APRIL is increased in serum of patients with brain glioblastoma multiforme

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ABSTRACT. A PRoliferation-Inducing Ligand (APRIL) is a cytokine with the ability to induce tumorigenesis. The aim of the study was to measure serum APRIL levels in patients with brain glioblastoma multiforme. Twenty five patients with brain tumor and a control group of 25 subjects took part in the study. APRIL was measured by the enzyme-linked immunosorbent method. The study showed increased APRIL levels in the serum of patients with brain glioblastoma multiforme compared to the control group ($p < 0.05$). However, there was no significant difference in the level of this cytokine between groups of patients divided according to their clinical state and tumor size ($p > 0.05$). Inflammation parameters such as C-reactive protein (CRP) and polymorphonuclear leucocytes (PMN) were also increased in patients with brain tumor compared to controls ($p < 0.05$). There was a significant correlation between APRIL and CRP and PMN ($p < 0.05$). Results from the study suggest that APRIL may play a role in the pathogenesis of brain glioblastoma multiforme. It is possible that anti-APRIL therapy might be useful in this disease. However, this cytokine cannot be regarded as a marker of tumor size or of severity of the clinical condition of patients.

Keywords: APRIL, cytokine, glioblastoma multiforme, serum, tumor

A PRoliferation-Inducing Ligand (APRIL), also named the TRDL-1 or TALL-2 is a new member of the tumour necrosis factor (TNF) family that is expressed in haematopoietic cells. It influences cellular pathways [1]. APRIL is cleaved intracellularly resulting in its secretion [2]. It is known that this cytokine and its receptors play an important role in regulating B cell homeostasis, tolerance and malignancy [3]. It can induce peripheral B cell maturation and survival, and stimulate tumor cell growth [4, 5]. Hahne *et a*l. [6] state that APRIL transcript levels in normal tissues are low, and that highest levels were observed in peripheral blood leucocytes. An increase in APRIL mRNA expression was found in tumor tissues [7] suggesting a role for this cytokine in the regulation of tumor growth.
The aim of the study was to measure APRIL levels in the serum of patients with brain glioblastoma multiforme, and to estimate whether serum APRIL may be a marker of severity of the clinical state of patients and of tumor size.

METHODS

Twenty five patients (13 males/12 females) with brain tumor (glioblastoma multiforme) and 25 (12 males/13 females) patients with tension headache acting as the control group took part in the study. The average age of the patients with brain tumor was 54 years, and that of the control group 52 years. The diagnosis of brain tumor was based on clinical symptoms, neurological examination, and results of computed tomography (CT) of the brain. Glioblastoma multiforme was confirmed by histopathology. Patients with brain tumor were divided into two groups according to the severity of their clinical condition; mild clinical state (without disturbances of consciousness and with mild or no hemiparesis); severe clinical state (with disturbances of consciousness and severe hemiparesis or hemiplegia). Patients were also divided into two groups according to tumor size (deduced from CT of the brain); small size (diameter - up to 30 millimetres); large size (diameter – over 30 millimetres). Patients with associated autoimmune diseases that could influence serum APRIL levels were excluded from the study. To estimate the potential autoimmune and/or inflammatory processes which may be associated with brain tumor, parameters such as C-reactive protein (CRP) and polymorphonuclear leucocytes (PMN) were measured. The study was approved by the local ethical committee and was conducted according to the principles established in Helsinki.

Blood samples were obtained from patients with brain tumor and from controls. The samples were centrifuged for 10 minutes and serum was stored at -70° C until analysis. APRIL and CRP levels were measured in duplicate by the enzyme-linked immunosorbent method using commercial ELISA kits for human APRIL (Bender MedSystems), and for human CRP (R&D Systems), in accordance with the manufacturer's instructions. The PMN count was estimated on the basis of clinical records of patients.

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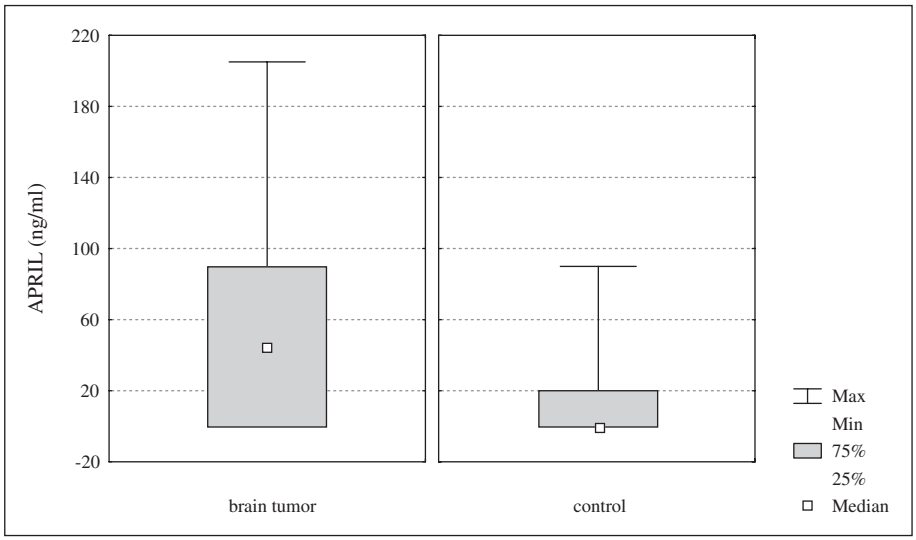

Figure 1
APRIL in patients with brain tumor and controls.

