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Research Article

Immunological Markers and Brain Derived Neurotrophic Factor in Schizophrenia Patients and Their First-Degree Relatives

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Abstract
Background: Functional outcomes in schizophrenia patients is more strongly correlated with cognitive impairments than psychotic symptoms. Patients with schizophrenia have been found to have elevated blood levels of chemicals that promote inflammation. A number of immune system imbalances can manifest, including those involving the innate system, the pro- and anti-inflammatory systems, and types 1 and 2 immunity. This research aimed to evaluate cognitive functions in patients with schizophrenia and their first-degree relatives in relation to these cytokines and brain derived neurotrophic factor serum level.
Methods: The research included 90 participants, aged from 18 to 40 years old, both sexes, and medication free Group I was 30 patients with schizophrenia, Group II: first-degree relatives of patients with schizophrenia and a group of 30 healthy individuals served as a control (Group III**).**
Results: Interleukin 2, 6, and 8, tumor necrosis factor-α, and C reactive protein levels were all greater in schizophrenia patients compared to control subjects. They had significantly lower amounts of Brain Derived Neurotrophic Factor in their serum as compared to the control. While first degree relatives showed level of some inflammatory markers midway between schizophrenic patients and control subjects. Immunological markers showed negative relationship with cognitive functions, while brain-derived neurotrophic factors showed positive relationship with both memory and problem solving in schizophrenia patients.
Conclusion: Schizophrenia patients and their first-degree relatives have higher levels of certain immunological markers, which is related to cognitive dysfunction, while BDNF showed lower levels in schizophrenic patients.

Keywords: Immunological markers, Brain-Derived Neurotrophic Factor, Cognitive Functions, Schizophrenia, First Degree Relatives

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1. Introduction:

Schizophrenia is a polygenic psychiatric disorder characterized by a complex of positive, negative, and cognitive symptoms. Hallucinations and delusions are examples of positive symptoms, while lack of expected response, as shown by symptoms like anhedonia, blunt affect, or social drive, are examples of negative symptoms. Individuals with schizophrenia also often suffer from cognitive difficulties that impact organized

thought processes, concentration, task completion, and memory. [1]

Morbidity and socioeconomic burden are already high enough for up to 30 percent of people with schizophrenia to be deemed "treatment-resistant". The financial burden of schizophrenia imposed on the health care systems imposed is considered heavy. The financial cost is estimated to exceed that of all cancers combined. [2]

Several domains of cognition are impaired in people with schizophrenia. [3] Among people who suffer from schizophrenia, cognitive deficits are more strongly linked to functional outcome than psychotic symptoms. [4]

Patients with schizophrenia have been found to have elevated amounts of cytokines and other proinflammatory chemicals in their blood and CSF. Many aspects of the immune system can be out of whack, including the innate system (monocytes, macrophages), the mechanisms that promote and inhibit inflammation, and the types 1 and 2 of immunity. [5]

When studying neuro-progression, it is important to take cytokine levels into account at various phases of the disease and during treatment. The data show that immunological function is affected by both the illness stage and the choice of antipsychotic. [6]

A number of research, including imaging, clinical trials, and preclinical investigations, point to brain-derived neurotrophic factors (BDNF) as a possible player in the pathogenesis of schizophrenia.[7]

The hypothesis was that people with schizophrenia display a different pattern of biomarkers compared to non-psychotic first degree relatives and healthy controls. These biomarkers include interleukin (IL) 2, IL6, IL8, C reactive protein (CRP), interferon gamma (INF γ), tumor necrosis factor alpha (TNF-α), and BDNF.

This research aimed to evaluate cytokines and BDNF serum levels in patients with schizophrenia and their first-degree relatives and their relationship with cognitive functions.

2. Patients and Methods:

2.1. Study design:

This cross-sectional case control study was carried out at Neuropsychiatry Department, Tanta University Center for Psychiatry, Neurology and Neurosurgery and Neuropsychiatry Department in Damanhour Medical National Institute.

2.2. Study subjects:

This current study was carried out on: group I: 30 patients aged from 18 to 40 years old, both sexes, diagnosed with schizophrenia according to the Diagnostic and Statistical Manual of Mental Disorders (DSM) IV, ability to read and write and medication-free (either never received antipsychotic treatment or stopped their medications for three months in case of long-acting medications or two weeks in case of oral antipsychotic drugs which is the drug washout period for antipsychotic drugs), group II: 30 first degree relatives of schizophrenic patients as Group II and 30 healthy volunteers as a control group (Group III).

The study was done from January 2019 through December 2021 after approval of the Ethical Committee of Tanta University Hospitals, Tanta, Egypt. Patient or from the next of kin of patients signed informed consent was acquired.

The following conditions were considered exclusion criteria: autoimmune illnesses (current or past), neurological disorders (current or past), significant physical health impairments, co-morbid substance use (except nicotine), current or past infections or allergies, pregnancy, or lactation.

