wiener and de-Girolamo 1995; Kaplan and Sadock 2003). In the present study, it was observed that the male patients had an earlier age of onset of schizophrenia (29.70±13.69 years) than the females (31.16 ±12.78 years) which is in accordance with earlier studies (Hafner et al., 1993; Gorwood et al., 1995; Takahashi et al., 2000). The late age of onset of schizophrenia in females may be due to the fact that most of the females have the tendency to remain at home and thus conceal the fact about their illness for a longer period of time (Chen et al., 1996). In addition, the late onset of schizophrenia in females may be due to the protective role of estrogen for schizophrenia in the female (Markham 2012; Riecher-Rössler 2017). In the present study, it was observed that most of the schizophrenia patients belong to rural areas. The finding is in accordance with the study conducted among the Chinese population (Liu et al 2015;

Wang et al., 2020). The reason behind the high prevalence of schizophrenia among the rural population is not clearly understood. However, in the Indian scenario it can be mentioned that the majority of the population in India live in the rural area (72.22%) than the urban area (27.78%) due to which a higher prevalence of schizophrenia is often observed among the rural population (Momi et al., 2016). In addition, the higher prevalence of schizophrenia in the rural population may be due to limited accessibility of health care services in rural areas, low socioeconomic status, and poor health and hygiene among the rural population (McCall-Hosenfeld 2014; Veerapu et al., 2016; Adjei et al., 2012). However, our result is not in agreement with the previous findings which reported an increased incidence of schizophrenia among the urban population (Torrey et al., 1991; O' Callaghan et al., 1995; Kelly et al., 2010). The disagreement in the findings may be due to the differences in the methodology of the study and the geographical differences of the study area (Lin and Goodman, 1992; Moreno-Küstner et al., 2014). The present study is the first of its kind to report one-year prevalence data of schizophrenia among the denizens of Siliguri, India. Our preliminary findings may help to develop an effective treatment, strategies to improve access to health services and may serve as important baseline information for future research. Future studies are warranted to validate our findings in the larger sample size of the population based on the multicenter study.

5.2. C-reactive protein and Interleukin-6 study

Several studies have observed the abnormalities in the immune system in schizophrenia patients (Horváth and Mirnics, 2014; Khandaker et al., 2015; Müller et al., 2016). Recently, Fond et al. (2017) proposed that chronic low-grade inflammation may play an important role in the etiopathology of schizophrenia. Therefore, various

studies have been undertaken to understand the role of inflammation in the etiopathology of schizophrenia using C-reactive protein, a well-known inflammatory marker. The role of the inflammatory process in the pathophysiology of schizophrenia got further strength when several meta-analyses reported elevated levels of CRP among schizophrenia patients (Miller et al., 2014; Ford et al., 2018). Recently, a study observed that the higher serum level of CRP is also associated with worse cognitive performance in patients with deficit schizophrenia (Pan et al., 2020).

To the best of our knowledge, the present investigation is the first attempt to quantitatively study the serum levels of CRP among India-born Bengalee schizophrenia patients. The serum levels of CRP were examined separately among the cohort of psychotropic medication free and anti-psychotic medicating patients, as the previous studies have reported the possible immunomodulatory effect of antipsychotic drugs in schizophrenia (Akanji et al., 2009). Our results show a significant increase level of CRP among antipsychotic medicating patients which are in agreement with the previous studies (Baptista et al., 2007; Carrizo et al., 2008; Mayer et al., 2009; Löffler et al., 2010; Diaz et al., 2010). In the present investigation, antipsychotic medicating patients were under neuroleptic drugs such as risperidone, clozapine, olanzapine and quetiapine. Among these drugs, olanzapine, quetiapine (Meyer et al., 2009), clozapine (Löffler et al., 2010) are suggested to cause elevated levels of CRP. Thus, elevated levels of CRP among medicating patients may be due to the immunomodulatory effect of olanzapine, quetiapine and clozapine. In addition, a population-based study suggested that antipsychotics drugs cause the elevation of CRP levels in those schizophrenia patients who had normal levels of CRP at the baseline (Suvisaari et al., 2011). However, in a recent study, CRP levels were not found to be increased after the initiation of antipsychotic treatment (Steiner et al.,

2020). Furthermore, a study found that antipsychotics do not affect CRP levels in individuals who already have elevated CRP levels, implying a probable higher general inflammation from the beginning in the patients (Kraemer et al., 2011). In the present study, the average length of illness for patients taking antipsychotic medication was 5.78 years, and the majority of the patients came from a low-socioeconomic background having a poor dietary supply of food. Thus, it can be suggested that patients included in the study may have subclinical inflammation from the beginning which could have led to elevated CRP levels among them (Pepys and Hirschfield, 2003). Further, a study reported that the use of antipsychotic drugs may cause excessive body weight gain (Baptista et al., 2007). Besides, in obese individuals, the invasion of macrophages into adipocytes may trigger inflammatory reactions and increase the CRP levels (Weisberg et al., 2003). In the present study, the BMI between antipsychotic medicating, medication-free patients and the controls were not found to vary. Thus, the influence of BMI in the levels of CRP can be ruled out in our study because the value of BMI in all the participants was in the normal range (18.5–24.9). In addition, the correlation between BMI and CRP was not observed. In contrast to our results, Henderson et al. (2009) found reduced CRP levels among antipsychotic medicating patients. The possible reason for disagreement of our findings with Henderson's study might be due to the different methods applied for the assay and the small sample size. Further, even though CRP levels were found to be higher in patients who were not under psychotropic medications, the difference was not statistically significant. Our results do not corroborate with a recent study in which higher levels of CRP were found in first-episode schizophrenia patients than the controls (Zhu et al., 2019; Bolu et al., 2019). The possible reason behind the nonagreement of the results may be due to differences in samples (plasma vs. serum),

assay methods, sensitivity of tests (ELISA vs. latex turbidimetry and pg/ml vs. mg/L), inclusion of controls (unrelated individuals vs. first-degree relatives), progression of the disease, and differences in ethnicity of the studied population under study. Furthermore, present results also do not agree with the study conducted among the South Indian population (Devanarayanan et al., 2017), which may be due to variation in the sample size, selection of only male patients, and ethnic differences between the studied population. Nonetheless, our results were in accordance with the study from Jaipur, India (Solanki et al., 2007) and Egypt (Fawzi et al., 2011). However, the present investigation has a lead over earlier investigations from India, for the inclusion of serum CRP and its modulating cytokines IL-6 for the study among the psychotropic medication-free and antipsychotic medicating patients. Furthermore, the sample size was comparatively more in the present investigation than in the previous two studies from India.

