

Inflammatory Markers in Patients with Frontotemporal Dementia

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Abstract

Purpose of the Study: The purpose of this study was to determine a level of inflammation markers in the blood of patients with frontotemporal dementia (FTD).

Materials and Methods: 34 patients with FTD were examined. The diagnosis of FTD was established according to the diagnostic criteria of the International Consortium 2011. The healthy group consisted of 35 people, comparable in age and sex with patients with FTD. Blood plasma was used for immunological studies. The enzymatic activity of leukocyte elastase (LE) and the functional activity of α 1-proteinase inhibitor (α 1-PI) were determined by spectrophotometric method, the concentration of interleukin-6 (IL-6), C-reactive protein (CRP) and the level of autoantibodies to S100b and myelin basic protein (MBP) were determined by enzyme immunoassay.

Results: Patients with FTD showed a significant increase in the activity of α 1-PI compared with the control ($p < 0.001$). Other indicators did not differ from the control ones. However, a significant variation of all studied parameters was revealed, both the excess of values and their decrease relative to the control. For example, LE activity was from 127 to 276.5 nmol/min-ml. According to this level, two immune phenotypes were identified: 47% of patients with FTD are characterized by a proinflammatory immune phenotype with an increase of inflammatory marker values, and 53% of patients with FTD have proinflammatory immune phenotype with insufficient neutrophil activity (decreased LE activity and increased level of other inflammatory markers). This immune phenotype was similar to that in patients with Alzheimer's disease.

Conclusion: The results obtained indicate the involvement of inflammation in the development of FTD and the immunological heterogeneity of this variant of dementia.

Keywords: Frontotemporal Dementia; Markers of Inflammation; Leukocyte Elastase; Interleukin-6; Acute-Phase Proteins

Introduction

Frontotemporal Dementia (FTD) is a complex of neurodegenerative diseases and syndromes of unknown etiology but shared morphological features of brain damage. Characteristic histopathological brain changes were first identified and described by Arnold Pick in 1892, then named after him (Pick cells and Pick bodies) and subsequently on the proposal of A. Alzheimer the clinical form of the disease became known as Pick's disease.

In 1994 the diagnostic group of disorders entitled Frontotemporal Dementia was created which, along with Pick's disease included primary progressive aphasia, semantic dementia and motor neuron disease. This group of disorders shares neurodegenerative damage of

frontal and temporal lobes, and in clinical picture has changes in personality and behavior; deterioration of language and other cognitive functions of “frontal” type [1]. Frontotemporal dementia is the third most common type of dementia in old age and the second among dementias with early onset, before the age of 65 [2]. Several FTD subtypes are allocated, their clinical differences depend more on anatomic localization of the disorder rather than on its morphological picture [3].

Recent research reveals the role of inflammation in neurodegenerative diseases. It is shown that activation of microglia (indicator of neuroinflammation) can be detected at early stages of frontotemporal dementia. It is also shown that development of dementias occurs with activation of inflammatory reactions on the periphery (in the bloodstream system) and change of proinflammatory cytokines level and other inflammatory markers are similar to those observed in brain. The most studied disease in this regard is Alzheimer’s disease [4-6].

The role of neuroinflammation and related systemic inflammation in pathogenesis of FTD is largely unknown. Neuroinflammation begins at early stages of FTD pathogenesis; it is characterized by innate immune system reactions in populations of immune cells and cytokines due to the influence of gene mutations, which leads to a change in the innate immunity [7].

The characteristic feature of FTD is the important role of genetic factors. Intensive genetic studies of FTD demonstrated the joint contribution of immune and genetic factors [8].

Purpose of the Study

The purpose of the present study was to determine a level of number inflammatory markers such as the enzyme activity of leukocyte elastase (LE), functional activity of alpha-1-proteinase inhibitor (α 1-PI), level of C-reactive protein (CRP), interleukin-6 (IL-6) and autoantibodies against neuro-antigens S100b and the myelin basic protein (MBP) in the blood of patients with frontotemporal dementia.

LE is one of the markers of neutrophil degranulation activity of the serine proteases family; it localizes within azurophilic granules of neutrophilic leukocytes [9,10]. The release of this enzyme from neutrophils into the extracellular space occurs when exposed to various soluble agonists of degranulation, as well as in neutrophil adhesion to vascular walls or their death. LE, through its proteolytic activity, can enhance vascular penetration and has cytotoxic effect on endothelial cells [11-14].

α 1-PI is an acute-phase protein that is synthesized mainly by hepatocytes. It is involved in LE inhibition and a number of other proteinases [15]. This protein controls proteolytic activity of LE and also creates conditions for limiting the site of inflammation and destruction.

