Original Article

Interleukin-2 Levels in Chronic Schizophrenia Patients

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Abstract

Introduction: Most research in interleukin activity in schizophrenia has been in Caucasian populations. We examined interleukin-2 (IL-2) levels and their relation to the duration of the illness, psychopathology and treatment effects, in chronic schizophrenia patients of Asian origin. Materials and Methods: Thirty chronic schizophrenia patients were recruited for the study and their demographic data and medication dosage were noted. Symptom severity was scored on the Positive And Negative Syndrome scale for Schizophrenia (PANSS) and blood sampling done. Ten healthy Chinese males were recruited as controls. Phytohaemagglutinin-stimulated production of serum levels of IL-2 were measured by enzyme-linked immunosorbent assay. Results: IL-2 levels (1327 ± 596.2) of all 30 patients were significantly lower than that of the Chinese controls (2420 ± 342.5). This effect was noted throughout the entire duration of the illness. Ethnic and age differences in IL-2 levels were not found. There was, however, a negative correlation with the duration of the illness and a positive correlation with the dosage of medication. Conclusions: The results of this study of a population of mostly Chinese patients with schizophrenia replicate an important finding. Data such as this has not been reported previously on Asians of this racial group.

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Introduction

Autoimmune processes have been implicated in the pathogenesis of schizophrenia. Several pieces of indirect evidence point towards a role of autoimmune processes in at least some cases of schizophrenia. The most widely studied of these have been cytokines, which comprise families of molecules such as interleukins, lymphokines, monokines, growth factors, interferons and chemokines.[1] Secreted by immune system cells and synthesised by neuronal tissue in the brain, their activities range from altering growth and differentiation to modulating neuronal and neuroendocrine activity.[2]

Abnormalities of interleukin activity have been reported in schizophrenia.[3-5] These include decreased lymphocyte production of interleukin-2 (IL-2) following mitogenic stimulation and increased serum IL-2 receptors.[6-9] Low IL-2 production has been consistently found in acute schizophrenia and has been noted to be independent of treatment.[10] Pollmacher et al[11] found that haloperidol treatment for 6 weeks had no effect on plasma levels of various cytokines. Ganguli et al[10] noted that low IL-2 production occurred early in the course of the disorder and was associated with an earlier age of onset and negative symptoms.

All these studies were done in Caucasian populations; only 2 studies have been done in non-Caucasian populations. Yong et al[12] found decreased IL-2 production and increased IL-2 serum levels in Korean schizophrenia patients, while Rapaport et al[7] reported that serum soluble IL-2 receptor concentrations were elevated in both Korean and Caucasian schizophrenia patients.

Some studies have also suggested that people with schizophrenia have a different rate of certain autoimmune diseases to the general population. Eaton et al[13] reported a lower-than-expected rate of rheumatoid arthritis among people with schizophrenia. Baldwin[14] found an excess of

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myxoedema among patients with schizophrenia in a record linkage study, while Finney[15] reported a deficit of schizophrenia among patients of insulin dependent diabetes mellitus. Sirota et al,[16] in a study of multicase families with schizophrenia, found that both patients with schizophrenia and their healthy first-degree relatives had an increased incidence of antinuclear autoantibodies as compared to controls. These findings suggest that the inheritance of schizophrenia is related to the inheritance of autoimmune diseases.

In this study, we examined the interleukin levels in chronic schizophrenia patients of Asian origin: the Chinese, Malays and Indians. These levels were studied in relation to the duration of the illness, psychopathology and treatment effects.

Materials and Methods

Approval was given by the hospital's Ethics Committee to undertake this study, which involved patients with chronic schizophrenia. After complete description of the study to the subjects, written informed consent was obtained. Only patients who gave written informed consent were entered into the study. The assessment involved weight and height measurements, collection of demographic data, a mental state examination and scoring on the PANSS and blood sampling.

Thirty male patients were entered into the study. There were 16 Chinese, 9 Malays, 4 Indians and 1 of mixed parentage (Chinese father and Indian mother). All patients fulfilled the DSM IV (Diagnostic and Statistical Manual of Mental Disorders, 4th ed) criteria for schizophrenia. Their medications remained unchanged for 6 months prior to study. Patients with comorbid psychiatric conditions and alcohol and substance abuse were excluded from the study. Patients with autoimmune disorders, infections and on other medications such as antibiotics and immunomodulatory drugs, were also excluded. Ten healthy Chinese males with no history of mental illness, substance abuse or physical illness were recruited as controls.