CRP belongs to the group of a protein called acute-phase protein which is produced by hepatocytes under the stimulation of IL-6. Moreover, it was suggested that IL-6 has trophic effects on oligodendroglia and glial cells which may result in the overexpression of the glial fibrillary acidic protein (Kahn et al., 1994). Further, it has been reported that IL-6 can also influence the synthesis of calcium through activation of N-methyl-D-aspartate receptor (NMDA) causing neuronal cell death (Kahn et al., 1998). Besides, IL-6 is a major cytokine involved in microglial activities which can exert a neurotoxic or neuroprotective effect (Eskes et al., 2002; Graver et al., 2003; Krady et al., 2008). Therefore, in the present study IL-6 was also investigated along with the CRP to understand its immunomodulatory role in schizophrenia. However, unlike the results of CRP the level of IL-6 was observed to be significantly high in both psychotropic medication free and antipsychotic medicating patients than the

controls. Our findings of elevated levels of both CRP and IL-6 in antipsychotic medicating patients were in agreement with the previous studies (Frydceka et al., 2015). Contrastingly, some of the previous studies were not in agreement with our results and reported the decreased levels of IL-6 in antipsychotic medicating patients (Sobi´s et al., 2014; Subbanna et al., 2020). Previously Singh et al. (2009) investigated the serum levels of IL-6 among the independent group of Bengalee schizophrenia patients and reported the decreased levels of IL-6, which is in contrast to the present findings (Singh et al., 2009). The possible reason for the discrepancy of our result with the previous investigation might due to the consideration of median value in the present investigation. In addition, the sample size was relatively small in the previous study. To conclude, the results of the present investigation provide evidence for the presence of inflammatory processes among schizophrenia patients.

5.3. Toll like receptor gene polymorphism study

It is well evident that the poor clearance of pathogens due to dysfunction of the immune system may lead to chronic inflammation. TLRs are important receptors of the innate immune system which recognized the PAMPs present in the pathogens and initiate a cascade of signaling pathways that orchestrate inflammatory responses (Broz and Monack, 2013). Reports suggest that polymorphic variant of TLR might affect host defense mechanisms and lead to poor elicitation of immune responses against the infectious pathogens. Recently, dysfunction of TLRs has been suggested to be one of the etiopathological factors for psychiatric disorders (McKernan et al., 2011). Since TLRs play a very important role in the orchestration of the inflammatory processes, it is hypothesized that SNPs present in the TLR gene may cause the functional change in the TLR receptors leading to depletion of the TLR pathway and dampen

inflammatory response triggering susceptibility to infections and psychiatric disorders such as schizophrenia (Venkatasubramanian and Debnath, 2013).

In the present investigation, AG genotype and G allele of TLR-4 rs4986790, CT genotype and T allele of TLR-4 rs4986791 were found to be positively associated with schizophrenia. Our findings are not in agreement with the previous two studies in which no direct association between the TLR gene polymorphism and schizophrenia was observed (Kang et al., 2013; Garcia-Bueno et al., 2016). TLR-4 is mostly present in monocytes, macrophages, dendritic cells and is known to recognize lipopolysaccharide (LPS). It is reported that 896 A/G base pair transition at TLR-4 rs4986790 SNP causes the substitution of aspartic acid by glycine at amino acid position 299 (Asp299Gly) and 1196 C/T base pair transition at TLR-4 rs4986791 SNP causes the substitution of threonine by isoleucine at amino acid position 399 (Thr399Ile). This polymorphism on the TLR-4 gene causes the functional changes in receptors and leads to the blunted responses to LPS (Arbour et al., 2000). In addition, the missense variation at TLR-4 rs4986790 and TLR-4 rs4986791 has been reported to alter the activity of TLR-4 in inflammatory and fibrogenic signalling pathways (Guo et al., 2009). Besides, these SNPs were also reported to alter the profile of the pro-inflammatory cytokine. A study reported that TLR-4 Asp299Gly polymorphism and TLR-4 Thr399Ile polymorphism may alter the pro-inflammatory cytokine profile of individuals and show high levels of IL-1, IL-6 and TNF-α (Hold et al., 2014). Thus, the TLR-4 polymorphism may alter the cytokine network leading to a pro-inflammatory phenotype. However, some other studies observed that TLR-4 rs4986790 polymorphism alone can increase the levels of pro-inflammatory cytokine (Ferwerda et al., 2007; Long et al., 2014). In our previous study, it was also observed that a high level of IL-6 among the patient with schizophrenia (Gurung et al., 2018).

Several studies have reported an association between the genetic variation in TLR-4 (rs4986790, rs4986791) with susceptibility to infectious and inflammatory diseases (Tal et al., 2004; Lagos et al., 2008; Papadopoulos et al., 2010; Vidyant et al., 2019). Thus, our results further add to the immune-inflammatory hypothesis of schizophrenia. The possible role of TLR-4 in schizophrenia has been strengthened by the finding where an increased percentage of TLR-4+ monocytes were observed among the drug-naïve schizophrenia patients who have severe cognitive deficits (Kéri, 2017). Since, schizophrenia is a multifactorial disorder with no clear genetic mode of inheritance, hence in the present study dominant, co-dominant, recessive, over-dominant, and log-additive genetic models were used to test for association between TLR SNPs and risk for schizophrenia. The TLR-4 rs4986790 in co-dominant, dominant and over-dominant models were observed to be associated with schizophrenia with the lowest Akaike's information criterion (AIC) values signifying the best fit model. Thus, our results further throw light on the possible role of TLR-4 in the immunopathogenic pathway of schizophrenia. In contrast to our study, Kang et al. (2013) reported the association of TLR-2 rs3804099 SNP with a poor concentration in the dominant model, while TLR-2 rs3804100 SNP was found to be associated with a poor concentration in both dominant and the co-dominant models in schizophrenia. The consideration of different SNPs of TLR-2 in both the present and previous studies may be the reason for the non-concordance of the findings.

In the present investigation, the frequency of C/T genotypes and T allele of TLR-9 rs352140 was found to be higher in patients than the controls, however, only T allele of TLR-9 rs352140 was found to be positively associated with schizophrenia. To the best of our knowledge, no prior studies have examined the TLR-9 rs352140 polymorphism among patients with schizophrenia. However, other studies have