Criteria for excluding first-degree relatives: Our study did not include subjects who were currently or previously diagnosed with a psychiatric disorder, had no known allergies or infections, or were taking any kind of psychotropic medication, hormonal agent, anti-inflammatory agent, anti-hypertensive, or anti-hyperlipidemic medication. Every individual underwent a thorough evaluation that included obtaining their medical history, conducting laboratory tests (including IL-2, IL-6, IL-8, CRP, TNF-α, INF, and BDNF), and a cognitive evaluation.

2.3. Psychometric assessment

2.3.1. Wisconsin Card Sorting Test 128 computerized version [8]:

We measured cognitive flexibility, problem solving, set shifting, utilization of feedback, inhibition of prepotent responses, and ability to change faulty methods using the Wisconsin Card Sorting Test (WCST). The participants indicated their preference by pressing the stimulus "card" that they felt best matched the description. The participant is left to their own devices to figure out the sorting category while receiving performance feedback from the computer.

2.3.2. Wechsler Memory Scale [9]:

Questions on orienting and information processing, eight tests of short-term memory, and four trials of delayed recall make up the Wechsler Memory Scale-Revised. The information and orientation section includes brief questions about the interviewee's background and experiences as well as general questions on the topic at hand. The Attention and Concentration score is based on tests for: Mental Control, Digit Span. Mental Control requires the subject to recite a series of numbers or letters. Digit spans consist of two parts and requires the subject to repeat digits forwards and backward

2.4. Laboratory assessment:

The following markers were measured from blood samples taken between 9 and 11 AM: IL2, IL6, and IL8, Tumor Necrosis Factor alpha and INF-γ, CRP, and BDNF. The subjects included patients, non-psychotic first degree relatives, and healthy control subjects.

2.5. Statistical analysis:

SPSS v27 (IBM©, Chicago, IL, USA) was employed to conduct statistical analysis. Histograms and the Shapiro-Wilks test were employed to assess if the data

distribution was normal. Our quantitative parametric data was analyzed using an ANOVA (F) test with a post hoc test (Tukey). The results were provided as the mean and standard deviation (SD). With quantitative non-parametric data shown as median and interquartile range (IQR), each group was compared using the Kruskal-Wallis test in conjunction with the Mann Whitney-test. The Chi-square test was used to assess the qualitative variables, which were laid out as percentages and frequencies. Using the Pearson moment correlation equation, we observed correlations between several variables. For statistical purposes, a two-tailed P value less than 0.05 was deemed significant.

3. Results:

With the exception of marital status and occupation, no significant differences were found in the patient characteristics among the groups. **Table 1**

Table 1: Patients characteristics the three studied groups

		Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	p
Age (years)		31.0±6.62	29.83±5.74	31.37±6.72	0.625
Sex	**Male**	10(33.3%)	11(36.7%)	13(43.3%)	0.718
	Female	20(66.7%)	19(63.3%)	17(56.7%)	
Marital status	**Married**	45(16.7%)	10(33.3%)	17(56.7%)	**^{MC}p= 0.017***
	Single	22(73.3%)	17(56.7%)	10(33.3%)	
	Divorced	3(10.0%)	3(10.0%)	3(10.0%)	
Education	**Low**	21(70.0%)	23(76.7%)	17(56.7%)	0.241
	High	9(30.0%)	7(23.3%)	13(43.3%)	
Occupation	**Manual work**	3(10.0%)	18(60.0%)	0(0.0%)	**^{MC}p <0.001***
	Governmental	1(3.3%)	0(0.0%)	21(70.0%)	
	Non employed	22(73.3%)	12(40.0%)	2(6.7%)	
	Student	4(13.3%)	0(0.0%)	7(23.3%)	
Residence	**Urban**	9(30.0%)	16(53.3%)	16(53.3%)	0.111
	Rural	21(70.0%)	14(46.7%)	14(46.7%)	
BMI (kg/m^2)		28.77±6.81	26.94±5.35	29.65±5.59	0.205
Smoking		11(36.7%)	5(16.7%)	9(30.%)	0.212
		Patients group (n=30)			
Duration of illness		5.0 (1.0 – 8.0)			
PANSS	**Positive**	20.97 ± 6.25			
	Negative	21.43 ± 6.66			
	General psychopathology	50.10 ± 8.45			

Data are presented as mean ± SD or frequency (%) or median (IQR). * Significant P value <0.05. MC: Monte Carlo, BMI: body mass index, PANSS: Positive and Negative Syndrome Scale.

