Section - 6

Comprehensive Discussion

6. COMPREHENSIVE DISCUSSION

Chronic diseases of the central nervous system including schizophrenia are suspected of having genetic, immunological and viral etiology by many investigators (Michels and Marzuk, 1993).

The argument in favour of an autoimmune basis for schizophrenia was popularized by Burch in the early 1960s. After analyzing the age-specific and sex-specific incidence rates, relapsing clinical course and prevalence of several conditions presumed to be autoimmune in origin, Burch concluded that schizophrenia may also have autoimmune basis. However, these evidences were not sufficient for establishing schizophrenia as an autoimmune disease. In 1950s, Witebsky and his colleagues proposed the criteria that could be used to determine whether a disease is actually autoimmune in origin. These criteria were more recently refined by Rose and Bona (1993). They proposed several levels of evidences for the autoimmunity: (i) direct evidence, that is transmissibility by lymphoid cells or antibody of the characteristic lesions of the disease from human to human or human to animal or reproduction of the functional defects characteristic of the disease *in vitro*, (ii) indirect evidence, that is reproduction of the autoimmune disease in experimental animals or isolation of autoantibodies or autoreactive T cells from the target organ and (iii) circumstantial evidence, that is the presence of markers that are descriptive of all autoimmune disease.

The present study has been carried out to investigate the circumstantial evidence of autoimmunity in schizophrenia. Several features are common to many autoimmune diseases which can be taken together as the circumstantial evidence of autoimmunity. These include association with other autoimmune diseases in the same individual or the same family, the presence of immune cells in the affected organ, association with particular MHC haplotype, high serum levels of autoantibodies, deposition of antigen-antibody complexes in the affected organ and improvement of disease symptoms with immunosuppression. In some cases of autoimmune diseases the alterations in the level of cytokines have also been observed.

The parameters we have taken for the present investigation includes HLA genes, Th-1 and Th-2 cytokine IL-2 and IL-6 respectively, CD4+ and CD+ cells and C- reactive

protein to study the immunological alteration in schizophrenia, if any. In addition, demographic characteristics were studied to investigate the role of environmental factors in the etiopathology of the disorder. The effort was also been given to shed light in the autoimmune basis of schizophrenia.

Study on the immunogenetic aspect of the disease is most useful in identifying not only the mode of inheritance of a particular disease process but also in understanding the immunopathogenic mechanisms underlying in it. The discovery of HLA associations with the specific disease implies that at least part of their genetic basis lies in the HLA region which suggests the possibility of determining the etiology. Incidentally, most diseases that show strong HLA associations are having unknown etiology and mode of inheritance, e.g. various autoimmune and rheumatological diseases.

Two general explanations for HLA and disease associations given by McDevitt (1985) is that there may be linkage disequilibrium between alleles at a particular disease associated loci and the HLA antigen associated with that disease; secondly the HLA antigen itself plays a role in disease manifestation by: (i) being a poor presenter of a certain viral or bacterial antigen , (ii) providing a binding site on the surface of the cell for a disease provoking virus or bacterium, (iii) providing a transport piece for the virus to allow it to enter the cell ,(iv) having such a close molecular similarity to the pathogen. It is most likely that all these mechanisms are involved, but to a varying extent in different diseases (Thorsby, 1977).

The first HLA association study was reported in schizophrenia by Cazzullo *et al.*, in 1974. More than 80 association studies have been reported since then. HLA and schizophrenia was first reviewed by McGuffin (1979), who commented that the MHC was a logical place to search for genetic markers for schizophrenia. This is because schizophrenia is similar to the diseases for which HLA association had been established as it was familial, had an imperfectly understood etiology, and had a postulated autoimmune pathogenesis (Burch, 1964).

Linkage analysis have also found the likely schizophrenia locus on chromosome 6p close to the human leukocyte antigen (Moises *et al.*, 1995; Schwab, 1995; Wang, 1995; Straub, 1995; Antonarakis *et al.*, 1995; Schizophrenia Linkage Collaborative

Group, 1996; Levinson *et al.*, 1996; Schwab *et al.*, 1998; Lindholm *et al.*, 1999; Li *et al.*, 2001; Schwab *et al.*, 2002). A recent study also found a significant increase in the frequency of a SNP in HLA-DOA in schizophrenia and a significant decrease in the frequency of a SNP in HLA-DRB1 region (Herbon, 2003). Furthermore genome-wide association study also found association of schizophrenia in chromosome 6p (The International Schizophrenia Consortium, 2009).