reported an association of TLR-9 rs352140 polymorphisms with inflammation-related diseases. Thus, a study reported an association of T allele and C/T genotypes of TLR-9 rs352140 with systemic lupus erythematosus (Xu et al., 2009). TLR-9 recognizes the unmethylated CpG dinucleotide motifs, present in bacterial DNA which is present in the endosomal compartments of cells (Hemmi et al., 2000; Medzhitov, 2001). TLR-9 can also mediate the cellular response to intracellular viral antigens (Lund et al., 2003; Varani et al., 2007) and trigger immune activation (Joshi et al., 2019). Although TLR-9 rs352140 variant is reported to be a synonymous coding variant, Karody et al. (2016) observed that T allele of TLR-9 rs352140 is associated with placental inflammation in new-born infants (Karody et al., 2016). Besides, it was observed that TLR-9 rs352140 polymorphisms can alter the plasma cytokine levels (Xu et al., 2018). Thus, it can be suggested that TLR-9 rs352140 polymorphisms can modulate the inflammatory process in the placenta by modulating the levels of cytokines in the fetal-maternal interface. However, the underlying mechanism of this association needs to be elucidated further. Nevertheless, in our study, I also observed elevated levels of IL-6 among schizophrenia patients (Gurung et al., 2018). Further, a study observed that CT or TT genotype of TLR-9 variant may increase the production of TNF-α in peripheral blood leukocytes (Karody et al., 2016). Besides, TLR-9 rs352140 polymorphism was found to be associated with the higher expression levels of the TLR-9 in IgM+ B cells (Kikuchi et al., 2005). In the present investigation, I further examined the association between TLR-9 genotypes and risk for schizophrenia based on the genetic model study. It was observed that TLR-9 rs352140 genotype C/C, C/T and T/T in co-dominant, dominant, recessive, over-dominant, and log-additive models were found to be significantly associated with schizophrenia. However, the AIC value suggested that the dominant model was the best fit model for schizophrenia. Studies

have observed an association of C/T genotype of TLR-9 rs352140 with human cytomegalovirus infection in the co-dominant, dominant and over-dominant model (Paradowska et al., 2016). Further, another study reported the protective effect of C/T and T/T genotype of TLR-9 rs352140 in the co-dominant, dominant and recessive model for Parkinson disease (Miri et al., 2020). On the other hand, in the present study a significantly high frequency of TT genotype and T allele of TLR-3 rs3775290, and AG genotype of TLR-7 rs179009 were found in the controls which suggests that these genotypes may confer protection to schizophrenia.

Haplotype-based association analysis is more informative than the SNPs, as specific haplotypes may modulate host defense mechanisms, resistance to infections, and influence the susceptibility to diseases (Stephens et al., 2001). Thus, in the present study haplotypes of different combinations of TLR-4, TLR-7, TLR-8 and TLR-9 SNPs were analyzed. Our results suggest that G-C and A-T haplotypes of TLR-4 (rs4986790 v/s rs4986791), and T-T haplotype of TLR-9 (rs5743836 v/s rs352140) may increase the risk for schizophrenia. Studies have reported the association of haplotype present in TLR-4 with an increased risk of high viral load (Pine et al., 2009). In another investigation, the TLR-4 A-C haplotype was found to provide an increased risk for inflammatory disease (Ortega et al., 2020). Besides, TLR-9 haplotype combination TG-TA was found to protect against *Chlamydia trachomatis* infection (Karimi et al., 2011). In contrast to the above studies, our findings show no effect of haplotypes A-A, A-G and G-G of TLR-7/TLR-8 (rs179009 v/s rs3764880) on schizophrenia susceptibility. The possible reason behind the non-concordance with the above studies may be due to the smaller sample size and fewer mutations observed in the studied TLRs.

It is hypothesized that prenatal maternal infection with pathogens (viz. influenza, herpes simplex virus type 2, cytomegalovirus) may cause neurodevelopmental abnormality leading to susceptibility to schizophrenia (Brown et al., 2004; Buka et al., 2008; Blomstromet al., 2012, Khandaker et al., 2013). Besides, viral infections during childhood have been reported to be associated with psychotic experiences and schizophrenia (Koponen et al., 2004, Khandaker et al., 2013). Studies have reported an association of SNPs such as TLR-4 rs4986790 with hepatitis B and C virus (Sghaier et al., 2019), TLR-4 rs4986791 with human immunodeficiency virus (Kim et al., 2021), and TLR-9 rs352140 with cytomegalovirus infection (Paradowska et al., 2016). Thus, these pieces of evidence suggest a link between the range of infections, TLR and schizophrenia. Consequently, it can be mentioned that a compromised innate immune system, possibly involving an inflammatory process may play an important role in the etiopathology of schizophrenia (Meyer, 2014; Khandaker, 2015; Muller, 2018).

Even though, I have observed an association of TLR-4 and TLR-9 in patients with schizophrenia, the underlying etiopathological mechanism of this association remains to be investigated further. It is reported that TLR-4 SNPs rs4986790 and rs4986791 may alter the extracellular domain of TLR which lead to receptor hyporesponsiveness in macrophages and mononuclear cells (Arbour et al., 2000). Consequently, it causes an alteration of the TLR-4 signaling pathway resulting in compromised clearance of pathogens leading to low-grade chronic inflammation in schizophrenia (Arbour et al., 2000; Liadaki et al., 2011). Mounting evidence suggests that peripheral inflammation may increase the permeability of the blood-brain barrier causing access of immune system components into the central nervous system (Engelhardt and Sorokin, 2009; Khandaker et al., 2016). Thus, the term “mild localized chronic encephalitis” has been proposed to describe the inflammatory process in schizophrenia (Bechter, 2001). To

boost the inflammatory hypothesis of schizophrenia studies have also shown the signs of inflammation in the schizophrenia brain (Korschenhausen et al., 1996). The results of the present investigation also provide evidence for potential genetic risk factors associated with TLR gene polymorphisms in the pathogenesis of schizophrenia among the Bengalee patients of Siliguri.

The present study has few limitations. The prevalence of schizophrenia was studied based on a monocentric single hospital-based study, which may have limited the exact estimation of the prevalence of schizophrenia. Besides, the sample size of the population included for CRP and IL-6 study was relatively small. Further, only specific SNPs present in TLR which were previously reported to be associated with viral diseases have been investigated in the present study due to which other TLR SNPs which may have an association with schizophrenia have been missed. In addition, an effort was not made to investigate the presence of virus-specific antigens/antibodies in the patients. Regardless of these limitations, our study provides evidence for the possible role of inflammatory processes in the etiopathology of schizophrenia. Our findings will encourage further research to decipher the underlying mechanisms of inflammatory processes in the etiopathology of schizophrenia.

CHAPTER-06

Conclusion

and

Future Direction

6. CONCLUSION AND FUTURE DIRECTIONS

The bidirectional communication between the nervous system and the immune system and vice versa has been well documented. This communication is brought about by the receptors which are common to both the system. It is now well evident that molecules of the immune system viz. toll-like receptors (TLRs) and cytokine receptors are also present on the neurons. Conversely, some neurotransmitters and their receptors are found to be expressed by immune cells. Hence, controlled bidirectional interactions between immune and neuronal systems are vital for maintaining homeostatic balance in the body. Moreover, an imbalance between these two systems due to pathogenic infections, stress, injury, etc. may cause alterations in immune responsiveness leading to susceptibility to infections and inflammation. It is suggested that the inflammatory process may interfere with neurotransmitter synthesis and neurotransmission which may lead to neuropsychiatric disorders such as schizophrenia (VanDerMast,1998). Nonetheless, increase norepinephrine is found to facilitate the inflammatory process and cause an increased expression of genes that may cause chronic low-grade inflammation (Boyle et al., 2007). Mounting evidence suggests that higher peripheral inflammation mediated by cytokines may increase the permeability of the blood-brain barrier causing access of immune system components into the central nervous system. The peripheral cytokine in the central nervous system (CNS) may facilitate neuroinflammation and impair normal brain functioning.