Group I had considerably greater levels of CRP, IL-6, IL-8, and TNF-α compared to groups II and III (P<0.001). Compared to group II, CRP levels in group III were substantially higher (P=0.007). Group II had considerably greater levels of IL-6, IL-8, and TNF-α compared to group III (P<0.05). The groups that were studied showed no significant difference in IL-2 and INF. There was no statistically significant difference in BDNF between the two groups. Group III had considerably higher BDNF levels than groups I and II (P<0.05). **Table 2**

Table 2: Laboratory investigations of the studied groups

	Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	Test	P
CRP	4.28±0.83	0.74±0.22	1.22±0.55	F=319.63*	**<0.001***
	P1<0.001*, P2<0.001*, P3=0.007*				
IL-2	23.16(19.80-25.73)	21.66(18.38– 34.23)	27.25(17.48–32.36)	H=1.063	0.588
IL-6	21.87±8.57	17.29±7.29	9.94±3.40	H=43.141*	**<0.001***
	P1=0.028*, P2<0.001*, P3<0.001*				
IL-8	20.0 (16.65–26.82)	15.49(12.37–18.04)	9.04(5.53–10.94)	52.738*	<0.001*
	P1=0.014*, P2<0.001*, P3<0.001*				
TNF-α	62.78±24.69	47.36±9.06	14.63±3.55	H=61.634*	**<0.001***
	P1=0.023*, P2<0.001*, P3<0.001*				
INF	123.6±36.93	106.0±34.66	114.9±52.16	H=3.787	0.151
BDNF	0.688(0.25–1.04)	0.709(0.53–1.06)	1.056(0.71–3.60)	--	**0.005***
	P1=0.431, **P2=0.002*, P3=0.018***				

Data are presented as mean ± SD or median (IQR). * Significant P value <0.05. P1: P value between group I and II, P2: P value between group I and III, P3: P value between group II and III, F: Fisher's exact test, H: hypothesis test, CRP: C -reactive protein, IL: interleukin, TNF-α: tumor necrosis factor-alpha, INF: interferon gamma, BDNF: brain-derived neurotrophic factor.

There was significant negative correlation between (BDNF and IL6). In groups of patients and first-degree relatives, there was a notable negative correlation between BDNF and TNF-α, but in the healthy control group, there was no such correlation. Only in the healthy control group did BDNF and IL8 show a significant negative correlation. **Table 3**

Table 3: Correlation between BDNF and different immunological markers each group

		CRP	IL2	IL6	IL8	TNF	INF
Group I							
BDNF	**r**	0.201	0.120	-0.393	0.090	-0.432	0.086
	P	0.287	0.528	0.032*	0.636	0.017*	0.653
Group II							
BDNF	**r**	-0.037	-0.146	-0.405	0.217	-0.434	0.165
	P	0.845	0.441	0.026*	0.250	0.017*	0.383
Group III							
BDNF	**r**	0.172	0.262	-0.364	-0.377	0.243	0.080
	P	0.362	0.163	0.048*	0.040*	0.196	--

r: Pearson Cofficients, * Significant p value <0.05, PANSS: Positive and Negative Syndrome Scale, BDNF: Brain derived Neurotrophic Factor, IL: interleukin, CRP: C-reactive protein, IFN-γ: interferon-gamma, TNF: tumor necrosis factor.

Significant differences were observed between the groups on the Wechsler Memory Scale. (P<0.05). There was a statistically significant difference in WST between the groups, with the exception of the group that failed to preserve their set. Compared to the patient and first-degree relative groups, the control group has a substantially higher general information subset means score; however, there is no difference between the latter two groups. In terms of the orientation subset mean score, the patient group performed worse than the first degree relative and control groups, while the latter two groups showed no significant difference. The mental control subset score, logical memory subset score, Digits total subset score (indicative of working memory), Digits total subset score (indicative of working memory), visual reproduction subset score (indicative of visual memory), associate learning subset score and the total memory score, all are significantly higher in the control group followed by the first-degree relative group, then the patient group. The number of categories completed mean (which reflects reasoning and problem solving), correct responses, percent of conceptual level response (which reflect initial conceptualization and capacity for abstraction), was higher in control group followed by 1st degree relatives then patient group. Regarding perseveration error mean (which indicates failure to inhibit a learning response despite receiving error information), was higher in the patient group, followed by first degree relative group, followed by control group. Regarding number of trials (which reflects visual learning) and failure to maintain a set (attention), The number of trials was much fewer among healthy controls compared to both patients and first degree relatives, but there was no significant difference between the two groups. Regarding perseveration response mean (which reflects processing speed) the levels were higher in the 1st degree relatives than both patients and healthy controls. There was no statistical difference between the patient group, first-degree relatives, and the healthy control group with regard to failure to maintain established mean, an indication of attention. **Table 4**

Table 4: Comparison of Wechsler memory scale and WCST between the three studied groups

