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Assessment the Serum Level of IL6, IL10 versus Neuregulin 1 among Schizophrenic Patients in Iraq

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Abstract

Background: *Schizophrenia is a severe and chronic neuropsychiatric disorder with a lifetime prevalence of about one percent throughout the world. Supporting neuro developmental models, many biological environmental factors are consistently shown to be involved in Schizophrenia development. Relationship of NRG1 and cognition is important to providing a theoretical mechanism to explain NRG1's potential role in Schizophrenia, it is thought to be associated with disruption of the cytokine milieu and the propensity for the production of proinflammatory cytokines.*

Aim: *The present study was carried out to investigate the association of selected pro-inflammatory markers (interleukin 6 and 10), and neuregulin 1 in the serum of selected sample of Iraqi patients with schizophrenia.*

Methods: *Blood samples were collected from participants and were immediately spun and frozen at −20°C. Inflammatory markers were assayed. Serum interleukin10 and 6 was measured by enzyme-linked immunosorbent assay, using Human IL10 &6 ELISA kit with its Components and remarks (KOMABIO-TECH- INC. Korea). And serum NRG1 were assayed using Human Neuregulin 1 ELISA kit with its Components and Remarks (Bioassay Technology laboratory China).*

Result: *No important or statistically significant differences in mean age was observed between the 3 study groups. In addition males were more frequent than females in the 3 study groups. Again no statistically significant differences in gender. Significant positive differences that favors cases as compared to both control groups were observed for serum NRG1 and serum IL6, P value= <0.001. IL10 on the other hand showed only a marginal and statistically insignificant increase in cases with schizophrenia when compared to both control groups P value= 0.74.*

Conclusion: *Schizophrenia is associated with higher serum IL6 and Neuregulin 1 but not IL10 level, than general population*

Keywords: *Schizophrenia, IL6, IL10, Neuregulin 1.*

Introduction

Schizophrenia (SZ) is a complex, heterogeneous behavioral and cognitive syndrome that seems to originate from disruption of brain development caused by genetic or environmental factors, or both, characterized by abnormal social behavior and failure to understand reality. It is believed to be a combination of environmental and genetic factors [1,2]. SZ is often described in terms of positive and negative (or deficit) symptoms [3]. Common positive symptoms include false beliefs, unclear or confused thinking, hearing voices, and negative symptoms which include reduced social engagement and emotional expression, and a lack of motivation[4]. About 0.3–0.7% of people are affected by SZ during their lifetime [5]. It causes approximately 1% of worldwide disability-adjusted life years [6]. Complex immune–brain interactions that affect neural development, survival, and function might have causal and therapeutic implications for many disorders of the CNS including psychiatric illness[7]. Meta-analysis of many cross-sectional studies show that SZ is associated with disruption of the cytokine milieu and the propensity for the production of proinflammatory cytokines [8]. Khandaker *et al.,* [7] suggest that increased serum concentration of the proinflammatory cytokine interleukin 6 (IL6) at age 9 years is associated with twofold increased risk of development of a psychotic disorder at age 18 years. One of the supportive findings that enforces the inflammatory role in SZ are the evidences which have shown that antipsychotic drugs can modulate components of the inflammatory-related pathways such as decreasing in serum levels of pro-inflammatory cytokines and raising the anti- inflamatory cytokines, IL-10, [9]. Schizophrenic disorders may be considered an inflammatory disorder because of elevated levels of proinflammatory cytokines in spite of lacking an overt inflammation [10]. Changes in serum IL-10 levels were significantly correlated with improvements in symptoms [11]. Interestingly, emerging evidence indicates that IL-6 level is increased already in subjects with at-risk mental state (ARMS) and might be a marker of transition from (at risk mental state) ARMS to SZ [12]. Neuregulin 1 (NRG-1) is an epidermal growth factor (EGF) like growth factor that plays critical roles in development of the central nervous system by influencing neuronal differentiation, regulation of neurotransmitter receptor expression, and oligodendrocyte development [13]. NRG1 proteins are implicated in the differentiation and myelination of Schwann cells and oligodendro-cytes, the migration of CNS neuronal precursors along radial glia, synaptogenesis, plasticity and regulation of neurotransmitter receptors [14]. NRG1 seems to play a major role in neurodevelopment both during fetal gestation and postnatal reorganization and myelination processes, which continue until early adulthood. Evidence shows that NRG1 signalling is altered in SZ [15]. Because *NRG1* is thought to modulate glutamate levels through its regulation of NMDAR, and because glutamate is thought to be important in multiple cognitive functions, a very common deficit in patients and their relatives, evaluating the relationship of *NRG1* and cognition is important to providing a theoretical mechanism to explain *NRG1*'s potential role in SZ [16]. So the present study aimed to investigate the association of selected pro-inflammatory markers (interleukin 6 and 10), and neuregulin 1 in the serum of selected sample of Iraqi patients with schizophrenia.