C-reactive protein (CRP), an acute-phase inflammation protein, is synthesized mainly by hepatocytes. The source of CRP may also be neurons, monocytes and lymphocytes [16]. This protein is controlled by proinflammatory cytokines (mainly IL-1 β and IL-6) [17].

Interleukin-6 is a proinflammatory cytokine, a glycoprotein, produced by both lymphoid and non-lymphoid cells [18,19]. T and B lymphocytes, eosinophils, mast cells, astrocytes and microglia can also release IL-6 [20-22]. IL-6 regulates the synthesis of other inflammatory mediators, participates in T-lymphocyte activation, and induces the synthesis of many acute-phase proteins: fibrinogen, α 1-antichemotripsin, haptoglobin, serum amyloid A, CRP, etc.

Normal elements of the immune system of any healthy person are natural autoantibodies against practically all antigens of a human body, including proteins of neural tissue. The natural contents and ratio of α 1- antitrypsin in blood serum fluctuates within certain limits specific to each age and can sharply change in various diseases. S100b - Ca⁺² binding protein of neural tissue is a trophic factor for serotonergic neurons, its boosted synthesis is indicative of astrocytes activation in response to neural tissue damage against a background of hypoxia or a hypoglycemia. MBP is involved in formation and maintenance of the structure integrity of myelin nerve fibers.

Materials and Methods

Patients

The research included 34 patients (18 men and 16 women; aged 62 ± 10.1 years), outpatients or those on treatment in Mental Health Research Centre. Frontotemporal dementia was the only criterion for inclusion of all patients signed informed consent. The diagnosis of FTD was confirmed according to diagnostic criteria of International Consortium, 2011 [23].

Patients with clinical and/or laboratory signs of infectious or autoimmune pathology at the time of the examination, or within 2 months prior to the examination, were not included in the study.

In most cases (27; 79.4%) the onset disease was before the age of 65, in other cases (7; 20.6%) it was after the age of 65. Average duration of the disease was 4.4 ± 3.4 years. According to MRI, clinically diagnosed mental and cognitive disorders of "frontal" type were confirmed by damage of frontal lobes in most cases (29 patients out of 34; 85.3%).

Among those examined two groups of patients with FTD were allocated: with mainly behavioral disorders and with mainly aphasic disorders (PPA). The diagnosis of behavioral variant of FTD met the international criteria for diagnosis of this form of disease [23].

16 patients (47%) with FTD were examined at the stage of mild dementia. The mini-test of mental state (MMSE) score, at the time of taking sample of blood, was between 21 and 29 points. In 11 patients (32.4%) dementia reached moderate stage, with a total MMSE score from 11 to 21 points. 7 patients (20.6%) suffered from severe dementia, their total MMSE score did not exceed 12 points. The average duration of mild dementia in the studied cases was 3 years, when the duration of moderate dementia stage reached 1.8 years. The duration of moderately severe dementia stage did not exceed 1 year.

35 healthy aged 60.8 ± 4.9 were enlisted in a control group, they underwent medical examination and did not find symptoms of mental disorders, somatic diseases or acute infection. The studied groups (patients - control) did not differ in the age ($p = 0.47756$).

Immunological indicators were defined in plasma of peripheral blood. After blood draw it was centrifuged at 750g for 15 minutes at 22°C, and then the selected plasma was used for the analysis. Blood plasma can be stored at the temperature 2 - 8°C within 24 hours or in a frozen state at the temperature from -18°C to -24°C within a month before the analysis.

The following immunological indicators were determined in blood plasma: enzyme activity of LE, functional activity of α 1-PI, concentrations of CPR and IL-6 and level of autoantibodies to S100b and MBP. Enzymatic method used to determine LE activity. Elastase activity of blood plasma (nmol/min-ml) related to the 90% presence of neutrophil elastase complex with α 1-PI was determined by enzymatic spectrophotometric method using a specific chromogenic substrate [24]. Spectrophotometric method was used to measure functional activity of α 1-PI in blood plasma (IU/ml) [25]. The concentration of IL-6 (pg/ml) and CRP (mg/L) was determined with ELISA using diagnostic kits by *Vector-Best (Russia)*. The level of autoantibodies to S100b and MBP was evaluated by the standard ELISA (optical density, OD).

Statistical methods were performed using Mann-Whitney U test to compare two independent groups. Data is represented as a median [Q1; Q3]. Values of $p < 0.05$ were considered statistically significant.

Results

The table 1 summarizes the results of comparisons of inflammatory markers in the blood of patients with FTD.