	Group I (n = 30)	Group II (n = 30)	Group III (n = 30)	Test of sig.	P
Wechsler memory scale					
Information	3.90±1.24	4.37±0.76	5.23±0.97	F= 13.420*	**<0.001***
	P_1=0.180, **P_2<0.001*, P_3=0.004***				
Orientation	4.23±1.04	5.0±0.0	5.0±0.0	F= 16.303*	**<0.001***
	P_1<0.001*, P_2<0.001*, P_3=1.000				
Mental control	5.0 ± 2.49	6.50 ± 1.66	7.77 ± 1.01	F= 17.331*	**<0.001***
	P_1=0.006*, P_2<0.001*, P_3=0.023*				
Logical memory	7.77±5.35	12.27±5.17	17.50±3.56	H= 37.328*	**<0.001***
	P_1=0.013*, P_2<0.001*, P_3<0.001*				
Digits total	7.03±2.31	8.97±1.22	10.97±2.01	F= 32.061*	**<0.001***
	P_1<0.001*, P_2<0.001*, P_3<0.001*				
Visual reproduction	8.50±3.34	10.23±2.05	12.93±1.17	F= 26.876*	**<0.001***
	P_1=0.015*, P_2<0.001*, P_3<0.001*				
Associate learning	8.0(3.0 – 12.0)	12.0(10.0 – 13.0)	15.0(13.0 – 18.0)		**<0.001***

	P_1=0.014*, P_2<0.001*, P_3=0.003*			H= 29.583*	
Total Percent of memory	78.7± 22.95	96.40± 18.51	124.7± 15.64	H= 46.658*	**<0.001***
	P_1=0.016*, P_2<0.001*, P_3<0.001*				
WCST					
Categories completed	0.50(0.0– 0.75)	0.86(0.67– 0.88)	1.0(1.0 –1.0)	H= 61.176*	**<0.001***
	P_1=0.006*, P_2<0.001*, P_3<0.001*				
Number of trials	127.8 ± 0.91	127.7 ± 0.69	120.9 ± 6.21	H= 50.636*	**<0.001***
	P_1=0.563, **P_2<0.001*, P_3<0.001***				
Correct responses	60.20 ± 23.30	82.47 ± 14.06	102.3 ± 4.35	F= 52.497*	**<0.001***
	P_1<0.001*, P_2<0.001*, P_3<0.001*				
Errors percentage	52.79 ± 18.42	36.44 ± 11.85	15.28± 2.45	F= 65.521*	**<0.001***
	P_1<0.001*, P_2<0.001*, P_3<0.001*				
Perseverative response	29.50(0.0– 53.0)	48.0(45.0 –55.0)	38.0(35.0– 41.0)	H= 21.226*	**<0.001***
	P_1<0.001*, P_2=0.460, P_3<0.001*				
Perseverative errors	38.23 ± 14.29	28.83 ± 8.65	11.87 ± 4.13	H= 54.808*	**<0.001***
	P_1=0.043*, P_2<0.001*, P_3<0.001*				
Number of Trials to complete 1st category	10.0(0.0– 13.0)	14.0(12.0– 19.0)	11.0(10.0–17.0)	15.181*	**0.001***
	P_1<0.001*, P_2=0.032*, P_3=0.082				
Failure to maintain set	1.0(0.0– 2.0)	1.0(0.0– 2.0)	1.0(0.0–1.0)	2.544	0.280
Percent Conceptual Level Response	25.75(11.70– 46.9)	60.25(39.10– 61.7)	78.85(77.80–81.0)	54.318*	**<0.001***
	P_1=0.010*, P_2<0.001*, P_3<0.001*				

Data are presented as mean ± SD or median (IQR). ***** Significant P value <0.05. F: F for One way ANOVA test, pairwise comparison bet. Each 2 groups were done using Post Hoc Test (Tukey), H: H for Kruskal Wallis test, pairwise comparison bet. each 2 groups were done using Post Hoc Test (Dunn's for multiple comparisons test), P1: P value between group I and II, P2: P value between group I and III, P3: P value between group II and III, WCST: Wisconsin Card Sorting Test.

The levels of BDNF and the number of completed categories, as well as IL6 and INF γ and the proportion of errors, were positively correlated in group I. Negative correlations were found between CRP and the percentage of correct responses at the conceptual level, IL6 and the number of trials needed to finish the first set, IL8 and the inability to maintain a set, and INF γ and the percentage of correct responses, the inability to maintain a set, and the percent of conceptual level. In group II, TNF-α and the number of trials to finish the first set, IL8 and the number of trials to complete the set and error percentage, and BDNF and categories completed were all significantly correlated positively. While a negative correlation was found between BDNF and the number of trials needed to complete the first category, CRP and the percentage of correct responses and the failure to maintain the first set, IL6 and the number of trials needed to complete the first set, and IL8 and the percent of correct responses and the failure to maintain the first set.

