Original Article

Serum endocan levels before and after surgery on low-grade gliomas

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Abstract

Background: Endocan has been shown to be a marker for several cancers and may show degree of malignancy. The aim of this study is to assess serum levels of endocan before and after surgery on low-grade gliomas (LGGs).

Methods: Endocan was assayed by commercially available enzyme-linked immunosorbent assay (ELISA) kits in a total of 19 patients and 12 controls. Serial serum samples were obtained before and after surgery (1st day, 1st week, and 1st month of surgery). Control samples were collected from cord blood during cesarean section. The results were compared with control brain tissues.

Results: Controls showed significantly lower serum endocan levels compared to before and after surgery ($P < 0.05$). There is a trend of increase in mean serum levels from before surgery and during the very early period after surgery (during first week); however, in the first month, mean serum levels became lower.

Conclusion: Endocan, a vital molecule for angiogenesis, is highly expressed before and after surgery in LGGs, but long-term data is needed. Furthermore, future studies should include high-grade gliomas to discuss whether endocan is associated with recurrence and response to treatment.

Key Words: Brain tumor, cancer, endocan, ESM-1, glioma, low-grade gliomas

INTRODUCTION

In neurosurgical practice, gliomas are commonly encountered brain tumors, and life expectancy in high-grade gliomas (HHGs) is very short. These most common brain tumors are re-classified as diffuse gliomas. According to this new classification, diffuse gliomas include World Health Organization (WHO) grade II and III astrocytic tumors, grade II and III oligodendrogliomas, grade IV glioblastomas, as well as the diffuse gliomas of

childhood. Different from the previous classification, the astrocytomas, which have a more benign growth pattern, are now distinct from the diffuse gliomas.[9] Total removal or removal as much as possible in gliomas is the goal of surgery, which has been proven to increase both progression free and overall survival times.[5,8] Despite advanced treatment modalities, including surgery and chemotherapy with respect to LGGs, we are still far from curative treatment in these common brain tumors. Furthermore, specific markers or a target does not exist to warn us whether there is progression of a residue or recurrence after treatment. Recent years showed that co-deletion of 1p19q makes oligodendroglioma more responsive to chemotherapy;[2] thus, as a routine, such gliomas are now treated by surgery and chemotherapy. Unfortunately, these treatment regimens also cannot prevent recurrence or upgrade of oligodendrogliomas. In clinical practice, LGGs, similar to other brain tumors, are followed by radiological imaging at regular intervals, with chance to monitor using a molecule the progression of these common brain tumors.

The rapidly expanding data related to endocan, previously known as endothelial cell-specific molecule-1 (ESM-1), has demonstrated that this soluble, freely circulating molecule in the blood could be used as a marker or could be a target not only in several tumors[4,10] but also in sepsis.[15] Endocan is a soluble dermatan sulfate proteoglycan (PG) and is present primarily on the cell surface, in the extracellular matrix, and body fluids.[7] Endocan is produced by endothelial cells and includes a protein core, dermatan sulfate.[1,7] Its overexpression in serum of patients with several cancers, including hepatocellular,[14] bladder,[6] colorectal,[4] and lung carcinomas,[7] has been demonstrated. Because this molecule is associated with neoangiogenesis, which is the *sine qua non* for tumor growth and progression, studies suggest that serum levels can be used as a marker that can alert clinicians about tumor progression More importantly, some studies have demonstrated that serum endocan levels can show malignancy; higher the grade of tumor, higher the serum levels of endocan.[4,12,14]

This molecule has been studied in serum or tumor tissues of patients or tumor lines of several tumors, however, there is little information regarding the levels of endocan in brain tumors. Thus, the current study is the first prospective study to show serum levels of endocan in patients with gliomas, LGGs here. A few studies related to endocan have been performed in pituitary adenomas;[11,13] only one study showed expressions of this molecule both at the mRNA and protein levels in human glioma cell lines and used glial tumor tissue sections to determine localization of endocan immunoreactivity *in situ*.[12] Thus, the aim of this prospective study is to show, for the first time, if serum levels of endocan are different from the controls and is there a change in serum levels after surgery during follow-up of patients operated upon for LGGs. For this purpose, serial serum levels of endocan were assessed before the surgery, and then on the first day, first week, and first month after surgery during the follow-up period.