From the present study, it is evident that an increased inflammatory response and immune reactions may play a very important role in the pathophysiology of schizophrenia. Further, our study suggests that an inflammatory mechanism involving some common pathophysiological pathways between the immune and neuronal systems may play a key role in the etiology of schizophrenia. As I have also observed

an association of virally associated SNPs present in the TLR genes with schizophrenia it can be concluded that mutation in the TLR genes may provide susceptibility to viral infections and may facilitate the inflammatory process in schizophrenia. However, it is still not well understood that whether inflammation may lead to the pathophysiology of schizophrenia or else the disorder itself causes the increased inflammatory response in schizophrenia. Nonetheless, the present work adds to the growing body of evidence that inflammation may play a significant role in the pathophysiology of schizophrenia.

To date, the exact mechanism of the role of inflammation in the etiopathology of schizophrenia is not clearly understood. Besides, from the present study, it is too early to speculate the exact pathophysiology involved in schizophrenia. Therefore, several issues remain to be addressed in future research to shed more light on the inflammatory hypothesis of schizophrenia. Firstly, future studies should be conducted in a larger cohort of samples using advanced molecular techniques to confirm our findings. Secondly, the presence of specific viral antigen/antibodies along with the polymorphism in TLR genes should be studied in the patients to evaluate the role of specific viral infection and TLR polymorphism in schizophrenia. Thirdly, future studies should focus on the effect of specific TLR SNPs on the expression of the cytokine to understand the modulatory effect of TLR polymorphisms in the cytokine levels in schizophrenia. Lastly, future studies involving specific signalling pathways of TLR are required to understand the underlying pathophysiological mechanisms of inflammation in schizophrenia. The unravelment of these issues might provide deeper insights into the role of inflammatory processes in the etiopathology of schizophrenia.

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8. Annexure-I

Ethical clearance certificate

Reference No: SU/IEC/2016/07 Date: 26/05/2016

To
Mr Jiwan Gurung
PhD Research Scholar
Department of Zoology
Sikkim University

Subject: Ethical Clearance

Dear Mr Gurung,

This has reference to the application of Dr Bisu Singh, Assistant Professor, Department of Zoology, Sikkim University dated May 24, 2016 for ethical clearance of your PhD research proposal titled "Study of C-reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia."

Following the ICMR guidelines for evaluation of research proposals, the Institutional Ethical Committee (EIC) approves your research proposal.

Member-Secretary
Institutional Ethical Committee
Sikkim University

9. ANNEX URE -II

Informed Consent Form

Nature and purpose of study: This study is entitled "An investigation of serum C–reactive protein and Toll like receptor gene polymorphism to study the inflammatory hypothesis of schizophrenia". The purpose of this study is to investigate the level of C-reactive protein and toll like receptor gene polymorphisms in the schizophrenic patients which can throw light in the inflammatory hypothesis of schizophrenia.

Foreseeable risks: For this study 5 ml of blood samples would be collected by the vein puncture method from the each participant which do not involve any risk to the health of the participant.

Duration: The duration of the study is five years and participants needs to donate the blood sample only once.

Benefits: The study may help to generate CRP and TLRs data which may serve as a preliminary reference for the role of inflammation in schizophrenia.

Procedures to be followed: CRP will be estimated by ELISA method and TLRs gene will genotype with the help of PCR-RFLP method. Test for each individual will be done purely for the scientific purpose and result of the study does not imply direct diagnostic or therapeutic value to the person subjected to the research.

Confidentiality: The result of the study will be kept fully confidential and it will be used only for the research purpose and not for the medical or therapeutic purpose and the data will be presented at the population level only.

The contact detail of the Supervisor and Co-supervisor investigator is given below:-

Dr. Bisu Singh (Supervisor)
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Sikkim University
Gangtok
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Email:bisusingh22@yahoo.co.in

Dr. Nirmal Kumar Bera (Co-supervisor)
Department of Psychiatry
North Bengal Medical College and Hospital
Siliguri, West Bengal
Contact No. 9434006972

Declaration

I understood the study procedure and I agree to donate my blood sample for this study. I am fully aware that the sample would be used for the research purpose only.

.....

Signature of the donor

10. ANNEXURE – III

Demographic data form

Dept. of Zoology , Sikkim University
Place of blood collection:
Serial No.- …….
Date: ……

Name: ……..

Address:……

DEMOGRAPHIC DETAILS

Date of birth: …… Age: ……

Sex: M/F:……. Ethnicity: ……

Subcast:……..

Occupation: ……

Height: ……. Weight: …….

Education:<class VIII/Class VIII/Madhyamik/H.S./Graduation/Masters/other:

Marital status: Marrid/Unmarrid/Widow/Divorced

Migration (if any): ……..

Any autoimmune disease in the family: ……

Any sort of substance abuse: …….

Any medical condition:…….

Blood Group :

Food habit:

Drug taken:

Pedigree:

11. ANNEXURE – IV

General Health Questionnaire

Please fill in the following questionnaire with rating of '0' denoting Not at all, '1' indicatingSeldom, '2' indicating Usual and '3' indicating More than usual,

1. Lost much sleep over worry
   0 1 2 3
2. Felt constantly under strain?
   0 1 2 3
3. Been able to concentrate on what you are doing?
   0 1 2 3
4. Felt that you are playing useful part in things?
   0 1 2 3
5. Been able to face up to your problem?
   0 1 2 3
6. Felt capable of making decisions about things?
   0 1 2 3
7. Felt you could not overcome your difficulties?
   0 1 2 3
8. Been feeling reasonably happy, all things considered?
   0 1 2 3
9. Been able to enjoy your normal day to day activities?
   0 1 2 3
10. Been feeling unhappy or depressed?
    0 1 2 3
11. Been losing confidence in yourself?
    0 1 2 3
12. Been thinking of yourself as a worthless person?
    0 1 2 3

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BRIEF REPORT

Elevated levels of C-reactive protein and IL-6 among the antipsychotic medicating schizophrenia patients of Siliguri, West Bengal, India

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ABSTRACT

Purpose: Chronic, low-grade inflammation is a proposed etiological factor associated with schizophrenia. Thus, various studies have been conducted to understand the role of inflammatory process in schizophrenia by using inflammatory maker C-reactive protein (CRP) with conflicting findings. Inadvertently, studies of CRP among the Indian schizophrenia patients are very few. Therefore, the present study was undertaken to investigate the role of inflammatory process among Indian Bengalee schizophrenia patients of Siliguri, using the marker CRP and its stimulating cytokine interleukin-6 (IL-6). In addition, the study also intended to investigate the immunomodulatory effect of antipsychotic medication on serum levels of CRP and IL-6.