In group III, the variables BDNF, categories completed, IL2, perseveration errors, and % of CLR were positively correlated. While there was a significant negative correlation between (CRP and both perseveration response and percent of CLR), (IL6 and number of trails to complete first set) and (INF γ and both numbers of trails to complete first set and correct responses). IL8 and TNF-α shows no relationship with any division of the test. **Table 5**

Table 5: Correlation between different markers with Wisconsin in each group

	BDNF		**CRP**		**IL2**		**IL6**		**IL8**		**TNF**		**INF**	
	r_s	**P**	**r_s**	**p**	**r_s**	**p**	**r_s**	**r_s**	**P**	**r_s**	**p**	**r_s**	**p**	**r_s**
Group I														
Categories completed	0.441	0.015*	0.250	0.183	-0.063	0.742	-0.344	0.063	0.182	0.334	-0.253	0.178	0.049	0.799
Number of trials	-0.075	0.693	-0.311	0.094	-0.097	0.612	-0.150	0.428	-0.311	0.094	-0.311	0.094	-0.290	0.120
Correct responses	0.192	0.309	0.074	0.699	0.106	0.577	-0.373	0.042*	-0.130	0.493	-0.098	0.605	-0.386	0.035*
Errors percentage	-0.191	0.311	-0.070	0.712	-0.105	0.581	0.371	0.044*	0.132	0.486	0.099	0.604	0.384	0.036*
Perseverative response	0.130	0.495	0.315	0.090	-0.086	0.652	-0.337	0.068	0.137	0.472	-0.061	0.748	-0.041	0.831
Perseverative errors	-0.142	0.455	0.180	0.341	0.116	0.541	-0.138	0.468	0.111	0.560	-0.087	0.648	0.045	0.815

Non-Perseverative errors	-0.211	0.262	-0.222	0.239	-0.042	0.824	0.382	0.037*	-0.032	0.866	0.026	0.891	0.196	0.300
Trial to complete 1st category	-0.167	0.377	-0.098	0.608	-0.022	0.909	-0.599	<0.001*	0.047	0.803	0.166	0.382	0.006	0.974
Failure to main set	-0.005	0.979	-0.193	0.308	0.300	0.107	-0.163	0.390	-0.458	0.011*	-0.184	0.329	-0.435	0.016*
Percent CLR	-0.048	0.801	-0.375	0.041*	-0.111	0.560	-0.210	0.265	-0.312	0.093	-0.067	0.724	-0.449	0.013*
Group II														
Cat completed	0.522	0.003*	0.129	0.497	-0.084	0.658	-0.325	0.079	0.011	0.953	-0.335	0.070	0.252	0.180
Number of trials	-0.142	0.455	0.164	0.386	0.204	0.280	-0.040	0.835	0.499	0.005*	0.266	0.155	-0.142	0.455
Correct responses	-0.121	0.523	0.002	0.993	0.058	0.760	-0.197	0.298	-0.364	0.048*	-0.061	0.747	0.176	0.351
Errors percentage	0.182	0.335	-0.153	0.421	0.014	0.940	0.248	0.186	0.484	0.007*	0.063	0.739	-0.245	0.191
Perseverative response	-0.127	0.503	0.179	0.344	-0.255	0.174	0.139	0.463	0.185	0.327	-0.023	0.905	-0.031	0.869
Perseverative errors	0.052	0.785	-0.039	0.837	-0.197	0.297	0.222	0.238	0.346	0.061	0.020	0.917	-0.168	0.374
Non-Perseverative errors	0.132	0.485	0.083	0.663	0.342	0.064	0.171	0.366	0.373	0.043*	0.053	0.782	-0.335	0.070
Number of Trial to complete 1st category	-0.368	0.046*	0.256	0.172	0.329	0.076	-0.378	0.039*	0.281	0.132	0.395	0.031*	-0.044	0.818
Failure to main set	-0.192	0.309	-0.378	0.039*	-0.154	0.416	0.388	0.034*	-0.462	0.010*	-0.068	0.721	-0.150	0.428
Percent CLR	-0.025	0.894	-0.371	0.043*	-0.227	0.228	-0.026	0.892	-0.183	0.333	0.104	0.584	-0.095	0.619
Group III														
Categories completed	0.437	0.016*	0.315	0.090	-0.019	0.920	-0.270	0.149	-0.045	0.814	0.315	0.090	-0.058	0.762
N of trials	-0.047	0.805	-0.116	0.541	-0.037	0.848	0.075	0.692	-0.043	0.822	-0.035	0.854	-0.473	0.008*
Correct responses	-0.075	0.693	0.191	0.311	-0.177	0.349	-0.009	0.961	-0.133	0.483	-0.181	0.338	-0.382	0.037*
Errors percentage	-0.230	0.222	-0.187	0.322	0.222	0.238	0.287	0.124	0.166	0.382	0.039	0.837	-0.003	0.989
Perseverative response	-0.015	0.939	-0.466	0.009*	0.035	0.856	-0.134	0.481	0.023	0.905	-0.071	0.710	-0.145	0.444
Perseverative errors	-0.077	0.687	0.029	0.880	0.423	0.020*	0.272	0.147	-0.176	0.351	0.036	0.852	-0.281	0.133
Non-Perseverative errors	-0.129	0.495	-0.290	0.120	-0.009	0.961	0.104	0.585	0.294	0.115	0.082	0.665	-0.080	0.674
Nomber Trials to complete 1st	-0.035	0.856	-0.310	0.095	0.159	0.400	-0.418	0.022*	0.142	0.455	-0.171	0.366	0.005	0.979
Failure to main	0.140	0.462	0.359	0.051	-0.181	0.339	-0.002	0.990	-0.180	0.342	-0.120	0.529	-0.249	0.185
Percent CLR	0.142	0.454	-0.424	0.019*	0.387	0.035*	0.024	0.899	-0.139	0.463	0.261	0.164	0.134	0.480