MATERIALS AND METHODS

Study population

This study included 19 patients who were operated on for LGGs and gave permission and met our inclusion criteria for the study. All patients or next of kin were fully informed, and ethical approval for this study was obtained from the Human Investigations Committee at Istanbul University, Cerrahpasa Medical Faculty. We excluded patients who had any kind of chronic or acute infection, immunological and metabolic diseases, neoplastic disease of other organ systems, any cardiovascular diseases, and recent major surgical procedure at the time of tumoral tissue collection, in which endocan status might be affected. Furthermore, any patients who received radio or chemotherapy and had surgery of any kind of cerebral disorders before admission were also excluded. Controls consisted of 12 healthy women who had caesarian section and multiple births; those with major congenital anomalies were excluded.

Serum samples: Patients

Serum samples from 19 patients were obtained before surgery during the routine work-up and during routine follow-up visits after surgery. Before surgery, blood samples were obtained once, however, after surgery, serial blood samples were collected on the first day, first week, and first month. Thus, a total of four blood samples were collected from each patient. Each blood was centrifuged and serum samples were stored at −80°C until assayed for endocan.

Serum samples: Controls

The control group consisted of 12 women whose cord blood was collected at the time of elective cesarean delivery with spinal anesthesia. Umbilical cord blood samples were taken into vacutainer tubes and separated by centrifuging the samples at 500 g for 10 minutes. Serum samples again were stored at −80°C until assayed for endocan. Blood samples from the controls were obtained only once.

Assay of endocan

Endocan levels were measured with commercially available enzyme-linked immunosorbent assay (ELISA) kits (YH Biosearch Laboratory, Shanghai, China) according to the manufacturer's instructions. Briefly, after pipetting the serum samples into a 96-well microplate coated with a monoclonal antibody (also known as capture antibody) specific to the C-terminal of human endocan, incubation for 1 hour was performed. Endocan present within a sample was bound by the capture antibody. After washing the remaining unbound molecules, a biotinylated secondary monoclonal antibody (specific to the endocan

N terminal) was added to the wells and allowed to incubate for 1 hour. Following the washing step, streptavidin-HRP (biotin-binding protein conjugated with polymers of horseradish peroxidase) was added and allowed to incubate in the dark for a further 30 minutes. Any remaining unbound material was again washed away. Chromogen solution was added and incubated in the dark for 10 minutes for converting the colorless solution into a blue solution, the intensity of which was proportional to the amount of endocan in the sample. Because of the acidic stop solution effect, the samples' color turned yellow. Then, the colored reaction product was measured using an automated endocan reader at 450 nm. The unit of serum endocan was determined as ng/mL.

Statistical analysis

We used a commercially available statistical software package (SPSS version 14.0 Inc., Chicago, IL, USA) for all the statistical analyses. The mean ± standard deviations (±SD) were calculated for each parameter. For all comparisons, the nonparametric Mann–Whitney U test was used as a statistical method. Differences were considered statistically significant if the probability value was less than 0.05.

RESULTS

Demographics and serum levels of endocan in both patients and controls are shown in Tables 1, 2 and Figure 1. The first result that we underline is that serum level in every control individual was very low compared to serum levels before and after surgery in patients. We found highly

significant differences between the mean level before surgery and controls ($P = 0.008$), first day after surgery and controls ($P = 0.002$), and first week after surgery and controls ($P = 0.00001$) during very early follow-up. However, at the first month after surgery, we did not find significant difference between the mean serum level at the first month of surgery and controls ($P = 0.2$). There seems to be a trend of increase in mean serum levels from before surgery and very early period after surgery (during first week), however, in the first month, mean serum levels became lower. Comparisons among the levels regarding patients showed that no significant differences were
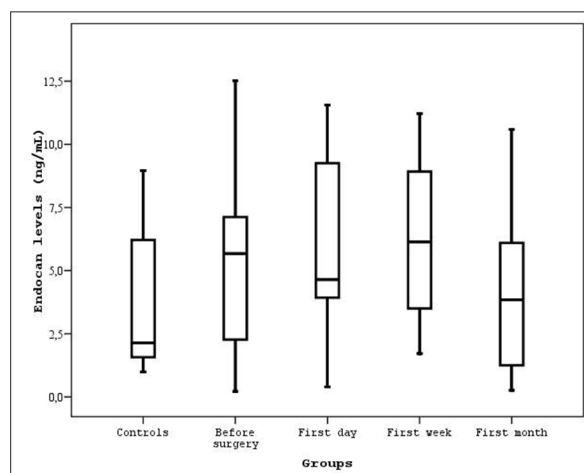

Figure 1: Summary of serum endocan levels in each group. Controls showed significantly lower levels compared to before and after surgery. The horizontal line inside the box is the mean, and the box represents the lower and upper quartiles. The whiskers indicate the minimum and maximum data values