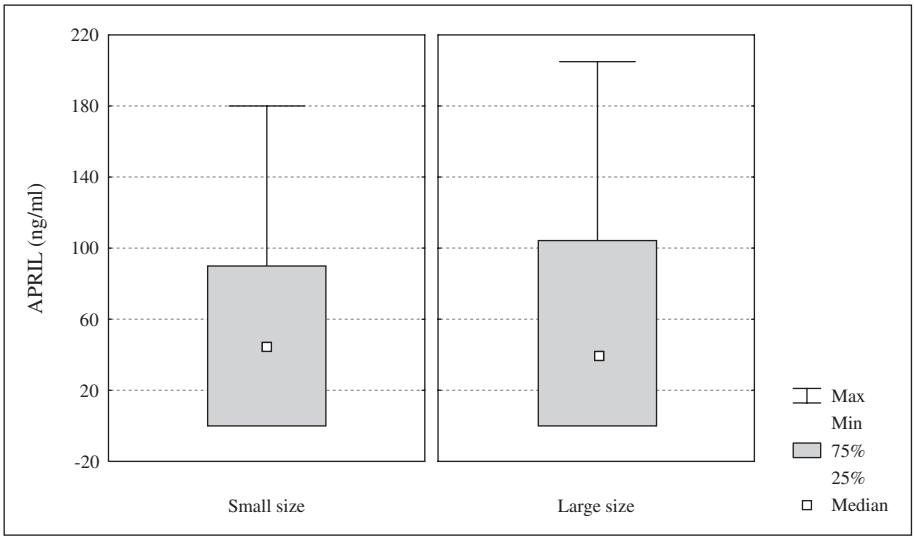

Figure 2
APRIL in patients with brain tumor in dependence on tumor size.

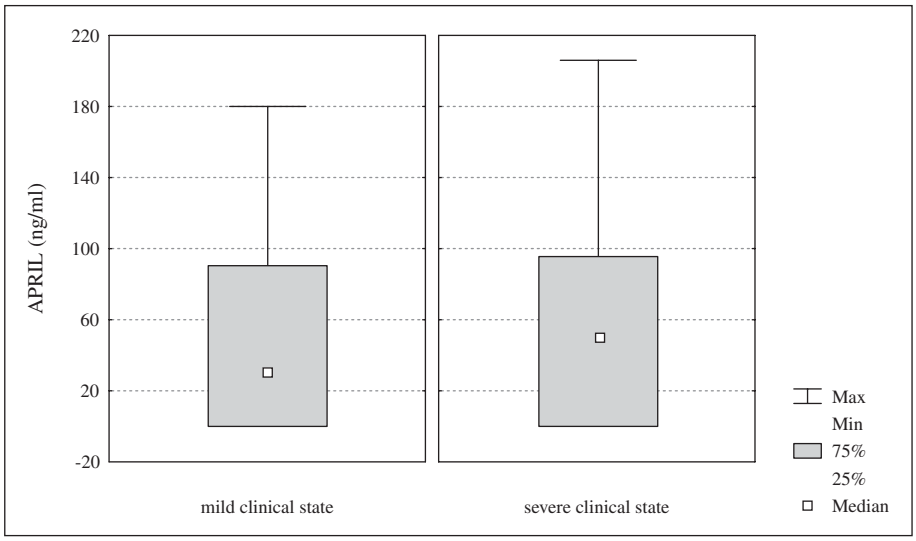

Figure 3
APRIL in patients with brain tumor in dependence on clinical state.