r: Pearson Coefficients, * Significant p value <0.05, BDNF: Brain derived Neurotrophic Factor, IL: interleukin, CRP: C reactive protein, IFN-γ: interferon-gamma, TNF: tumor necrosis factor.

In group I, BDNF and total memory percentage on WMS were positively correlated with IL2 and visual reproduction. While there was a significant negative correlation between (CRP and total memory percentage of WMS) and (INF γ and both orientation and associate learning). Otherwise, none of the other markers (IL6, IL8 and TNF-α) had any relationship with the subsets of WMS.

In group II, A strong positive connection was found between BDNF and the overall percentage of digits and total memory on WMS, while (TNF-α and general information). When it came to (IL8 and both logical

memory and associate learning) and (CRP and overall memory percentage of WMS and positive link with associate learning), there was a statistically significant negative correlation. Otherwise, none of the other markers (IL2, IL6 and INF γ) had any relationship with the subsets of WMS.

In group III, there was a significant negative correlation between (BDNF and logical memory, total digit and total memory percentage on WMS), (CRP and total memory percentage of WMS and positive relationship with general information, logical memory, total digit and visual reproduction), (IL6 and total digits subset in WMS) and (IL8 and total memory percentage). Although (IL2 and both total digit) and (INF γ and associate learning) were significantly correlated positively. In the control group, there was no correlation discovered between TNF-α and any of the WMS subcategories. **Table 6**

Table 6: Correlation between different markers and subsets of WMS in each group

	BDNF		CRP		IL2		IL6		IL8		TNF		INF	
	r_s	P	r_s	p	r_s	P	r_s	p	r_s	p	r_s	P	r_s	p
Group I (n = 30)														
Information	0.247	0.189	0.243	0.196	0.089	0.640	-0.255	0.173	0.032	0.868	-0.288	0.123	-0.283	0.130
Orientation	-0.119	0.531	0.064	0.736	0.278	0.137	-0.015	0.936	-0.158	0.403	-0.113	0.552	-0.428	0.018*
Mental control	0.030	0.874	0.180	0.342	0.263	0.160	-0.337	0.069	-0.053	0.780	0.134	0.480	-0.177	0.349
Logical memory	0.244	0.194	0.084	0.657	0.165	0.384	0.059	0.757	0.277	0.139	0.113	0.553	-0.121	0.526
Digit total	0.100	0.601	0.281	0.132	0.128	0.499	-0.261	0.164	0.079	0.679	0.131	0.489	-0.096	0.612
Visual reproduction	0.062	0.744	-0.056	0.769	0.410	0.024*	-0.238	0.205	-0.273	0.145	0.032	0.865	-0.233	0.216
Associate learning	-0.032	0.869	-0.071	0.708	0.299	0.108	-0.030	0.875	-0.025	0.897	0.094	0.622	-0.397	0.030*
Percent of memory	0.470	0.009*	-0.461	0.010*	0.025	0.895	0.024	0.900	0.045	0.815	-0.151	0.426	0.003	0.988
Group II (n = 30)														
Information	0.108	0.569	0.109	0.568	0.229	0.224	-0.075	0.693	-0.055	0.774	-0.395	0.031*	-0.159	0.401
Orientation	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mental control	0.233	0.215	-0.027	0.886	0.194	0.306	-0.077	0.686	-0.004	0.982	-0.215	0.255	-0.031	0.872
Logical memory	-0.014	0.942	-0.111	0.559	0.017	0.928	0.145	0.444	-0.494	0.006*	-0.120	0.528	-0.200	0.289
Digit total	0.471	0.009*	0.084	0.660	0.030	0.877	-0.098	0.608	0.116	0.541	-0.237	0.208	0.136	0.473
Visual reproduction	-0.197	0.298	0.105	0.581	0.052	0.785	0.305	0.101	-0.331	0.074	-0.014	0.943	-0.180	0.342
Associate learning	-0.348	0.060	0.571	0.001*	0.161	0.395	-0.155	0.413	-0.396	0.030*	0.055	0.772	0.145	0.444
Percent of memory	0.587	0.001*	-0.368	0.046*	-0.282	0.131	-0.035	0.854	0.058	0.761	-0.350	0.058	0.034	0.858
Group III														
Information	0.277	0.138	0.582	0.001*	-0.034	0.859	0.102	0.593	-0.197	0.297	0.188	0.319	-0.001	0.995
Orientation	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Mental control	0.134	0.480	-0.107	0.572	0.262	0.161	-0.223	0.236	-0.257	0.170	-0.099	0.604	-0.085	0.654
Logical memory	0.596	0.001*	0.399	0.029*	0.200	0.289	-0.154	0.415	-0.297	0.111	0.181	0.337	0.163	0.391
Digit total	0.515	0.004*	0.517	0.003*	0.398	0.029*	-0.391	0.033*	-0.342	0.064	0.115	0.546	0.152	0.422
Visual reproduction	0.289	0.122	0.613	<0.001*	0.148	0.435	-0.134	0.481	-0.104	0.585	0.041	0.829	0.146	0.442
Associate learning	0.321	0.083	0.245	0.193	0.300	0.107	-0.047	0.807	-0.147	0.438	0.031	0.871	0.538	0.002*
Percent of memory	0.397	0.030*	-0.363	0.049*	0.418	0.022*	0.111	0.558	-0.419	0.021*	-0.001	0.996	0.143	0.450