Materials and methods: The serum levels of CRP and IL-6 were measured by Enzyme-Linked Immunosorbent Assay (ELISA) among 67 antipsychotic medicating, 28 psychotropic medication-free schizophrenia patients, and it was compared with 72 age, sex and ethnicity-matched controls.

Results: A significantly higher level of CRP and IL-6 were recorded among the antipsychotic medicating patients. Although CRP was found to be higher among the psychotropic medication-free patients than the controls, it was not found to be significant. However, a significantly higher level of IL-6 was observed in this group.

Conclusions: The results provide the evidence for a possible immunomodulatory effect of antipsychotic drugs on CRP. Future investigations including the study of antipsychotics separately may help to understand the differential effects of individual antipsychotics on CRP level. Additional studies with a larger sample size of psychotropic medication-free patients may help to verify the role of inflammation in schizophrenia patients of this region.

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KEYWORDS
C-reactive protein; interleukin-6; schizophrenia; Siliguri

1. Introduction

Schizophrenia is a multifactorial psychiatric disorder characterized by positive, negative and cognitive symptoms [1]. Despite tireless research, etiology of schizophrenia is still concealed [2]. Increasing evidence suggests that dysregulation of the immune system and inflammatory processes, along with the genetic and environmental factors contribute to etiology and pathophysiology of schizophrenia [3–5]. Recently, the study of immunoinflammatory process in schizophrenia has received an increase attention from the researchers. One of the well-known inflammatory markers which have been intensively studied in schizophrenia is C-reactive protein (CRP). It is synthesized by hepatocytes during the inflammatory process [6] under the direct stimulation of interleukin-6 (IL-6) [7]. However, the findings of CRP in schizophrenia till date are not in agreement with each other. Thus, some of the studies reported its increased level [8–13], whereas others observed no associations [14–18]. The reasons for inconsistencies of the findings may be attributed to small sample size [8,9,19,20], the absence of controls [10,21–23], lack of simultaneous studies of CRP along with its direct stimulatory cytokine such as IL-6 [18,24,25], and none consideration of medication status [22,26,27] in the previous investigations.

Various studies have been carried out till date among the schizophrenia patients to understand the modulatory effect of antipsychotic drugs on the CRP levels. Thus, some of the studies reported higher levels of CRP after treatment with clozapine [28], olanzapine [14], quetiapine [22] and atypical antipsychotics [29]. Nonetheless, in most of the previous investigations, either the controls were not included in the study or else have considered only first-degree relatives of the schizophrenia patients. However, the comparison of CRP among the patients and their relatives may not be free from biases as studies have reported 40% heritability of the baseline CRP levels [30]. Moreover, the variation in gene encoding CRP was found to be associated with circulating CRP levels [31,32]. Furthermore, studies suggest that CRP gene polymorphisms are independently associated with increased or decreased levels of CRP [33].

Most of the studies of CRP in schizophrenia have been carried out in the Caucasian [13,34] and some Mongoloid population [24,35]. The literature review suggests that only two studies [12,20] have been conducted so far in India to

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BRIEF RESEARCH COMMUNICATION

A 10-year retrospective study of suicide in Sikkim, India: Sociodemographic profile and risk assessment

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ABSTRACT

Objective: The present study had been undertaken to investigate the sociodemographic profile of individuals who had committed suicide in Sikkim which may throw light on the vulnerable groups.
Materials and Methods: Ten-year suicide data (2006–2015) obtained from Police Headquarters, Crime Branch, Gangtok, have been statistically evaluated to study the sociodemographic profile.
Results: The results showed that out of 1604 suicide cases recorded for the past 10 years, 1051 were males (65.5%) and 553 (34.5%) were females. Suicide was found to be common among the age group of 21–30 years (24.4%), Rai community (15.8%), population of rural areas (82.6%), and among the population of eastern districts (50.6%). Hanging (94.8%) was found to be the most common method adopted for suicide.
Conclusion: The study provides preliminary information about the vulnerable groups for suicide in the state which may be vital for taking necessary steps for its prevention shortly.

Key words: Bhutia, Lepcha, Nepali, Sikkim, suicide

INTRODUCTION

Worldwide, about 2% of deaths are attributed to suicide.[1,2] In comparison to developed and developing nations, the rate of suicide is found to be very high in Estonia, Lithuania, Belarus, and the Russian Federation.[3]

According to the National Crime Report Bureau (NCRB) report, 2014, more than one lakh people die every year due to suicide in India.[4] Moreover, the suicide rates vary widely across the different states of India, ranging from as high as 40.4 in Puducherry to as low as 0.6 in Nagaland. According to the latest report of the NCRB during the past 10 years (2004–2014), the number of suicide has increased to 15.8%. Suicide among the states of India shows the highest incidence from Maharashtra (16,307) (12.4%) followed by Tamil Nadu (16,122) (12.2%), West Bengal (14,310) (10.9%), Karnataka (10,945) (8.3%), and Telangana (9,623 suicides) (7.3%) which together accounts for 51.1% of the total national incidence and the rest 48.9% comes from 24 states and 7 union territories.[4] As far as the rate of suicide is concerned, the national average rate exhibits 10.6 during the year 2014 with Puducherry reporting to be the highest (40.4) followed by Sikkim (38.4), Andaman and Nicobar Islands (28.9), Telangana (26.5), Kerala (23.9), and Tamil Nadu (23.4).[4]

Even though Sikkim has one of the highest suicide rates in the nation, very few studies have been conducted to understand this grave problem of the state.[5] Moreover, previous studies had relied heavily on the NCRB data which

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Study of the mosquito larval diversity in the artificial breeding habitat of Gangtok, Sikkim

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Abstract
With the growing urbanization, the number of discarded containers and construction of drains has increased considerably in Gangtok which may provide a conducive breeding habitat for mosquito larva. Therefore, the present study was undertaken to investigate the mosquito larval diversity in the artificial breeding habitat of Gangtok. A random sampling was done from the discarded buckets, tanks, tyres, drains at seven different sites during the months of July to October 2017. A total of 247 mosquito larvae was collected from seven sampling sites. The species observed in the different sampling sites were *Aedes albopictus*, *Aedes atropalpus*, *Ochlerotatus japonicus*, *Culex pipiens*, *Culex territans*, *Ochlerotatus taeniorhynchus*, and *Ochlerotatus trivittatus.* Among the observed species *Ochlerotatus japonicus* was found to have the maximum abundance (32%). Correlation analysis tests showed the significant positive correlation between the larval abundance and the volume of water (r=0.94, P=0.002). As per the Shannon-Wiener index, highest diversity was observed at Fifth mile (1.37) and least in Gangtok town (0.44). Pielou's Evenness index indicated that species were evenly distributed in all the sites except Gangtok town (0.63). Bray-Curtis index revealed the highest species similarity between Ranipool and Tadong (0.82). The present study gives elementary information about the species diversity of mosquitoes in the artificial breeding sites of Gangtok. The presence of potent disease vectors like *Culex* and *Aedes* shows the vulnerability of this region for the outbreak of mosquito-borne diseases in the near future as Gangtok is a famous tourist destination.