Venous blood was collected in heparinised tubes on the day of assessment. Aliquots of venous blood were mixed with RPMI 1640 (Roswell Park Memorial Institute) media in a ratio of 1:9 (vol/vol). The media was supplemented with 10 mmol/L HEPES (N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid) buffer, 2 mmol/L glutamine and 50 ng/ml streptomycin. All the chemicals used in the study were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). To generate IL-2 production, 0.5 ML of diluted blood was stimulated with phytohaemagglutinin in a final concentration of 5 mL/mL in 24 well-plates for 48 hours at 37^{o}C in 5% carbon dioxide.

At the end of the incubation period, the media in each well was placed in polypropylene tubes and freeze-thawed twice in liquid nitrogen to obtain the supernatant, which was stored at –80^{o}C until assayed. The IL-2 obtained was quantified using a commercially available enzyme-linked immunosorbent assay kit (Research and Diagnostic System, Minneapolis, Minnesota, USA).

The 1-Sample Kolmogorov-Smirnov test was used to check the normality of the continuous variables. If the normality assumptions were satisfied, the parametric t-tests/ANOVA were used; otherwise the Mann-Whitney U test or Kruskal-Wallis test was applied. Multiple regression was applied for the primary response variable IL-2, adjusting for relevant covariates. A P value <0.05 was considered statistically significant.

Results

The schizophrenic patients ranged in age from 20 to 69 years, compared to 20 to 49 years for the controls (P = 0.158, Mann-Whitney U test). Both groups were also comparable in weight (P = 0.972, 2-sample t-test) and height (P = 0.888, 2 sample t-test). The characteristics of the patients and controls are given in Table 1.

No differences in IL-2 levels were detected amongst the different ethnic groups (P = 0.378, Kruskal-Wallis test). Thus, we were able to compare the patients (regardless of race) versus the controls (only Chinese).

The IL-2 levels (1327 ± 596.2) of all 30 patients were significantly lower (P <0.001, Mann-Whitney U test) than that (2420 ± 342.5) of the controls. Similarly, for all patients, regardless of the duration of their illness, the IL-2 level was significantly lower compared to that of the controls (P = 0.003, Kruskal-Wallis Test). The trend tended towards lower levels with a longer duration of illness (Table 2) but this was not statistically significant. (P = 0.90, Kruskal-Wallis test). However, there were no significant differences among the different age groups. Patients on higher Chlorpromazine (CPZ) doses of neuroleptic medication had higher levels of IL-2 compared to those on lower doses of CPZ equivalents (Table 2), but this was not statistically significant (P = 0.90, Kruskal-Wallis Test). Using multiple regression analysis and taking into account age, total PANSS score and CPZ equivalent doses as covariates, the IL-2 levels of the patients were significantly lower than that of the controls throughout the duration of the illness (P <0.001).

The Chinese patients were comparable with the 10 Chinese controls in height and weight, except for age (P = 0.04, Mann-Whitney U test). The patients had significantly lower IL-2 levels when compared to controls (P <0.001, 2-sample t-test) regardless of the duration of the illness (P = 0.01, Kruskal-Wallis test). There were no

Table 1. Characteristics of Patients and Controls

	Patients		Controls
	All Races (n = 30)	Chinese Only (n = 16)	Chinese Only (n = 10)
Age groups (%)			
20-29	6.7	0.0	20.0
30-39	43.3	31.3	50.0
40-49	33.3	56.3	30.0
50-59	6.7	12.5	0.0
60-69	10.0	0.0	0.0
Weight (kg)			
Mean	64.1	63.3	63.9
SD	13.1	13.9	6.1
Range	45.0-92.0	51.0-92.0	53.0-72.0
Height (m)			
Mean	1.67	1.66	1.67
SD	0.07	0.07	0.06
Range	1.50-1.80	1.54-1.79	1.58-1.80
Duration of illness (y)			
0-9	36.7	37.5	-
10-19	40.0	37.5	-
20-29	16.7	18.8	-
30-39	6.7	6.3	-
Interleukin-2 (pg/mL)			
Mean	1327.0	1431.1	2420.0
SD	596.2	528.2	342.5
Range	742.7-3379.7	820.3-2526.6	1900-2900
CPZ level			
Mean	508.3	487.5	-
SD	354.9	310.0	-
Range	50-1225	50-1150	-
PANSS Score (%)			
30-59	0.0	0.0	100.0
60-89	20.0	25.0	0.0
90-119	80.0	75.0	0.0

CPZ: chlorpromazine; PANSS: Positive and Negative Syndrome Scale for Schizophrenia