r: Pearson Coefficient, * Significant p value <0.05, BDNF: Brain derived Neurotrophic Factor, IL: interleukin, CRP: C reactive protein, IFN-γ: interferon-gamma, TNF: tumor necrosis factor.

4. Discussion

In terms of the results from the laboratory, we discovered that compared to the control group, patients and their first-degree relatives had considerably higher levels of CRP, IL6, IL8, and TNF α. The three groups did not differ in terms of serum IL2 and INF-γ levels. Patients and their first-degree relatives had reduced BDNF levels compared to the healthy control group.

This aligns with the findings of numerous research, Fawzi et al. [10] determined that CRP levels were considerably greater in the control group compared to the schizophrenia group. While Upthegrove et al. [11] found no significant difference between patients and control subjects. Al-Hakeim et al. [12] detected much greater IL6 levels in the study group compared to the control group. He et al. [13] verified that schizophrenia patients had elevated levels of IL8. Upthegrove et al. [11] discovered that TNF-α had a significantly significant impact, indicating that these cytokines are elevated in first-episode psychosis, independent of the effects of treatment. Borovcanin [14] discovered no statistically significant difference between the control and psychotic patient groups.

Regarding cognitive function, Except for the general information division, where no significant difference was found between patients and relatives, the patient, first-degree family, and healthy control groups all had considerably lower scores across different divisions and total scores. Concerning the category completed, perseveration errors, correct responses and percent of conceptual level response mean were higher in control group followed by 1st degree relatives then patient group. Concerning the trials to completion, the patients group had a considerably higher first category mean than the control and 1st degree relative groups; however, there was no significant difference between the 1st degree relative group and the healthy control group. There was no statistically significant difference in the number of trials or inability to maintain a set between patients and first-degree relatives, while the healthy controls exhibited a substantially lower number of trials compared to both groups.

Previous research suggests that cognitive impairment is a hallmark of schizophrenia. [15]

Ye Yang et, al. [16] discovered no statistically significant difference between patients with persistent and first-episode symptoms.

Concerning the relation between cognitive functions and laboratory results, we found that BDNF showed positive relationship with total memory percentage on WMS in the three studied groups, with total digit percentage in first degree relatives and control subjects and with logical memory only in the healthy controls. We also found positive relationship with number of categories completed in the three studied groups. CRP showed negative relationship with total memory percentage of WMS in the three groups while positive relationship with associate learning the first degree relatives, and positive relationship with logical memory, total digit and visual reproduction in the control group only. We also found negative relationship with percent of conceptual level response in the three studied groups and with failure to maintain set only in the relatives. IL2 showed positive relationship with visual reproduction in the patient group only and positive relationship with total digit and total memory percentage. IL6 showed negative relationship with total digit only in healthy groups. The number of attempts to finish the first category was positively correlated with failure to maintain set in the patient and relative groups, whereas the number of trials to complete the first category was negatively correlated across all three groups. IL8 showed negative relationship with logical memory and associated learning in the first-degree relatives and negative relationship with total memory percentage in the control group. There was also negative relationship with failure to maintain set in both patient and relative groups, positive relationship with non-perseveration errors in the relative group. TNF α showed negative relationship with general information only in the first-degree relative group. While there was positive relationship with number of trials to complete first category only in the relative group. INF-γ showed Negative relationship with orientation and visual reproduction in the patient group and positive relationship with associate learning in the control group. A negative correlation was found between the percentage of patients' answers that were at the conceptual level and the number of accurate answers overall, as well as between the patient group and the control group.