Materials and Methods

Subject: The current case- control study conducted during the period from first of April 2015 to the first of December 2015, 60 patient who attended the outpatient department of psychiatry were diagnosed by a consultant psychiatrist in the psychiatry department in Al-Diwaniya teaching hospital on the basis of the structured clinical interview (DSM-IV) criteria for schizophrenia (American psychiatric association), and 60 healthy control individuals (30 healthy first degree relative and 30 random unrelated healthy individuals with no family history of psychiatric

illness), were included in the study. The study population were assessed by verbal questionnaire regarding personal history age, sex, marital status, duration of illness (in the study group),and the coexistence of other chronic diseases, explanation and verbal consent was taken from the study population regarding the inclusion in the study and the retrieval of the required amount of blood sample.

Enzyme Linked Immunosorbent Assay: Blood samples were collected from participants and were immediately spun and frozen at −20°C. Inflammatory markers were assayed. Interleukin 10 and 6 was measured by enzyme-linked immunosorbent assay, using Human IL10 &6 ELISA kit with its Components and remarks (KOMABIOTECH- INC. Korea). And serum NRG1 were assayed using Human Neuregulin 1 ELISA kit with its Components and Remarks (Bioassay Technology laboratory China).

Statistical Analysis: Data were translated into a computerized database structure. Statistical analyses were computer assisted using SPSS version 21 (Statistical Package for Social Sciences). The statistical significance, direction and strength of linear correlation between 2 quantitative variables, one of which being non-normally distributed was measured by Spearman's rank linear correlation coefficient. P value less than the 0.05 level of significance was considered statistically significant.

Result

The results presented were based on the analysis of a random sample of 60 cases with an established diagnosis of schizophrenia. Their ages ranged between 14 and 75 years with a mean of 37.4 years. A paired sample of 30 healthy controls was selected from first degree relatives of 30 cases. Each of 30 randomly selected case had one matched healthy control. Their ages ranged between 21 and 65 years with a mean of 38.9 years. Another random sample of 30 general population healthy controls (group matched on age and gender) was included in the study. Their ages ranged between 15 and 60 years with a mean of 34.1 years. No important or statistically significant differences in mean age were observed between the 3 study groups. In addition males were more frequent than females in the 3 study groups. Again no statistically significant differences in gender distribution were observed between the 3 study groups, table (1). Half of the cases with schizophrenia (50%) were 20-29 years of age at diagnosis of the condition, while those younger than 20 years and older than 40 years constituted around one fifth of the cases. A positive family history of the disease was obtained in 70% of the cases. About (35%) of cases had their disease for less than 5 years and only (23.3%) had the condition for more than 14 years, table (2). Significant positive differences that favors cases as compared to both control groups were observed for serum NRG1 and serum IL6. The mean serum NRG1 was significantly higher in schizophrenic group (34.1 ng/ml) compared to both control groups (16 and 15.8 ng/ml in general population and relatives group respectively) P value= <0.001 (table 3).The median serum IL6 was significantly higher in schizophrenia group (309.5 pg/ml) compared to both control groups (1 pg/ml in general population and relatives groups) P value= <0.001(table 4). IL10 on the other hand showed only a marginal and statistically insignificant increase in cases with schizophrenia when compared to both control groups P value= 0.74, (table 4).

Table (1): Age and Gender Differences Between the 3 Study Groups.

		General population Controls		First Degree Relatives (Controls)		Cases (Schizophrenia)	
		NO.	%	NO.	%	NO.	%
1.	Gender						
	Female	9	30.0	13	43.3	14	23.3
	Male	21	70.0	17	56.7	46	76.7
	Total	30	100.0	30	100.0	60	100.0
	P= 0.15[NS]						
2.	Age group (years)						
	<30	12	40.0	7	23.3	19	31.7
	30-49	14	46.7	18	60.0	30	50.0
	50+	4	13.3	5	16.7	11	18.3
	Total	30	100.0	30	100.0	60	100.0
	Range	(15-60)		(21-65)		(14-75)	
	Mean	34.1		38.9		37.4	
	SD	11.9		11.3		12.7	
	SE	2.16		2.06		1.64	
	N	30		30		60	
	P=0.29[NS]						

Table (2): Frequency Distribution of Cases with Schizophrenia by Age of Onset, Duration of the Disease and Family History.

		NO.	%
1.	Age of onset groups (years)		
	<20	7	11.7
	20-29	30	50.0
	30-39	17	28.3
	40+	6	10.0
2.	Family history		
	Negative	18	30.0
	Positive	42	70.0
	Total	60	100.0
3.	Duration of the disease (years)-categories		
	<5	21	35.0
	5-9	11	18.3
	10-14	14	23.3
	15+	14	23.3
	Total	60	100.0

Table (3) The Difference in Mean and Median of Serum Neuregulin 1 (NGR1) Between the 3 Study Groups.