Keywords: Artificial habitat, Gangtok, Mosquitoes

1. Introduction
Gangtok is the capital city of Sikkim and is a popular hill station of North East India. It has a temperate climate throughout the year with an annual rainfall of about 2739 mm (Census of India. Sikkim. 2011) [7]. As such, water gets accumulated in the containers scattered in different localities which are being discarded by the locals and the tourists (Kumar *et al.* 2009) [14]. With the growing urbanization, the number of discarded containers is increasing day by day in Gangtok which provides suitable breeding habitat for mosquitoes. Studies revealed that with the extended urbanization around the world, preference of mosquitoes for artificial containers for breeding has increased to a large extent, which implies that the distribution of larval mosquitoes is greatly influenced by human ecology (Chen *et al.* 2009) [8]. Thus, many of the studies have been conducted to investigate the mosquito larval diversity in artificial breeding sites. Studies have reported that the species of mosquitoes such as *Aedes aegypti*, *Aedes albopictus*, *Aedes niveus*, *Culex quinquefasciatus*, *Anopheles* and *Culex* generally breed in artificial containers (Chen et al. 2009; Adeleke *et al.* 2008; Dass *et al.* 1998; Suganthi *et al.* 2014) [8, 1, 10, 21]. Further, it has been observed that in the urban areas the environment has a major effect on the incidence of *Aedes albopictus* larvae (Li *et al.* 2014) [15]. In addition, studies have also observed the existing and disappearing species of mosquitoes along with their pattern of distribution (Asha *et al.* 2014)4[4]. It has been reported that artificial containers provide a good habitat for the vector of Dengue (Rajesh *et al.* 2013) [18]. So far, the study of mosquito diversity in the Himalayan region of Sikkim has been meagre. A survey conducted on the haematophagous arthropods in the Himalayan regions of West Bengal and Sikkim revealed the existence of 29 species of mosquitoes (Bhat 1975) [6]. Similarly, Aditya *et al.* (2006) studied the mosquito larval habitats (temporary pools and cemented water storage tanks) and their temporal variation in the town of Darjeeling, which revealed the existence of four mosquito genera belonging to *Aedes*, *Armigeres*, *Culex* and *Toxorhynchites* with

Research Article

An Evaluation of *In vitro* anti-inflammatory Activity of Ethnomedicinal Plants *Dendrocnide sinuata* (Blume) Chew and *Chenopodium ambrosioides* (L.)

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ABSTRACT

Traditionally *Dendrocnide sinuata* and *Chenopodium ambrosioides* are used by Assamese people to treat inflammation related ailments. The aim of the present study was to investigate and validate the *in-vitro* anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides.* The anti-inflammatory property was studied by Human red blood cell (HRBC) membrane stabilization method. The phytochemicals were analyzed according to the techniques given elsewhere. Both the plant extracts were found to have membrane stabilizing activity with *Chenopodium ambrosioides* showing higher efficacy than *Dendrocnide sinuata* as compared to standard drug diclofenac. The activity of the extracts was dose-dependent and highest at 5,000 µg/ml for both the plant extracts. The phytochemical analysis showed the presence of alkaloids, flavonoids, triterpenes, saponins, cardiac glycosides, resins. The study shows the anti-inflammatory efficacy of both the plants.

Keywords: Inflammation, diclofenac, HRBC, *Dendrocnide sinuata, Chenopodium ambrosioides.*

INTRODUCTION

Inflammation is a non-specific defence of the body in respond to tissue malfunction and is a property of both innate and adaptive immune system to keep in check any pathogenic intruders.[1] However, chronic inflammation leads to autoimmune diseases and cancer.[2,3] The anti-inflammatory drugs to treat ailments are known to come with a lot of side effects like mucosal damage, bleeding, renal failure etc.[4] The side effects, cost of medication, combined with an interest in returning to natural or organic remedies, has led to an increase in the use of plants as medicines.[5] Phytoconstituents of plants like flavonoids and triterpenoids are potent anti-inflammatory agents.[6] Therefore, one of the latest trends in biological research is to scientifically extract these phytoconstituents that may be a prospect for drug development in near future.[7]

Assam, one of the states of North East India has 200 plants documented and scientifically validated to have medicinal property.[8,9,10] Recently, the research and documentation of traditional knowledge of North Eastern states have increased, but looking at the vast source of indigenous knowledge, still there is dearth of study.[11] Many of the ethno medicinal plants have been investigated for validation of their anti-inflammatory property by *in vivo* and *in vitro* methods. Plants such as *Balanites aegyptiaca*,[12] *Ipomoea staphylina*,[13] *Mikania micrantha*,[14] *Ficus hispida*,[15] *Mikania glomerata* and *Mikania laevigata* [16] was found to have anti-inflammatory property by *in vivo* carrageenan induced rat-paw oedema method. On the other hand, by *in-vitro* human RBC membrane stabilisation method plants such as *Cassia occidentalis*,[17] *Centella asiatica*,[6] *Gendarussa vulgaris*,[18] *Erioglossum rubiginosum*,[19] *Centratherum punctatum*,[20] were found to have anti-inflammatory property. In our previous study two ethnomedicinal plants of Sikkim *Viscum articulatum* and *Acorus calamus* were observed to have anti-inflammatory property by human RBC membrane stabilization method.[7]

Dendrocnide sinuata is used by various ethnic communities of North East and has been found to have anti-microbial activity.[21] Both *Dendrocnide sinuata* and *Chenopodium ambrosioides* have been explored for anti-inflammatory property by carrageenan induced rat-paw oedema method.[22,23] However, both these plants have not been explored in *in-vivo* models. Even though *Chenopodium ambrosioides* have been investigated previously by TrivellatoGrassi et al. (2013)[22] from Nigeria but it has not been investigated from Assam and it is expected that phytoconstituents of a plant species may vary considerably according to its geographical location which are involved in anti-inflammatory property.[24] Therefore, the present study was undertaken to investigate the anti-inflammatory property of *Dendrocnide sinuata* and *Chenopodium ambrosioides* from Assam by human RBC membrane stabilization method. The assay is useful for studying anti-inflammatory property as the RBC membrane resembles the lysosomal membrane[25] and rupture of RBC membrane resemble the burst of the lysosomes in inflammatory reaction.[26]

MATERIALS AND METHODS

Plant collection and processing

Leaves of *Dendrocnide sinuata* and *Chenopodium ambrosioides* were collected from the Sivasagar district of upper Assam during the month of December 2017 and January 2018. The cleaned leaves were dried in the shed

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Short population report

HLA-A -B and -DRB1 distribution in Kami: A caste population of Gorkha community from the sub-Himalayan region of West Bengal, India

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ABSTRACT

The present investigation is the first of its kind to understand the HLA profile of Kami population from the Indian Gorkha community of sub-Himalayan West Bengal, India. A total of 158 individuals from Kami population were genotyped at first field resolution by HLA ABDRB1 PCR SSP typing kit. The genotype assignment to the individuals was performed by Ready Gene V.1.0.0.0′ software. The data were analysed by PopWin32-0.7.0 software. All the loci typed were in Hardy-Weinberg equilibrium. The genotype data is accessible at Allele Frequencies Net Database with the name India, sub-Himalayan West Bengal, Kami number 3720.