Jing Zhu et al. [17] found that CRP in the blood were positively correlated with cognitive impairment in individuals with first-episode schizophrenia, suggesting that this marker could be used as a quick and objective way to assess cognitive impairment in this population. Bulzacka, et, al. [18] impaired General Intellectual Ability and Abstract Reasoning were found to be associated with abnormal CRP levels. Additionally, the decline of all components of working memory and a wide range of other impaired cognitive functions, such as memory, learning abilities, semantic memory, mental flexibility, visual attention, and speed of processing, was associated with abnormal CRP levels. Mustafa M. Amin, [19] cross-sectional analytic research was conducted, in which a total of 100 subjects were collected and categorized into two groups: patients with schizophrenia (PwS) and control group). The TNF-α level was significantly lower in the PwS group than in the control group. However, there was no significant correlation between the TNF-α level and the MMSE (Mini Mental State Examination) score of the PwS.

Regarding correlation between BDNF and cognitive functions, Safwat et al. [20] found a positive correlation in General Psychopathology scale PANSS test and BDNF concentration in schizophrenic group which is consistent with our findings. Zhang et al. [21] found that schizophrenic patients performed worse than normal on the majority of cognitive tasks, and BDNF levels were substantially lower in patients than in controls. For male patients, BDNF was significantly associated with general psychopathology sub score and the negative symptom subscore: on the contrary of our results. Nevertheless, no statistically significant correlation was observed between BDNF and any cognitive

performance measure. For female patients, immediate memory and the RBANS total score were significantly correlated with BDNF.

Regarding first degree relatives, we found that BDNF level was lower in 1st degree relatives than healthy group and It was not significantly different in the patient group than in the 1st degree relative group. We also discovered that the 1st degree relative group exhibited higher levels of IL6, IL8, and TNF-α than the healthy subject group. However, there were no differences in the levels of CRP, IL2, and INF-γ between group II and group III. Hou et al. [22] had comparable outcomes. Compared to the healthy control group, patients with schizophrenia exhibited substantially elevated serum levels of proinflammatory cytokines (sIL-2R, IL-6, TNF-α, INF-γ). Similarly to the corresponding schizophrenia patients, the unaffected first-degree relatives exhibited alterations in proinflammatory cytokines (sIL-2R, IL-6, and TNF-α), albeit at a lower level than the healthy control group. Lizano, et al. [23] discovered a substantial negative correlation between BDNF and Perseverative Errors (percent) in the schizophrenic group Additionally, there was a negative correlation between BDNF and Perseverative Errors (percent) in the first-degree relatives' group, as well as a positive correlation between BDNF and Categories completed.

Limitations:

Limitations of this study included a limited number of inflammatory and immune markers. There is no genetic information available for the participants in the current study, particularly with respect to the val66met BDNF polymorphism. Although we have made every effort to account for all potential confounders in the study, there are still numerous factors that could potentially impact serum cytokines and BDNF levels, such as stress, physical activity, and sleep difficulty. These factors were not able to be controlled in this study and necessitate additional research. There was only one HC cohort, and no other group of patients with severe mental disorders, such as bipolar disorder, was available for comparison. Consequently, it is impossible to conclude that these markers are specific to schizophrenia. This study sample was collected from January 2020 till December 2021 concomitant with the Covid 19 pandemic which itself may represent a conflict of interest for inflammatory cytokines.

Conclusion

Schizophrenic patients showed higher levels of inflammatory markers (CRP, IL6, IL8 and TNF-α) than control subjects which suggests the inflammatory etiopathogenesis of schizophrenia. Schizophrenic patients showed lower levels of serum BDNF as compared to control subject. First-degree relatives of schizophrenia patients exhibited inflammatory marker levels (IL6, IL8, and TNF-α) that were intermediate between those of schizophrenic patients and healthy control subjects. First-degree relatives of schizophrenia patients exhibited lower levels of BDNF in comparison to control subjects. In comparison to healthy control subjects, first-degree relatives of patients with schizophrenia exhibited lower cognitive functions, including working memory, attention, executive functions, visual learning, verbal learning, reasoning, and problem-solving. BDNF showed positive relationship with both memory and problem solving in patients with schizophrenia. The negative correlation between BDNF and both IL6 and TNF-α in schizophrenia patients may be indicative of a pathological mechanism that involves an interaction between insufficient neurotrophin compensation and inflammatory injury.

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Ethics approval and consent to participate

The research was approved by the scientifc and ethical committee of the Faculty of Medicine, Tanta University. The coded and secure patient data could only be accessed by the principal and co-investigator on a personal computer.

Consent for publication

All authors of the manuscript have provided their consent for submission and publication.

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