Variable analyzed	Experimental groups				
	Control (n = 35)	FTD all (n = 34)	Mild dementia (n = 16)	Moderate dementia (n = 11)	Severe dementia (n = 7)
LE nmol/min·ml	213,8 [197,6; 220,3] 181-235,9	218,6 [195,3; 245,8] 127 -276,5	225,5 [202,8; 245,2] 179,3-276,5	239,8 [212,1; 248,2] 175 -269,5	192,7 [138,8; 218,2] 127 -219,1
α1-PI IU/ml	39,2 [34,3; 41,6] 24-48,9	46,6**** [41,0; 51,3] 15,6-67,2	44,5* [41,6; 49,4] 22,7-56,8	49,4** [41,0; 55,3] 15,6-67,2	48,6* [32,8; 52,6] 31,0-54,4
CRP mg/l	2,93 [1,15; 6,7] 0,12-13,4	4,4 [1,8; 9,5] 0,20-23,8	3,2 [0,53; 6,9] 0,20-12,7	5,3 [1,8; 12,1] 0,46-17,9	7,4 [4,1; 11,6] 2,5-23,8
IL-6 pg/ml	3,73 [2,94; 4,4] 0-5,95	3,9 [3,4; 5,0] 2,6-31,1	3,8 [3,4; 4,7] 2,6-8,4	3,8 [3,2; 4,5] 2,8-10,4	5,5 [4,0; 18,4] 2,6-31,1
S100b OD	0,70 [0,60; 0,78] 0,4-1,1	0,70 [0,62; 0,86] 0,44-1,25	0,75 [0,66; 0,90] 0,61-1,1	0,64 [0,51; 0,79] 0,44-1,16	0,66 [0,59; 0,75] 0,55-1,25
MBP OD	0,72 [0,64; 0,80] 0,55-0,93	0,72 [0,63; 0,85] 0,44-1,06	0,74 [0,65; 0,85] 0,6-1,0	0,73 [0,48; 0,77] 0,44-1,06	0,66 [0,62; 0,88] 0,53-0,91

Table 1: Immune indicators in blood plasma of patients with FTD and control.

* $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ compared to control.

As can be seen from table 1 in patients with FTD as a whole, only the increase of α1-PI activity at all stages of the disease compared to the control group ($p < 0.0001$) was statistically significant. The remaining indices, such as enzyme activity of LE, levels of CRP and IL-6, as well as level of AAT to S-100b and MBP in this sample of patients were not different from the control. A decreasing tendency in LE activity ($p = 0.09$) and an increasing trend in CRP and IL-6 levels ($p = 0.087$ and $p = 0.082$, respectively) in case of severe dementia were observed. The absence of statistically significant differences showing the change in indicators with severe dementia appears to be due to a small sample of patients in this group. In the whole group of patients, the negative correlation between LE and CRP levels ($r = -0.365764$) was detected. Similar correlation was found in patients with Alzheimer’s disease [4]. There was a considerable variation in all the indicators studied (See table 1) in the group of patients with FTD, i.e. both exceedance and decline of values in relation to the control were observed. For example, variation in LE activity ranged from 127 to 276.5 nmol/min·ml.

Previously, when examining patients with Alzheimer’s disease decrease of LE activity related to the severity of dementia and the degree of cognitive decline was found [4]. At the same time, the increase in LE activity was typical for old patients with endogenous psychoses [26], an increased functional activity of acute phase protein α1-PI was revealed in the blood of all old patients with dementias, which indicated activation of inflammatory reactions.

Lack of distinctions shown in the studied indicators, depending on the age of FTD onset, confirms not only clinical, but also pathogenetic common neurodegenerative pathology in early and late forms of this disease. Due to multidirectional (compared to the control) changes of LE activity, the general group of patients with FTD was divided into two immunological subgroups (immune phenotypes) (Table 2): subgroup A - 47%, whose LE activity was higher than the upper reference limit; subgroup B - 53%, whose LE activity was lower than the lower reference limit or inside the reference range. Increase of α 1-PI functional activity in comparison with the control was characteristic of both subgroups.

Variable analyzed	Experimental groups		
	Control (n = 35)	FTD patients with immune phenotype A (n = 16)	FTD patients with immune phenotype B (n = 18)
LE nmol/min-ml	213,8 [197,6; 220,3]	246,4***# [235,4;257,0]	197,0** [188,0;209,5]
α 1-PI IU/ml	39,2 [34,3; 41,6]	48,6*** [42,5;55,8]	45,0* [36,2;49,4]
CRP mg/l	2,93 [1,15; 6,7]	3,2 [0,39; 6,8]	5,7 [3,6; 11,6]
IL-6 pg/ml	3,73 [2,94; 4,4]	3,8 [3,4; 4,7]	4,0 [3,1; 5,6]
S100b OD	0,70 [0,60;0,78]	0,71 [0,62;0,82]	0,70 [0,61; 0,89]
MBP OD	0,72 [0,64;0,80]	0,72 [0,64;0,76]	0,72 [0,62;0,86]

Table 2: Immune-biochemical parameters in patients with FTD with isolated immune phenotypes A and B (compared to control).

