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Altered IL-2 and IL-10 serum levels in schizophrenic patients

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Abstract

Schizophrenia (SZ) is severe mental disorder and characterized by fundamental disturbances in thinking, perception and emotions. Immune deregulation has been postulated to be one of the mechanisms underlying the pathogenesis of schizophrenia. This study hypothesized that interleukins would have a link with schizophrenia patients. The serum IL-2 and IL-10 levels were examined by enzyme-linked immunosorbent assay (ELISA) in schizophrenia patients (n=60) and healthy controls (n = 30). The results showed that serum IL-2 and IL-10 levels were significantly different among schizophrenia patients. The observations indicate a significant decrease ($P < 0.05$) in schizophrenic patients serum levels of IL-2 compared with healthy control. Whereas detection of IL-10 in the schizophrenic patients serum showed significantly increase level ($P < 0.05$) compared with healthy control. The results supported that immune disturbance is related to the schizophrenia patients and might play an important role in the pathophysiology of schizophrenia.

Keywords: Schizophrenia, IL-2, IL-10, ELISA.

تغير مستوى الحركيات الخلوية (IL-2 و IL-10) في مرضى فصام الشخصية

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الخلاصة

يعتبر مرض الفصام (SZ) اضطراباً عقلياً شديداً، ويتميز باضطرابات في التفكير والإدراك والعواطف. وقد افترض الاضطراب المناعي ليكون واحد من الآليات الكامنة وراء التسبب في مرض الفصام. افترضت هذه الدراسة أن الحركيات الخلوية سيكون لها ارتباط مع المرضى بالفصام. تم فحص مستوى الحركيات الخلوية (IL-2 و IL-10) في مصل المرضى الذين يعانون مرض انفصام الشخصية (n=60) والأصحاء (n=30) باستعمال تقنية الأمتزاز المناعي المرتبط بالإنتزيم. وأظهرت النتائج أن مستوى الحركيات الخلوية (IL-2 و IL-10) كانت مختلفة بشكل كبير بين مرضى الفصام. حيث أظهرت النتائج إلى وجود انخفاض معنوي ($P > 0.05$) في مستويات مصل مرضى الفصام للأنترلوكين IL-2 مقارنة مع عينات الأصحاء. في حين كشف مستوى IL-10 في مصل مرضى الفصام ارتفاعاً معنوياً ($P > 0.05$) مقارنة مع الأصحاء. هذه النتائج تدعم أن الاضطراب المناعي له علاقة بمرض الفصام، وربما يلعب دوراً هاماً في الفيزيولوجيا المرضية لمرض انفصام الشخصية.

Introduction

Schizophrenia (SZ) is characterized by delusions, hallucinations, disorganized speech and behavior, and other symptoms that cause social or occupational dysfunction. SZ entity was first

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explained by Emil Kraepelin in 1878. For a diagnosis, symptoms must have been present for six months and include at least one month of active symptoms. The prevalence of SZ is estimated to be about 0.5 - 1.0 %, being slightly more frequent in the male gender [1].

The etiology of SZ is currently unknown; it could be result from a complex combination of genetic, environment, and immunological factors [2]. While the etiology and pathogenesis of SZ are poorly understood, there is evidence that immune system abnormalities are associated with symptoms in a substantial number of affected individuals [3]. Immune dysfunction plays a major role in the pathophysiology of SZ [4]. Owing to their multiple roles in mediating and modulating inflammatory processes in the CNS [5, 6], a great deal of interest has been centered upon the role of microglia in SZ [7]. A decrease in IL-2 production is one of the most frequently confirmed immunological phenomena in SZ [8]. Low IL-2 production has been consistently found in acute SZ and has been noted to be independent of treatment [9,10].

IL-10 is secreted under different conditions of immune activation by a variety of cell types, including T cells, B cells, and monocytes/macrophages [11,12]. Although IL-10 is classified as a Th2-type cytokine, it has been shown to suppress a broad range of inflammatory responses and is known to be an important factor in maintaining homeostasis of overall immune responses [13-15]. There was a strong relationship between an increased IL-10 level and negative symptoms of SZ were observed [16].

Interleukin-10 is a pleiotropic cytokine that inhibits cell mediated immunity while enhancing humoral immunity. It also inhibits the synthesis of a number of cytokines such as IL-2. [17].

Materials and Methods

Patients and Controls

Two groups of subjects were enrolled in the present study during the period December 2013 - March 2014. The first included 60 cases of Schizophrenia (SCZ) from both genders: 31 male and 29 female, with an age range of 21-72 years. For the purpose of comparison 30 age and gender matched cases were enrolled as a control including: 19 male and 11 female of healthy individuals. From each participating subject, venous blood sample (3 ml) was obtained. The serum obtained by putting the blood samples in a clean dry plain plastic tube and allowed to clot at room temperature (20-25 C) for 15 minutes before centrifugation. The tubes centrifuged at 5000 rpm for 5 minutes, serum was collected and kept in freezer until used. The laboratory methods included assessment of serum for cytokines; Interleukins (IL-2 and IL-10) were determined by enzyme-linked immunosorbent assay (ELISA) method.

