

# Increased Natural Killer Cells Activity in Schizophrenic Patients

Mohammad Vodjgani\*, Hedieh Matloubi, Abbas Ali Nasehi, Mohammad Hossein Niknam, Anoushirvan Kazemnejad, Eisa Salehi, Tahereh Aboufazeli, Zahra Gheflati

Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

## ABSTRACT

**Background:** Schizophrenia has been associated with altered immunity. Different studies regarding natural killer cell activity (NKA) in schizophrenic patients have shown inconsistent results. **Objectives:** To evaluate NK cell activity in schizophrenic patients in comparison with healthy control individuals. **Methods:** 30 medication-free schizophrenic patients and 41 healthy sex, age and smoking status matched individuals were included in this study. NK cell activity of case and control subjects was measured by Methyl-Thiazol-Tetrazolium (MTT) test. Statistical analysis of the data was done using SPSS 11.5 software. **Results:** NK activity of patients and normal subjects had a mean of  $36.94 \pm 26.15$  (Mean  $\pm$  SD) and  $22.31 \pm 17.92$ , respectively. A significant increase in NK activity in schizophrenic patients compared to controls ( $P = 0.011$ ). Among patients, NK activity of smokers was significantly lower than that of non-smokers ( $P = 0.02$ ). Other demographic factors didn't show any influence on NK activity. **Conclusion:** The higher activity of NK cells in the schizophrenic patients as compared with the control population could explain the low incidence of cancer in these patients. Decreasing the effect of smoking on NK activity in the patients could be one of the responsible factors for the inconsistency in the results of different studies.

**Keywords:** Methyl-Thiazole-Tetrazolium Test, Natural Killer Cell activity, Schizophrenia

\*Corresponding author: Dr. Mohammad Vodjgani, Department of Immunology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran. Tel/Fax: (+) 98 21 6641 9536, e-mail: vojganim@sina.tums.ac.ir

## INTRODUCTION

As a severe psychiatric illness, Schizophrenia has been associated with different alterations in the immune system. Increased levels of IL-1, IL-2, IL-6 and their soluble receptors (sIL-2R, sIL-6R), decreased production of IL-2 by lymphocytes, increased levels of nonspecific and specific autoantibodies against CNS in serum and CSF of schizophrenics are the most common findings in schizophrenia (1,2). But the role of Immunology in the etiology of the disease is still unknown. One of the studied immunological factors in schizophrenia is the cytotoxic activity of NK cells. What makes studying NK activity (NKA) in schizophrenia more interesting is the low incidence of lung cancer and other malignancies in schizophrenic patients, despite the high frequency of smoking among them (more than 3/4 of patients) (3,4). The results of NKA studies, like most other immunological factors associated with schizophrenia, have been very controversial. Some studies found no difference in NKA measures in schizophrenics compared with the normal population, while others showed increased or decreased NKA in these patients.

## SUBJECTS AND METHODS

**Subjects.** 30 patients with schizophrenia including 26 men and 4 women between 22-48 years of age who referred to the clinic of Razi psychiatry hospital between the June and the October of 2004 and 41 healthy volunteers from Tehran Blood Transfusion Organization who were matched with patients in age, sex and smoking status were studied.

The patients were diagnosed by a psychiatrist according to axis I of DSM-IV (The fourth edition of the Diagnostic and Statistical Manual of Mental Disorders) criteria. None of the patients were diagnosed with axis II of DSM-IV (mental retardness or personality disorder). Taking neuroleptics or any other medication for at least two weeks which could possibly affect the immune system, having long-acting injections within the past two months, accompanying somatic illnesses and drug abuse were exclusion criteria for the patients. Any psychiatric diagnosis was considered an exclusion factor for control individuals.

**K562 Cell Culture.** K562 cells (NCBI: 122) were obtained from the cell bank of Pasteur Institute of Iran as a target for NK cell cytotoxic activity. The cells were cultured in a medium of RPMI-1640 + 100 U/ml Penicillin + 100 µg/ml Streptomycin + 2 gr/lit Sodium bicarbonate + 0.03 gr/ml L-Glu + 10% FBS (Fetal bovine serum) and were incubated in a moistened 37°C incubator with 5% CO<sub>2</sub>. The cells were passaged every other day.

**Preparation of Peripheral Blood Mononuclear Cells (PBMC).** Ten ml heparinized blood were obtained from all the subjects in total sterile condition. Blood was diluted 1:1 with incomplete RPMI medium (without FBS and L-Glu) and was centrifuged with Ficoll-Hypaque (1077) at 900g for 20 minutes in 24°C. Collected buffy coats were washed twice by incomplete RPMI, each time centrifugated at 280g for 20 minutes. Cell suspension was diluted to  $2 \times 10^6$  cells/ml with complete RPMI medium.

**Measuring NK Cell Activity by MTT (Methyl-Thiazol-Tetrazolium) Test.** MTT test was performed as described previously (5,6). Briefly, PBMC together with K562 cells (in a concentration of  $2 \times 10^5$  cell/ml) were incubated in the wells of a flat bottom 96 well sterile cell culture plate (Effector: Target ratio of 10:1) with a total volume of 100µl in each well (ET). 100µl of the same concentration of PBMC (E), 100µl of the same concentration of K562 (T), and 100µl of RPMI medium (B) were incubated in different wells as controls and blank, respectively. This test was done in triplicates.