In our study, initially we have reported an association of HLA-A*03 with the sample size of 50 schizophrenic patients (Singh *et al.*, 2008). In the same study we have also reported the negative association of some of the HLA genes such as A*25, A*31 and B*51. Subsequently we have analyzed our findings in the large cohort of patient and control sample where we got the significantly higher frequency of HLA-A*03 genes in patient group. Our results also showed the significantly lower frequency of HLA A*31 and B*51 but not A*25 among the patient group. The association of HLA-A*03 found in the present investigation was in accordance with the previously reported study of our laboratory by Debnath *et al.*, 2005. However the sample size of the previous study was small and the study comprised only of the paranoid schizophrenics. Both the present findings and the findings of Debnath *et al.*, (2005) corroborated with the study by Rudduck *et al.*, (1984). On the other hand, in the present study the frequency of HLA-A*31 and B*51 are significantly lower which is the new findings of the present study. The most significant haplotype HLA A1-B8 observed among the patient is associated with the several autoimmune diseases such as celiac disease, autoimmune active chronic hepatitis, myasthenia gravis, adrenocortical hyperfunction-cushing's syndrome and systemic lupus erythematosus (Dorman *et al.*, 1990; Khalil *et al.*, 1992; Pugliese *et al.*, 1995; Brewerton *et al.*, 1973). Thus our results hint towards the autoimmunological background of the disorder. At this moment it is difficult to propose the mechanism of association of HLA with schizophrenia. Correlations with several other factors are also need to be established such low birth weight, viral infections, prenatal infections etc. which are at least common in Indian population to further shed light in this respect. Our result may be considered preliminary as the results had so far not been correlated with these factors.

Nevertheless, our result suggests the possible existence of a susceptibility locus for schizophrenia within the HLA region. The study further strengthens our earlier

finding of association of HLA-A*03 with schizophrenia along with the negative association of HLA A*31 and B*51. The study further suggests the susceptibility locus to schizophrenia in chromosome 6 close to the HLA locus.

Several immunological findings also suggest that immunological dysfunctions may have relevant implications for the etiology of schizophrenia. Accumulating evidence suggests that in some cases, schizophrenia is accompanied by changes in the immune system, such as the presence of anti brain antibodies in serum (Henneberg *et al.*, 1994), an altered distribution of T-cell subsets (Muller *et al.*, 1993), reduced mitogen-induced lymphocyte production of interleukin-2 (IL-2) (Bessler *et al.*, 1995, Ganguli and Gubbi, 1997), alteration in serum levels of interlukin-2 soluble receptor α (IL-2sRα) (Hornberg *et al.*, 1995), interleukin 2 (Theodoropoulou *et al.*, 2001; Zhang *et al.*, 2002 ; Zhang *et al.*, 2005) and interleukin 6 (IL-6) (Shintani *et al.*, 1991; Ganguli *et al.*, 1994; Frommberger *et al.*, 1997). These findings indicate that aberrant immune function in schizophrenia may be associated with the manifestation of the clinical phenotype and disease processes (McAllister *et al.*, 1997).

In the present study we have studied the serum level of Th-1 and Th-2 cytokines IL-2 and IL-6 respectively in schizophrenic patients and compared with the matched controls. The purpose of our study was to: (i) study the immunological alteration in schizophrenia, (ii) investigate the Th-2 shift hypothesis and (iii) investigate the immunomodulatory effect of the antipsychotic medicine. For this, we have divided the patients into psychotropic medication free and medicating groups. The serum level of IL-2 and IL-6 was measured by ELISA method. In the result we have observed the decreased level of IL-2 and IL-6 in both the groups. Further, we found the lower level of both the interleukins in the medicating patients than the psychotropic medicine free patients. Our results were in agreement with Theodoropoulou *et al.*, (2001) but in contrast to Ebrinc *et al.*, (2002), Zhang *et al.*, (2005) and Kim *et al.*,(2000). Taking this into account our results suggest some kind of immunological abnormalities in the schizophrenic patients and further hints the heterogeneity of schizophrenia which has also been suggested by Graver *et al.*, (2003). The unique finding of the present study is the lower level of IL-6 in the serum of the patients which we have reported first time in the world (Singh *et al.*, 2009). On the other hand our findings are not in agreement of the Th-2 shift hypothesis in schizophrenia. The lower level of

interleukins in the antipsychotic medicating patients suggests the immunomodulatory affect of the antipsychotic drugs.

The result of our present findings strengthens the hypothesis of immune system dysregulation in schizophrenia which may be one of the etiological factors for the disorder. Further, studies are needed to throw light on the exact mechanism of the changes in the serum level of IL-2 and IL-6 in schizophrenia.