Assessment of Serum for Cytokine Levels (IL-2 and IL-10)

Before carrying out the assay procedure of IL-2 and IL-10, the kit was left at room temperature (18-25°C) for 30 minutes to equilibrate, as suggested by the manufacturer. After that, assay was carried out following the instructions in the kit's leaflet (PeproTech; USA):

- a) The wells of the plate were coated with capture antibody by dispensing 100 µl of anti-human of IL-2, IL-10 and antibody in each well, and the plate was sealed and incubated overnight at room temperature (18-25°C).
- b) After 24 hours, the contents of the wells were discarded and each well was washed four times with washing buffer (300 µl / well / wash), and then the plate was inverted to remove residual buffer and blotted on towel paper.
- c) In each well, 100 µl of block buffer was dispensed and the plate was incubated at room temperature for 60 minutes, and then the washing step was repeated (step b).
- d) An aliquot (100 µl) of standards IL-2 (125, 250, 500, 1000, 2000 and 4000 pg/ml), IL-10 (78.13, 156.25, 321.5, 625, 1250 and 2500pg/ml), or serum samples was dispensed into respective wells. The plate was incubated at room temperature for two hours and then the washing step was repeated (step b).
- e) An aliquot (100 µl) of detection antibody (biotinylated anti-human IL-2, IL-10, or antibody) was dispensed in each well. The plate was incubated at room temperature for two hours, and then the washing step was repeated (step b).
- f) An aliquot (100 µl) of avidin-HRP conjugate was dispensed in each well. The plate was incubated at room temperature for 30 minutes, and then washing step was repeated (step b). Finally, 100µl of

substrate solution was added, and color development was monitored with ELISA plate reader and absorbance was measured at a wave length of 405 nm. Three readings were done (3, 6, and 9 minutes) and the mean absorbance was considered for calculations. The sample results were calculated by interpolation from a standard curve that was performed in the same assay as that for the sample by using standard curve fitting equation (Figures-1, -2 for IL-2 and IL-10 respectively).

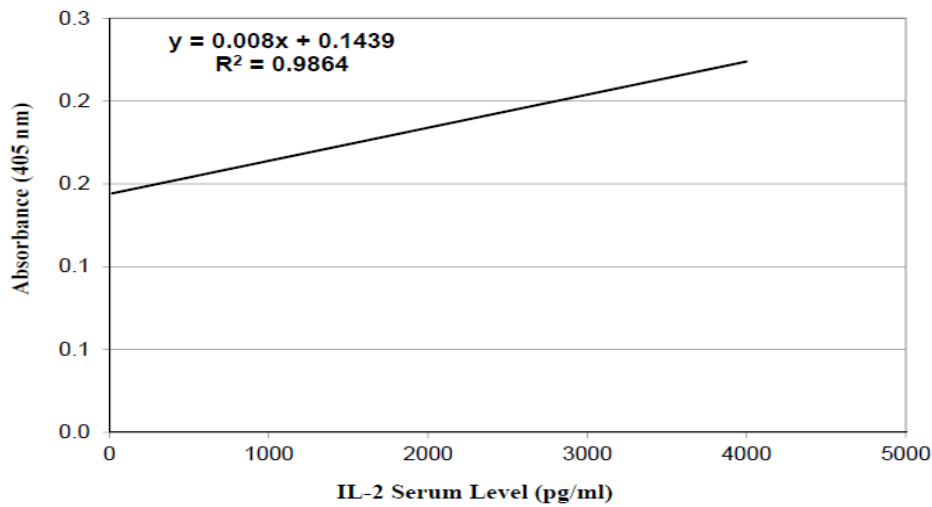


Figure 1-Standard Curve of IL-2 Serum Level.

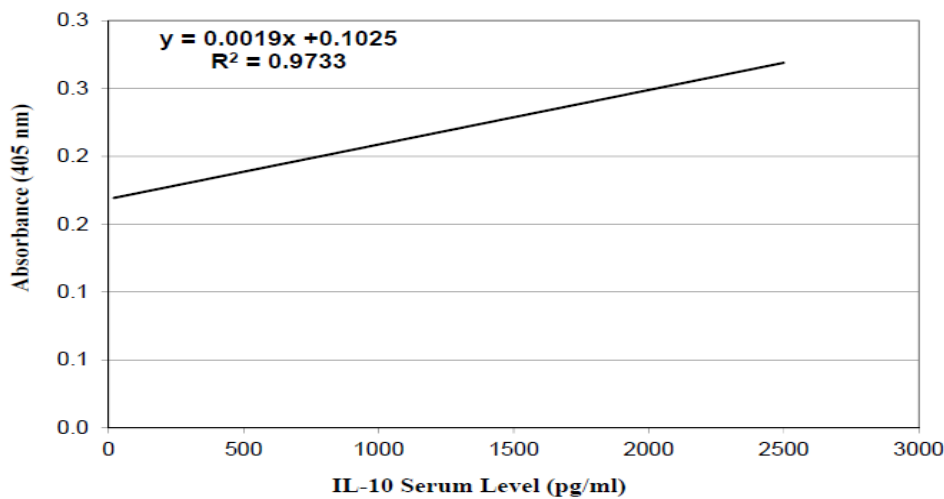


Figure 2-Standard Curve of IL-10 Serum Level.