After 4 hours of incubation in a moistened 37°C incubator with 5% CO<sub>2</sub>, 10µl of MTT solution (5mg/ml PBS) was added to each well. After 3 hours of incubation, 100µl of an acidified

SDS solubilizer (10% SDS in 0.01 N HCl) were added to each well. The plate was incubated for another 16 hours in the same incubator. Optical density of wells was read at 570 nm against reference wavelength of 630 nm by an ELISA reader (Behring). Percentage of NK activity was calculated as:

$$\text{NKA}\% = \left[ 1 - \frac{\text{OD}_{(ET)} - \text{OD}_{(E)}}{\text{OD}_{(T)}} \right] \times 100$$

**Data Analysis.** Results were analyzed by SPSS 11.5 statistical software. Independent student t-test and Pearson Correlation test were used. Differences with P value less than 0.05 were considered significant.

## RESULTS

The mean age of patients and healthy subjects were  $36.77 \pm 5.99$  and  $36.86 \pm 6.6$ , respectively. There was no significant difference in age and sex distribution between the two groups

**Table 1. Sex, age and smoking status distribution in the case and control group**

		Case	Control
Sex	Men	26	35
	Women	4	6
Age	Minimum	22	22
	Maximum	48	49
	Mean	36.77	36.86
	SD	5.99	6.60
Smoking Status	Smoker	13	15
	Non-smoker	16	26

(Table 1). Within the patient group, mean duration of illness was  $13.75 \pm 7.31$  and mean age of onset was  $22.67 \pm 7.29$ . Among 30 patients, 20 were paranoid, 2 catatonic, 4 undifferentiated and 3 residual. The subgroup of one of the patients was undiagnosed (Table 2).

Thirteen patients were smokers and 16 were non-smokers. Smoking status of one patient was unknown. Within the control population, 15 were smokers and 26 were non-smokers. Smoking status distribution between the two populations was not significantly different (Table 1).

The mean NKA among patients was  $36.93 \pm 26.15$  while the control population had a mean of  $22.31 \pm 17.92$ . Analysis of these results by Independent t-test showed significant increase of NKA in patients compared with healthy subjects ( $p = 0.011$ ).

Effects of sex and smoking status on the NKA were evaluated by independent t-test. No significant difference existed between men and women in the case or control groups. Within the case group there was a significant decrease of NKA in smokers as compared with non-smokers ( $p = 0.02$ ). There was no significant difference between the smoker patients and the control group. No significant difference was found between smokers and non-smokers within the normal subjects (Table 3).

**Table 2. Distribution of subgroups in the case group**

Disease Subgroup	Number	Percentage
Paranoid	20	69
Catatonic	2	6.9
Undifferentiated	4	13.8
Residual	3	10.3
Total	29	100

Pearson Correlation test showed no correlation between age, age of onset and duration of illness and NKA. No significant difference in NKA measures was shown among different subgroups or between paranoid and non-paranoid categories.

**Table 3. Comparison of mean NK activity between patients and controls**

	Patients	P Value	Controls	P Value
	36.93 ± 26.15		22.31 ± 17.92	0.011
Smoker	33.16 ± 20.99	0.02	23.52 ± 10.25	0.75
Non Smoker	44.27 ± 21.11		23.78 ± 21.28	

## DISCUSSION

The results of present study showed significant increase of NKA in schizophrenics compared with healthy subjects. This might be an evidence of higher immunological surveillance in these patients. But as in other immunological fields, results of NKA in schizophrenic patients have been varied and contradictory. Some researchers such as Wang et al., Ghosh et al., and Yovel et al. also showed increased NKA in schizophrenics as compared to the normal population. Like our investigation, Wang et al. worked on drug-free patients, while Ghosh studied medicated patients (7,8). Yovel reported increased NKA per NK cell in hospitalized schizophrenic patients compared to healthy subjects and showed that neuroleptic therapy normalizes NKA measures in patients. They also showed that NKA was lower in smokers and in women than in non-smokers and men within the case and control groups (9), although the effect of smoking did not reach statistical significance. Besides, most of the patients in his study had been on neuroleptic therapy. Small sample size for the drug-free patients and the possible effect of hospitalization on the immune system are limitations and possible confounding factors of his study. The present study showed significant decrease in NKA in smokers compared to non-smokers in the patient population. However, there was no such significant difference in NKA within the control group. Excluding the smoking patients, there was no significant difference between the case and the control groups. NKA difference between men and women in the current study failed to reach significance, probably due to the low number of women in the study. Some studies did not find any significant difference in NKA between schizophrenics and normal individuals. McDaniel's study had a small sample size for schizophrenics. Therefore, he reported his results with a SD larger than the Mean (10). Caldwell studied drug-naïve paranoid in patients. He had a small sample size and besides, all of his patients had a late age of onset and were in their first episode of schizophrenia (11).

In most studies done prior to that of Caldwell, the effects of neuroleptics and demographic factors were not considered (12-14).

Abdeljaber et al. showed significantly decreased NKA in schizophrenics compared to healthy individuals. He had chosen a large sample size and eliminated the effect of neuroleptics in his study, but all of the patients were classified among the undifferentiated subgroup (15). Koliaskina et al., Vasileva et al. and Sasaki et al., also reported significant decrease in NKA in schizophrenics compared with the healthy population (16-19).

In the present study, we eliminated the confounding effects of medication, smoking, accompanying diseases and drug abuse on NKA. Thus, it seems that the higher NKA among the patients is a characteristic of the disease, not an epiphenomenon. Other than our study, Yovel

was the only researcher who considered smoking as a confounding factor, and in both of these studies specially the current one, with larger sample size for drug-free patients, smoking influence on NKA has been proven. It is quite likely that by controlling the effect of smoking and medications in other studies, much of the discrepancies shown could be clarified.

In conclusion, more research with large sample sizes for each disease subgroup, plus a comprehensive control for possible confounding factors such as neuroleptic medications and smoking status is suggested.

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