	General population control	First Degree Relatives (Controls)	Cases (Schizophrenia)	P Value
Serum NGR1 (ng/ml)				<0.001
Range	(9-19.9)	(6.2-19.5)	(24.6-46.7)	
Mean	16	15.8	34.1	
SD	3.1	3.4	5.3	
SE	0.56	0.62	0.69	
N	30	30	60	
Bonferonni t-test for difference in mean between:				
Cases (Schizophrenia) x Unrelated Controls<0.001				
Cases (Schizophrenia) x First Degree Relatives (Controls)<0.001				

Table (4): The Difference in Mean and Median of Serum Interleukin 6 (IL6) and Interleukin (IL10) Between The 3 Study Groups

	General population control	First Degree Relatives (Controls)	Cases (Schizophrenia)	P Value
Serum IL6 (pg/ml)				<0.001
Range	(1-11.1)	(1-11.1)	(13.5-821.4)	
Median	1	1	309.5	
Range	(1-1)	(1-1)	(78.2-615.6)	
N	30	30	60	
Mean Rank	29	32	90.5	
P (Mann-Whitney) for difference in median between:				
Cases (Schizophrenia) x Unrelated Controls<0.001				
Cases (Schizophrenia) x First Degree Relatives (Controls)<0.001				
Serum IL10 (pg/ml)				0.74[NS]
Range	(71.2-640.3)	(98.8-490.3)	(98.8-490.3)	
Mean	310.8	302.5	322.3	
SD	145.2	110.2	106.3	
SE	26.52	20.12	13.73	
N	30	30	60	

Discussion

Immune dysfunction represents one of the most important questions in the field of the pathophysiology of schizophrenia because the immunological mechanism would mediate the relationship between genetic vulnerability and environmental factors [17]. Inflammatory markers such as pro-inflammatory cytokines are well-known etiological factors for psychiatric disorders, including schizophrenia. Inflammation in the central nervous system is closely related to neurodegeneration [18]. The median serum IL6 was significantly higher in Schizophrenia group (309.5 pg/ml) as compared to both control groups P<0.001. Results are highly agreeable with a large bulk of studies with variable respects to the clinical stage of the disease and status of medical treatment or associated other parameters, among these Potvin *et al.*, [19] who conducted meta-analysis for data from 62 studies involving a total sample size of 2298 schizophrenia patients and 1858 healthy volunteers. Another study accomplished by Khandaker *et al.*, [7] conducted a prospective general population birth cohort study based in Avon County, England. They have studied a subsample of approximately 4500 individuals from the cohort with data on childhood IL-6 serum level base line measurement

later psychiatric assessments ,they concluded that higher levels of the systemic inflammatory marker IL-6 in childhood are associated with an increased risk of developing schizophrenia in young adulthood. Chiang *et al.,* [20] found that the IL6 level in schizophrenic patient, and control subjects were not significantly distinguishable P= 0.93. On the other hand serum IL10 showed only a marginal and statistically insignificant increase P=0.74, in cases with schizophrenia mean 322.3pg/ml when compared to both healthy and relative groups (mean 310.8 and 302.5 pg/ml) respectively P=0.74. Marco *et al.,* [21] reported a parallel results to our study, the mean serum IL-10 (pg/ml) was 1.5 ± 0.2 compared to 1.9 ± 0.6, $P = 0.5$, values proved to be not significantly different between patients and controls for serum IL10. Potvin *et al.,* [19] depending on meta-analysis of data obtained from several studies, they concluded that the difference in serum IL10 of patient with schizophrenia and control was not significant. However many other studies document quit different results, Kunz *et al.,* [22] and Schwarz *et al,* [23]. While De Witte *et al.,* [9] documented an increase in serum level of IL10 in schizophrenic patients as compared to control. In contrary some other studies reported a significantly lower serum levels of IL10 in schizophrenic patients as compared to control [24].

In the current study, results of the serum neuregulin 1, dictated significantly higher mean serum NRG1 in Schizophrenic group (34.1 ng/ml) when compared to both control groups (mean 16 and 15.8 ng/ml) in general population and relatives group respectively P<0.001).However other studies Wang *et al.,* [25] and Shibuya *et al.,* [26] reported in contrast, a lower level of immunoreactivity measurement of serum neuregulin 1 in schizophrenic patients as compared to control. Despite of expanding data regarding the important role of neuregulin 1 in the pathogenesis and etiology of schizophrenia most of the researches has extensively dealt with the issue of *neuregulin 1* gene and the variation in the in *SNP* of the *neuregulin 1* gene, few studies evaluate the expression of the *NRG 1* gene or the actual plasma level of neuregulin 1 in the schizophrenia researches.

Conclusion

Schizophrenia is associated with significantly higher serum levels of IL6 and neuregulin 1 while the levels of IL 10 in the serum are not different in schizophrenic patients from general population.

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