Table 1
APRIL, PMN and CRP levels in individual patients with brain tumors and controls

No.	Controls			Brain tumors		
	APRIL [ng/ml]	PMN cells/µl	CRP [ng/ml]	APRIL [ng/ml]	PMN cells/µl	CRP [ng/ml]
1.	0	6,4	340	180	11,8	5480
2.	70	7,2	765	100	8,7	2340
3.	24	8,1	576	0	6,8	1399
4.	20	5,7	633	0	13,8	126
5.	30	7,6	245	30	10,2	2778
6.	20	4,5	680	30	9,4	1890
7.	10	6,0	122	90	6,2	4670
8.	17	2,8	354	0	4,9	679
9.	20	2,6	670	0	8,9	360
10.	90	9,3	145	120	12,7	2438
11.	0	6,9	266	68	10,6	700
12.	0	4,8	355	45	9,1	345
13.	0	3,9	67	0	2,9	6777
14.	0	5,6	145	0	11,7	820
15.	0	6,9	386	0	8,5	4500
16.	0	6,9	544	0	9,4	2672
17.	0	7,2	347	140	10,6	2317
18.	0	7,9	137	50	8,9	1765
19.	0	4,8	654	205	10,5	4524
20.	0	6,3	344	104	9,2	2210
21.	15	7,0	145	85	7,1	1555
22.	0	2,3	655	0	4,7	348
23.	0	6,7	240	0	6,4	1278
24.	5	5,6	146	49	8,3	2766
25.	34	6,8	243	70	10,6	3891
	Median (range)			Median (range)		
	0 (0-90)	6,4 (2,3-9,3)	344 (67-765)	45 (0-205)	9,1 (2,9-13,8)	2210 (126-6777)

Table 2
APRIL levels in individual patients with brain tumors in dependence on tumor size and clinical state of patients

	APRIL leveles [ng/ml]			
	Brain tumor size		Clinical state of patients	
No.	Small	Large	Mild	Severe
1	180	0	180	0
2	100	0	100	0
3	0	0	0	0
4	0	140	0	140
5	49	50	30	50
6	90	205	30	205
7	0	104	90	104
8	0	85	0	85
9	68	0	0	0
10	45	0	120	0
11	0	30	68	49
12		70	45	70
13		120	0	
14		30		
	Median (range)			
	45 (0-180)	40 (0-205)	30 (0-180)	49,5 (0-205)

For statistical analysis, the nonparametric Mann-Whitney U test was used because data were not distributed normally. The correlation analysis was performed using the Spearman's correlation coefficient. P values < 0.05 were considered significant.
APRIL and CRP levels were expressed in ng/mL. PMN was presented as number of cells per 1 μL.

RESULTS

The study showed significantly increased APRIL levels in the serum of patients with glioblastoma multiforme compared to the control group ($p = 0.03$) *(figure 1)*. CRP levels and the PMN count were also significantly increased in patients with brain tumor compared to controls ($p = 0.000001$, and $p = 0.00008$; respectively) (data not shown). APRIL, PMN and CRP in individual patients with brain tumor, and in controls are presented in *table 1*.
There was no significant difference in APRIL levels between group of patients with small or large tumor size or between patients with mild or severe clinical state ($p = 0.60$, and $p = 0.80$; respectively) *(figures 2, 3)*. APRIL levels in individual patients of these groups are presented in *table 2*.
There was a significant correlation between APRIL and CRP and PMN in patients with brain tumor ($p = 0.02$, and $p = 0.04$; respectively). However, the correlation between CRP and PMN in patients with brain tumor was not significant ($p = 0.90$). There was also no significant correlation between APRIL and tumor size ($p = 0.53$) (data not shown).

DISCUSSION

Previous studies have shown that APRIL is upregulated in different autoimmune diseases. Thangarajh *et al*. [8] observed increased expression of APRIL mRNA in monocytes and T cells in patients with multiple sclerosis (MS), and suggested that MS may be associated with increased transcription of factors promoting B-cell survival, including APRIL, in peripheral blood. Seyler *et al*. [9] revealed that this cytokine plays a role in rheumatoid arthritis through regulation of B cell and T cell function, probably via its pro- and antiinflammatory activities. Jonsson *et al*. [10] observed increased serum APRIL levels in patients with Sjogren's syndrome. Data from the literature showed that serum APRIL levels correlated positively with musculoskeletal manifestations among patients with systemic lupus erythematosus and correlated inversely with clinical disease activity [11, 12].
Results from this study indicate that APRIL participates in the pathogenesis of human glioblastoma multiforme that confirm a previous experimental study conducted on a glioblastoma cell line. Roth *et al*. [13] observed that APRIL causes upregulation of the X-linked inhibitor of apoptosis (XIAP), which influences the activity of caspases, resulting in inhibition of apoptotic cell death. Additionally, APRIL can promote tumor cell proliferation. Deshayes *et al*. [14] showed that APRIL is produced in human glioblastoma cell lines and may induce their proliferation.
Patients with concommitant autoimmune diseases were excluded from the recent study. However, the autoimmune and/or inflammatory processes may be associated with brain tumor. The increase in CRP levels and PMN count observed in patients with brain tumor in this study confirm this suggestion.
In conclusion, the recent study conducted on human serum supports previous experimental investigations indicating that APRIL may play a significant role in the tumorigenesis of brain glioblastoma multiforme; it cannot be excluded that anti-APRIL therapy might be useful in this disease. However, this cytokine cannot be regarded as a marker of tumor size or of the severity of the clinical condition of patients.

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