* $p < 0,05$, ** $p < 0,001$, *** $p < 0,0001$ compared to control; # - $p < 0,00001$ - significant difference between immune phenotypes A and B.

Next, an attempt was made to identify a possible relationship between the isolated immune phenotypes and clinical forms of FTD (Table 3).

Immune phenotypes	FTD clinical types	
	Behavioural variant (behavFTD) n = 25	Primary progressive aphasia (PPA) n = 9
A	10 (40%)	6 (66,7%)
B	15 (60%)	3 (33,3%)

Table 3: The Distribution of immune phenotypes A and B in different clinical variants of FTD.

The provided data indicate that both allocated immune phenotypes can be found in each clinical subgroup of patients. Immune phenotype A was identified in 10 patients with behavioral variant of FTD and in 6 patients with primary progressive variant, i.e. with prevalence of high rates of LE activity in blood plasma (40 and 66.7% respectively), which is characteristic of patients with endogenous psychoses. Immune phenotype B, characteristic of patients with Alzheimer’s disease, was identified in 60% of patients with behavioral variant of FTD and in 33.3% of patients with PPA.

As illustrated in the clinical assessment of the patients with FTD, behavioral variant included two subtypes of disorders. Prevalence of disinhibited behavior can be seen in 13 out of 25 patients with this variant of FTD, in other 12 patients their mental state was predominantly determined by apathy and asponaneity. 9 patients from the FTD whole group were allocated to verbal variant, 8 out of them were diagnosed as primary progressive aphasia and 1 as semantic dementia.

At allocating immune phenotypes it turned out that immune phenotype A was identified in 3 patients (18.8%) with apathetic subtype of behavioral variant of FTD, in 7 patients (43.8%) with disinhibited behavior variant and in 6 patients (37.5%) with predominant language deterioration (all with PPA). In turn, immune phenotype B was identified in 6 patients (33.3%) with disinhibited behavior subtype of behavioral variant of FTD and in 10 patients (55.6%) with the prevalence of apathy, while in 2 patients (11.1%) this immune phenotype was observed with the predominance of aphasic disorders. The similarity of immune phenotype A identified in FTD with the one found in endogenous mental pathology and, on the contrary, immune phenotype B with immune-biochemical indicators of Alzheimer's disease makes it difficult to conduct differential diagnosis in FTD with other endogenous diseases, especially at initial stages. It is known that FTD is not always diagnosed immediately when behavioral disorders, characteristic of FTD, resemble the symptoms of mania with disinhibition manifestations; apathy and asponaneity show formal similarity to manifestations of indifference and lack of motivation in depression, and signs of emotional impoverishment guards concerning deficit in schizophrenia.

Discussion and Conclusion

Preliminary data was obtained when considering the representation of various phenotypes (A and B) in clinically different subtypes of FTD. The study finds the involvement of inflammatory reactions in FTD development, in addition the heterogeneity of this dementia type on immunological indicators (immune phenotypes) is revealed. 47% of patients with FTD have proinflammatory immune phenotype characteristic of patients with endogenous psychoses [26]. At the same time 53% of patients with FTD have the immune phenotype common for patients with AD, which distinctive feature is a significant decrease in enzyme activity of LE that can be connected with the change of neutrophil degranulation activity, the most important cellular component of innate immune system, participant of the process of inflammation and phagocytosis [5].

This is a pilot study and some restrictions are obvious. Small sample size, small number of relatively homogeneous FTD manifestations, make it difficult to conduct meaningful comparison to obtain statistically significant results. However, this work has clear advantages in view of modern methods underlying the study of several immune-biochemical indicators showing the involvement of neuroinflammation in FTD pathogenesis. The data obtained in the present study confirm the results of earlier conducted researches in other neurodegenerative diseases. It should be acknowledged that the number of works studying various aspects of FTD, both in Russian and foreign science, is small and this problem is only being developed.

Novelty of the research lies in the obtained ideas of variability of proinflammatory immune phenotypes (A and B). The dichotomy of immune phenotypes A and B coincides with clinical views of FTD heterogeneity and proximity of initial clinical manifestations in different variants of FTD, in some cases with Alzheimer's disease, in other cases with endogenous diseases, which in turn can reflect features of hereditary load. Preliminary data should be verified on more numerous and homogeneous samples, but the results are definitely of interest when used in early detection and further study of FTD pathogenesis.

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