1. Introduction

The sub-Himalayan region of West Bengal, India extends between 27°2′ N latitude and 88°15′E longitude. It is bordered by Nepal, Sikkim and Bhutan and covers a surface area of 3149 km^2 with a population of around 18.47 lakh mainly composed Gorkha (also called Gurkha), Lepcha and Bhutia community. The current demographic pattern of Gorkhas in the sub-Himalayan West Bengal is largely due to migration across the Indo-Nepal border since 1700 CE. The principal factors for migration were extremely severe policies in Nepal, developing tea industry, the establishment of Darjeeling Himalayan Railways, development of Darjeeling into a hill resort by Britishers and recruitment of Gorkha soldiers in British Army from outside the borders of Nepal [1–4]. Historically the word Gorkha is associated with khasas from northern India [5]. Moreover, after the establishment of the Gorkha dynasty by Drabya Shah the word Gorkha was used for the inhabitants of the principality where the Gorkhas ruled [6]. However, nowadays the term 'Gorkha' is used to define a community or is used to differentiate Indian citizens of Nepali ethnicity from citizens of Nepal [6]. The Gorkhas as a community can be divided into three ethnic groups; (i)'Kiratis' which include Rai, Magar, Limbu, Lepcha, Tamang, (ii)'Newaris' or 'Newars', and (iii) 'Tagadharis' who are the Nepali counterpart of Indian Hindus including Bahuns, Chettri, Kami, Damai and Sarki [5]. Each of these community is unique in their language, script, culture and customs.

Kami are Indo-Aryan language speaking Hindu socio-ethnic caste group from Gorkha community mostly confined to the sub-Himalayan region in the state of West Bengal, and sporadically to the other states of India. They are essentially artisans involved in metalwork and makers of famous 'Khukuri' knives used by the Gorkha army. Besides, they are also involved in making traditional Nepali drum called 'Madal' and exponent of 'Maruni Nritya', a traditional folk dance. They are mostly monogamous however; polygamy was also evident in the past. Kami are classified into 54 exogamous clans and the marriage among the different clans is a common phenomenon [7]. However, the marriage with other populations within and outside the Gorkha community is rare. According to 2011 census Kami population is about 52,178 in West Bengal. Very little is known about the origin of Kami population. However, it has been mentioned that predecessors of Kami have emigrated from different places of India such as Punjab, Rajputana, Kashmir and neighbouring places between 10th and 15th Centuries AD to Nepal [7].

The 158 individuals belonging to Kami population were recruited from Darjeeling, Kalimpong and Jalpaiguri district of sub-Himalayan West Bengal, India. After explaining the study procedure, written consent was taken from the individuals for voluntarily donating their blood samples and participation in the study. The three-generation pedigree charts were prepared for each individual to assure their unrelatedness, and those having the history of inter-caste marriage within the studied pedigree were excluded. The study was approved by the Institutional Ethical Committee, Sikkim University. All the laboratory experiment was conducted at the Molecular Biology Laboratory, Department of Zoology, Sikkim University, Gangtok, India. The extraction of genomic DNA was performed by a kit (Qiagen) and the first field HLA Class I and Class II genotyping were performed by HLA ABDRB1 PCR SSP typing kit (Inno-Train Diagnostik, Germany) as per allele list based on release

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HLA Profile of Kami Population Refutes the Earlier Proposition of Exclusive Closer Genetic Affinity of All the Gorkhas to Mongoloids

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Keywords
Human leucocyte antigen · Kami population · Gorkha population · Khukuri knife · Indo-Aryan population

Abstract
Objective: Based on the HLA profile of Indian Gorkhas, Debnath and Chaudhuri (2006) proposed that Gorkhas are genetically closer to Mongoloids, and they may have originated from Mongolians or Tibetan stocks. However, the major limitation of the earlier study was that Gorkhas comprise 2 broad groups, i.e. Tibeto-Burmans and Indo-Aryans. Besides, Gorkhas have an assemblage of many sociocultural and linguistically distinct populations such as Rai, Magar, Limbu, Tamang, Newar, Bahun, Kami, and so on. Thus, the generalization of the findings on Gorkhas by considering them as a single homogenous population may not be free from biases. Therefore, the present study aims to understand the genetic affinity of a constituent population from the Gorkha community, i.e. Kami, based on HLA polymorphism. ***Methods:*** First field HLA typing was performed among 158 Kami individuals by PCR-SSP methods. ***Results:*** The most frequent genes observed were HLA-A*11, HLA-B*15, HLA-DRB1*15. The frequency of HLA-DRB1*15 reported here is the highest recorded among the North Indian population to date, which is a noteworthy finding of the study. The hierarchical cluster analysis and principal component analysis showed that the Kami population lies within the cluster of the Indian subcontinental population. ***Conclusion:*** The study refutes the earlier proposition of exclusive belongingness of all the Gorkhas to Mongoloids.

Introduction

Kamis are an Indo-Aryan language-speaking socio-ethnic caste group from the Gorkha community. Presently the Indian Kami population is mostly confined to the sub-Himalayan region of West Bengal, India, and sporadically in the other parts of the country. Kamis are essentially Hindu and worship different Hindu deities of the pantheon. However, few of them have converted to Christianity or other religions. By tradition, Kamis are artisans involved in metalwork and hold the legacy of making the famous "Khukuri" knives used by the Gorkhas. Monogamy is prevalent among the Kamis, even though polygamy was also common in the past. There are 54 exogamous clans among the Kamis, and marriage among the different clans is a usual phenomenon [1]. Sporadic intercaste marriage of Kamis with other populations from the Gorkha community is evident. However,

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An investigation of traditional uses and anti-inflammatory property of *Clematis buchananiana* De Candolle and *Tupistra nutans* Wall. ex Lindl.: Native ethnomedicinal plants from Sikkim, India