Several researchers have suggested some kind of inflammatory process involved in schizophrenia (Rapaport and Lohr, 1994; Sirota, *et al.*, 2005). Therefore, in the present investigation we have studied a well known inflammatory marker C-reactive protein (CRP) in the schizophrenic subjects. Simple latex agglutination test was followed for determining the elevated level of CRP. CRP was treated as categorical variable, undetectable or normal (<6mg/L) and detectable or elevated (≥6mg/L). The results showed the elevated level of CRP in the drug naïve patients. The elevated level of CRP in this study provides further evidence of the involvement of inflammatory processes behind the etiopathology of schizophrenia. The elevated level of CRP in our study is in accordance to the findings of Fan (2007) and Dikerson (2007). In the study by Ohaeri *et al.*, 1993, the elevated level of CRP was found in the patient who was experiencing psychotic symptoms. In the follow up study of non-psychotic state, the level of CRP was found to be normal. In this respect the present study suggests that the antipsychotic drug may perhaps down regulate the inflammatory process which in turn brings the CRP level to the normal state. It is however not clearly understood whether the elevation of the level of CRP is the by-product of the pathophysiology of schizophrenia or directly contributes to the clinical manifestations of the disorder. Moreover our findings suggest that the inflammation may be another possible mechanism in the etiopathology of schizophrenia. Additional studies using the highly sensitive techniques like ELISA with longitudinal follow up studies in the large cohort of samples would be required to further strengthen the present study.

Another important marker which can highlight the immunological state of an individual is the T-lymphocyte. It has been suggested that the changes in the T-lymphocyte ratio reflects the changes in the metabolism of central nervous system cells and could be used as neural markers in the analysis of psychiatric disorders

(Gladkevich *et al.*, 2004). The appropriate CD4/CD8 lymphocyte ratio is expected to be 2:1. Ratio below 1:1 indicates serious disorder of the immune system (Kouttab *et al.*, 1989). Therefore, we investigated the percentage of CD4+ and CD8+ cells in the schizophrenic patients and compared with the normal controls. The result of our study did not show any abnormality in the percentage of CD4+ and CD8+ cells among the patients when compared with the control. On the other hand the CD4+ and CD8+ subset ratio also did not show any significant deviation from the control groups. The result of our findings corroborate with the work of Villemain *et al.*, (1989), Achirion *et al.*, (1994), Baskak *et al.*, (2008) and Sperner-Unterweger *et al.*, (1999) who also failed to find any differences in T lymphocyte subsets. However our result is in contrast to the findings of Zhang *et al.*, (1996) (2002), Cosentino *et al.*, (1996), Ganguli *et al.*, (1987) Henneberg *et al.*, (1990), Muller *et al.*, (1993), Cazzullo *et al.*, (1998), Cazzullo *et al.*, (1998) and Villemein *et al.*, (1999). Our findings on CD4+ and CD8+ ratio is not in agreement to Sperner-Unterweger *et al.*, (1999) who have found higher CD4/CD8 ratio than healthy controls.

When we compared our results with our own findings on IL-2 and IL-6. It was complete contrast and unlike the findings of Zhang *et al.*, 2002 who reported lower number of CD4+ cells and lower IL-2. In our study we found lower level of IL-2 and normal CD4+ cell percentage. Our result suggests the abnormal production of ILs is not due to the abnormal number of CD4+ cells but it may by due to some abnormality in the CD4+ cells or else there may have some other factors responsible for this phenomenon. Moreover knowledge about Th17 cells and Treg is growing day by day and it may help to understand the role of CD4+ and CD8+ in schizophrenia.

The demographic data show the study comprises of more number of male schizophrenics and they have long duration of illness compared to females which hints the higher vulnerability of men to this disorder, at least in this region. A comprehensive study should be done in order to shed light in this respect. Most of the patients included in the study did not have any family history of the psychiatric and autoimmune disorder. As far as smoking and other substance abuse is concerned, there is decrease incidence of smoking and other substance abuse among the patients. The present findings of significant association of schizophrenia with the patients who are not the first child was in accordance to the study of Sham *et al.*, (1993). The study suggests that in addition to the genetic predisposition some environmental factors

such as viral infection may play a pivotal role on the onset of the disorder and the older children in the family may act as a source of viral infection for developing fetus in the mother. Thus the present study strengthens the hypothesis "younger children in a family has a significantly increased risk of later developing schizophrenia". On the other hand the analysis of the season of birth among the patients and the control subjects did not show any correlation between them.

From our study it is still too early to speculate the autoimmune etiopathology of schizophrenia but the study definitely strengthens the hypothesis of immune dysregulation in schizophrenia which may be one of the etiological factors for the disorder. Our study also supports the hypothesis of increase risk of developing the disorder among the younger children of the family. Additional studies are needed to reveal the mechanism of the changes in the immune system in schizophrenia.

There are several strengths in this study. First, clinical assessment was rigorously conducted. The most refined assessment procedure was used for diagnosis. The assessment of psychotic symptoms was performed on the basis of interview data and supplementary data from family information as well as data from medical records. Great care was taken for selecting the control subjects. Modern molecular methods were used for the studies which are most reliable. To evaluate the significance of the results, the calculations were corrected for the fact that we performed multiple tests.

To conclude with, the present study provides suggestive, but not conclusive evidence for autoimmune basis of schizophrenia. Moreover our study suggests the immunological dysfunction in schizophrenia which may be one of the etiological factors for the disorder. The findings which have been reported in this study should be regarded as preliminary since they are based on only a small number of individuals and awaits further research.