Table 2. IL-2 Levels with Duration of Illness and CPZ Levels

Variable	Patients		Chinese Controls (n = 10)
	All Races (n = 30)	Chinese Only (n = 16)	
Duration of illness (y)			
0-9	1335.0 ± 566.0 (742.7-2526.6)	1544.2 ± 657.5 (820.3-2526.6)	
10-19	1408.4 ± 768.8 (742.7-3379.7)	1427.8 ± 566.6 (975.4-2449.0)	2420.0 ± 342.5 (1900-2900)
20-29	1192.6 ± 292.2 (897.9-1673.4)	1337.3 ± 293.6 (1130.5-1673.4)	
30-39	1130.7 ± 109.9 (1053.0-1208.4)	53.0 ± 0.0 (1053-1053)	
CPZ level			
50-449	1262.8 ± 442.8 (742.7-2526.6)	1518.3 ± 507.7 (1053-2526.6)	
450-849	1374.3 ± 582.8 (820.3-2449)	1350.3 ± 634.7 (820.3-2449)	
≥850	1453.7 ± 994.9 (742.7-3379.7)	1324.4 ± 493.6 (975.4-1673.4)	

Values are mean ± SD (range)

Clinical implications:

1. Cytokine dysregulation may be ongoing in schizophrenia.
2. Duration of illness is an important contributing factor.
3. Neuroleptic medication has little effect in reversing cytokine dysregulation.

Limitations of the study:

1. Small sample and control group size.
2. The study was cross-sectional. Longitudinal assessments and blood sampling might show differences in interleukin-2 over time.
3. Available literature on interleukin-2 in schizophrenia is small.

significant association between IL-2 levels and CPZ equivalent doses of neuroleptic medication (P = 0.56, Kruskal-Wallis test).

In this subgroup too, IL-2 values after adjusting for age, total PANSS score and CPZ levels, for all patients with different durations of the illness were significantly lower (P <0.001) as compared to the controls.

Discussion

The association between schizophrenia and an autoimmune reaction has been reviewed previously.[17] However, laboratory findings have not been regularly replicated, possibly due to differing techniques.

Our study found that IL-2 production was low in chronic schizophrenia patients of Asian origin. This confirms the finding in Caucasian schizophrenic populations namely, that IL-2 production is below normal in schizophrenia patients. There were also no differences amongst the Malay, Chinese and Indian ethnic groups, although the sample size was admittedly small. Our findings also indicate that those with a longer duration of illness had significantly lower IL-2 levels, indicating that cytokine dysregulation may be ongoing. Changes in cytokine concentration with the duration of illness was reported by Ganguli et al[18] in their study done on serum interleukin-6 (IL-6) concentration in schizophrenia patients. They reported significantly higher levels of IL-6 in patients as compared to controls, and comparisons within the patient group revealed that serum IL-6 was significantly correlated with duration of illness. They postulated that elevated IL-6 levels in schizophrenia develop during the course of illness and may be related to either treatment or to disease progression.

Patients on higher CPZ equivalent doses of neuroleptic medication had higher levels of IL-2. This is in contrast with earlier findings by Ganguli et al[10] and Pollmacher et al[11] that IL-2 production in acute schizophrenia was

independent of treatment effects. There is a possibility that medication reverses the immunological response. Bessler et al[19] found that neuroleptic treatment resulted in a 61.9% increase in IL-2 production compared to schizophrenic patients who were not on treatment and the levels reached did not significantly differ from controls. Leykin et al[20] have tested the effects of antipsychotics on phyto-haemagglutinin stimulation of peripheral blood lymphocytes from normal subjects determined by ^{3}H-thymidine uptake and cytokine production. They demonstrated a clear suppression of both ^{3}H-thymidine uptake and IL-2 secretion thereby leading them to suggest that neuroleptics can act as immunomodulators. In our case, the longer duration of the illness could have resulted in a lack of increase in IL-2 production even after therapy. This may indicate that prolonged illness could cause irreversible changes in cytokine response. However, we were unable to show any correlation between the total PANSS score, an indication of symptom severity and IL-2 level.

The limitations of this study include the small sample size and control group size. The study was also cross-sectional; longitudinal assessments and blood sampling might show differences in IL-2 levels over time. Nevertheless, the findings have significant clinical implications. It appears that cytokine dysregulation may be ongoing in schizophrenia and that the duration of the illness is an important contributory factor. Neuroleptic medication appears to have little effect in reversing cytokine dysregulation.

While the findings are not completely new, they represent a replication of an important finding in a small Asian population of mostly Chinese origin. Data such as this has not been reported previously on Asians of this racial group.

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