Statistical Analysis

Data were entered and analyzed by using SPSS (Statistical Package for Social Sciences) version 21 for Windows. Descriptive statistics (frequencies, percentages, tables, graphs) and inferential statistical were used. Independent T-test used to compare mean between cases and controls. P-value ≤ 0.05 was considered Statistically Significant.

Results and Discussion

Interleukin-2

The results showed a significant decreased in the schizophrenic serum level of IL-2 was recorded (10.55 ± 0.69 vs.15.65 ± 13.3) pg/ml compared with the healthy control group (P≤ 0.05) as shown in Table-1.

Table 1- Serum Level (pg/ml) of IL-2 in schizophrenic patients and control.

Group	N	IL-2 level Mean \pm SE (pg/ml)	95% Confidence Interval for Mean		P \leq
			Lower Bound	Upper Bound	
Schizophrenia	60	10.5 \pm 0.69*	9.16	11.93	0.05
Control	30	15.6 \pm 1.12	13.30	17.91	

* = significant difference (P \leq 0.05) between means.

The current study results matched with of Mahendran and Chan [10], who reported that IL-2 production was low in chronic SZ patients. Moreover, Potvinet *al.*, [18], showed a decrease in IL-2 levels in SZ patients relative to controls. Furthermore, Wilke [19] and Aroltet *al.*, [20], confirmed the association between acute SZ and low IL-2 production.

In patients with SZ, decreased IL-2 production and increased level of the soluble IL-2 receptor (sIL-2R) have been observed [20]. On the other hand, studies conducted by Cazzullo *et al.*, [21], have found significantly higher production of IL-2 in schizophrenic patients than in controls. Paul *et al.*, [22], concluded that low levels of IL-2 in paranoid SZ is due to the variations in promoters of IL-2 genes may contribute to the development and clinical course of the disease. Maes [23], proposed the macrophage T-lymphocyte theory, which states that IL-2 produced by chronically activated macrophages and T-lymphocytes, are the fundamental mediators of SZ.

In the latter study, a highly significant positive correlation was found between age-of-onset and IL-2 production. This is particularly important, since it suggests an association between lowered IL-2 production and the structural brain abnormalities which are supposed to be associated with these clinical features [24].

Interleukin-10

The study observations indicated a significant increase (P \leq 0.05) in SZ serum level of IL-10 (12.91 \pm 0.96 pg/ml) compared with the healthy control (6.03 \pm 0.64 pg/ml) as shown (Table 2).

Table 2- Serum Level (pg/ml) of IL-10 in schizophrenic patients and control.

Group	N	IL-10 Level Mean \pm SE (pg/ml)	95% Confidence Interval for Mean		P \leq
			Lower Bound	Upper Bound	
Schizophrenia	60	12.91 \pm 0.96*	10.99	14.84	0.05
Control	30	6.03 \pm 0.64	4.70	7.36	

* = Significant difference (P \leq 0.05) between means.

These results suggest that IL-10 (anti-inflammatory and regulatory cytokine) may play a role in the pathogenesis of SZ. This is matching Al Asmary *et al.*, [25], who noticed that there was elevated level of cytokine IL-10 in the serum of patients with SZ and one of the mechanisms attributed to the role of IL-10 in reducing inflammation in SZ is suppression of pro-inflammatory cytokines.

On the other hand, Ajami *et al.*, [24], has concluded that the increase of TNF- α and decrease of IL-10 may have an important role in psychopathology of SZ.

There was a strong relationship between an increased IL-10 level and negative symptoms of SZ were observed [16]. Upthegrove and Barnes [26], reported that IL-10 produced by Th2 and Treg lymphocytes and its role as anti-inflammatory: inhibits IFN- γ production and involved in compensatory anti-inflammatory response syndrome (CARS) is increased in schizophrenic patients compared with healthy control.

Dimitrovet *al.*, [27], reported that the reviewing of the fetal brain cytokine imbalance of SZ it was report that IL-1 β is most capable in inducing the conversion of rat mesencephalic progenitor cells into a dopaminergic phenotype and that IL- 6 is highly efficacious in decreasing the survival of fetal brain serotonin neurons. It was interesting that enhanced levels of IL-10 during prenatal development are sufficient to prevent the emergence of multiple behavior abnormalities [28].

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