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In the traditional medicinal system of Sikkim *Clematis buchananiana* and *Tupistra nutans* is used extensively to treat various ailments, however, they have not been validated for their anti-inflammatory property by *in vitro* method. Therefore, the present study was carried out to investigate specific ethnomedicinal uses, *in-vitro* anti-inflammatory property, and phytochemical constituents of *Clematis buchananiana* and *Tupistra nutans*. The ethnomedicinal usage was studied by calculating the value for fidelity level, use-value, and informant consensus factor. Stabilization of human red blood cell membrane and protein denaturation method was used to study anti-inflammatory property. The phytochemicals were analysed by the methods described elsewhere. *Clematis buchananiana* was found to be used more frequently for sinusitis, headache, cold and *Tupistra nutans* for high blood pressure, diabetes and stomach-ache. Both *C. buchananiana* and *T. nutans* was found to inhibit the HRBC membrane and protein denaturation effectively in a dose-dependent manner. However, inhibition of haemolysis and protein denaturation by *C. buchananiana* was found to be higher than *T. nutans* at all doses. The phytochemical screening revealed the presence of anti-inflammatory metabolites such as flavonoids and phenolics in both the plants. The results provide evidence for the anti-inflammatory property of *C. buchananiana* and *T. nutans.*

Keywords: *Clematis buchananiana*, Ethnomedicine, Inflammation, Sikkim, *Tupistra nutans*

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Inflammation is a common protective immunological reaction in response to tissue injury which is characterised by the accumulation of polymorphonuclear leukocytes and macrophages at the sites of tissue injury[1]. These cells contain large numbers of lysosomal granules in their cytoplasm which on release during inflammatory reactions can destroy pathogens as well as the normal cells and tissues leading to pathological condition[2]. Thus, stabilization of lysosomal membrane holds an important prospect to inhibit an inflammatory response[3]. Furthermore, denaturation of protein has been well documented in inflammation and arthritic diseases which results in the production of auto-antigens[4,5]. Although nonsteroidal anti-inflammatory drugs (NSAIDs) are often used to treat inflammation they come with various side effects[6,7].

In traditional medicine practices of Sikkim, about 420 plants are used to treat various ailments[8]. In the previous studies, elaborative documentation of medicinal use of plants from Sikkim has been made, out of which some are reported to be used to treat inflammatory diseases[9]. Moreover, only a handful of plants from Sikkim have been investigated for documentation of its anti-inflammatory property by experimental evidence-based method[10-13]. Among many of the plants used to treat various inflammation-related diseases, *Clematis buchananiana* belonging to the family Ranunculaceae and *Tupistra nutans* of the family Asparagaceae is being used in the traditional medicinal system of Sikkim. The genus *Clematis* was reported to be used for rheumatoid arthritis, bone disorder, chronic skin disease, muscle aches, colds, headaches, respiratory ailments etc.[14,15]. In the previous studies, the species of *Clematis* such as *Clematis erecta*[16], *Clematis chinensis*[17], *Clematis simensis*[18], *Clematis pickeringii*[19], *Clematis vitalba*[20] and *Clematis flammua*[21] have been investigated for anti-inflammatory property. Moreover, the anti-inflammatory property of *Clematis buchananiana* has not been performed to date. On the other hand, the genus *Tupistra* has been reported to be used for diabetes[22,37]. Moreover, only a few studies have been

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Short Research Communication

Species composition of mosquito breeding in bamboo stumps in Sikkim, India

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ABSTRACT

Background & objectives: Sikkim is a part of Eastern Himalayan biodiversity hotspot of India rich in bamboo flora harbouring over 30 different bamboo species. The present study was aimed to investigate the larval mosquito diversity in the bamboo stumps of Gangtok, Sikkim. Besides, efforts were also made to evaluate the propensity of particular species of mosquito towards specific bamboo species (if any).

Methods: A total of 75 bamboo stumps of four genera were surveyed and screened at five different sampling sites of Gangtok from July to October 2017. Mosquito species similarity between the five sampling sites and the four varieties of bamboo species was calculated using the Bray-Curtis similarity index.

Results: A total of 216 larvae were collected from 25 different bamboo stumps studied. The species identified were *Aedes albopictus, Ae. atlanticus, Ae. aegypti, Orthopodomyia signifera, Oclerotatus japonicus, Oc. taeniorhynchus, Armigeres subalbatus,* and *Toxorhynchites splendens*. The *Oc. japonicus* (34.5%) was found to be the most abundant species having distribution in *Phyllostachys assamica*, *Dendrocalamus hamiltonii* and *Bambusa nutans*. On the other hand, genus *Armigeres subalbatus* and *Tx. splendens* were found to breed only in the stumps of *P. assamica*. Based on Bray-Curtis similarity index highest species similarity was recorded between *D. hamiltonii* and *P. assamica* bamboo species.

Interpretation & conclusion: The study may help to understand the bioecology of the mosquito larvae which may help to devise suitable mosquito control programmes. Future studies including the survey of large number of bamboo stumps both in urban and rural areas of Sikkim may provide better insight into the mosquito diversity in the bamboo stumps of Sikkim.

Key words Bamboo stumps; eastern Himalaya, mosquitoes; Sikkim

INTRODUCTION

Sikkim is a part of the Eastern Himalayan biodiversity hotspot of India, covering an area of 7096 km^2 and lies at 27° 00' 46" and 28° 07' 48" N latitude and 88° 00' 58" and 88° 55' 25" E longitude. The topography of the state is quite diverse and the altitude ranges from 200–8598 m[1]. Sikkim is rich in bamboo flora harbouring over 30 different bamboo species[2]. Its capital city Gangtok is one of the famous holiday destinations for its unique ambience and attracts many tourists every year. The city experiences heavy rainfall during the months of May to September with the average annual rainfall being 2739 mm. The rainfall accumulates in natural and artificial habitats which create a varied environment favouring mosquito breeding. The temporal changes in the number of habitats and the heterogeneity are important factors that affect habitat use of species and community organization[3]. Such a situation can be seen in the bamboo stumps, that hold small aquatic pools which harbour communities of aquatic organisms consisting of several taxa including mosquito larval breeding[3].

Studies have found that bamboo stumps provide ideal breeding habitat for mosquitoes under Diptera: Culicidae, comprising the genus *Wyeomyia, Toxorhynchites*[4], *Trichoprosopon* spp.[5], *Anopheles vagus*[6], *Culex pseudovishnui, Cx. whitmorei*[7], *Armigeres*[8], *Cx. quinquefasciatus, Aedes aegypti*[9], *Ae. albopictus*[10], *An. barbirostris,* and *An. vagus*[11]. As far as the literature review is concerned, only one study has been conducted in Sikkim to study the mosquito diversity in the water samples of roadside seepage pools and channels[12]. Despite the fact that the region is rich in bamboo fauna, till date no study has been conducted to investigate the diversity of mosquitoes in the bamboo stumps of Sikkim. Therefore, the present study was carried out to investigate the larval mosquito diversity in the bamboo stumps in and around the city of Gangtok, Sikkim with the