Table 1: Summary of demographic data of the patients studied here

No	Age (yrs)	Sex	Presenting symptom	Tumor side	Tumor site	Pathological diagnosis	Follow-up (mos)
1	28	F	Seizure	Left	Insula	Oligoastrocytoma (grade-II)	31
2	37	M	Seizure	Right	Frontal	Oligoastrocytoma (grade-II)	30
3	44	F	Seizure	Left	Frontal	Astrocytoma (grade-II)	29
4	47	M	Seizure	Left	Fronto-insula	Oligoastrocytoma (grade-II)	28
5	37	M	Headache	Left	Insula	Oligodendroglioma (grade-II)	21
6	21	F	Headache	Right	Uncus	Gangliocytoma (grade-I)	21
7	30	F	Headache	Left	Frontal	Low-grade glioma (grade-II)	20
8	32	M	Seizure	Left	Frontal	Low-grade glioma (grade-II)	19
9	62	M	Seizure	Right	Fronto-insula	Oligodendroglioma (grade-II)	19
10	47	F	Seizure	Right	Fronto-insula	Oligodendroglioma (grade-II)	15
11	29	M	Headache	Left	Fronto-insula	Astrocytoma (grade-II)	14
12	25	M	Seizure	Right	Parietal	Astrocytoma (grade-II)	13
13	48	F	Headache	Left	Parietal	Astrocytoma (grade-II)	13
14	25	M	Seizure	Right	Fronto-insula	Astrocytoma (grade-II)	12
15	26	F	Headache	Left	Frontal	Ganglion cell tumor (grade-I)	11
16	29	M	Seizure	Right	Frontal	Low-grade glioma (grade-II)	10
17	24	M	Seizure	Left	Temporo-insula	Astrocytoma (grade-II)	8
18	26	F	Seizure	Left	Fronto-insula	Oligodendroglioma (grade-II)	6
19	23	M	Seizure	Left	Temporal	Glioneuronal tumor (grade-I)	6

F: Female; M: Male; mos: Months; yrs: Years.

Table 2: Serum endocan levels (ng/mL) in both patients and controls

Patient no	Before surgery	After surgery			Controls
		First day	**First week**	**First month**	
1	7.40	4.64	9.84	10.04	1.65
2	6.83	8.85	7.96	10.59	2.87
3	6.19	9.74	10.28	7.96	1.69
4	6.22	4.10	4.35	4.61	1.54
5	7.75	4.77	11.21	3.94	1.37
6	8.95	4.53	6.13	7.39	1.82
7	1.56	9.49	6.32	3.84	2.13
8	5.67	9.90	7.99	1.95	1.57
9	1.79	2.74	1.85	1.41	2.18
10	5.87	9.0	5.27	10.03	1.46
11	2.74	3.75	2.93	3.77	0.98
12	12.5	11.5	4.07	3.93	2.27
13	4.97	0.92	6.82	1.0	-
14	3.23	10.0	10.91	1.19	-
15	0.60	4.26	10.88	1.30	-
16	5.20	0.44	5.25	0.65	-
17	0.22	0.39	1.71	0.26	-
18	10.91	4.43	2.27	4.80	-
19	12.02	5.57	2.19	0.45	-
Mean	5.25±3.44	5.74±3.52	6.22±3.30	4.16±3.47	1.79±0.49

obtained between before and first day of surgery ($P = 0.86$), before and first week of surgery ($P = 0.35$), before and first month of surgery ($P = 0.26$), first day and first week of surgery ($P = 0.60$), and first day and first month of surgery ($P = 0.18$). Interestingly difference between the first week and first month of surgery reached a significant level ($P = 0.03$).

DISCUSSION

It is very difficult to discuss or compare our preliminary results obtained from the present study and with the current literature because there were no reports showing serum endocan levels in brain tumors. Serum endocan levels have been assessed in gastric cancer,[10] bladder cancer,[6] colorectal cancer,[4] and hepatocellular carcinoma.[14] All these studies suggested that serum endocan levels can be used as a potential serum marker for early detection of recurrence and assessment of prognosis. Regarding brain tumors, there are very limited reports to assess endocan levels in common brain tumors, especially glial tumors, and all studied tumor tissues, not serum.[11-13] This suggests that further studies should aim to assess serum levels before and after surgery to examine whether serum endocan levels could be used as a marker for prognosis or recurrence with respect to brain tumors. Maurage et al.[12] demonstrated by immunohistochemical analysis that endocan expression was always found on the endothelial cells of newly formed vessels and tumor cells of glioblastoma multiforme, the most aggressive HGG, and associated with extensive angiogenesis and proliferating multilayered capillary vessels. Interestingly, they found no endocan on LGGs and normal cerebral tissues far from the tumor. Tumor invasiveness and poor prognosis were also demonstrated by immunohistochemical analysis of tissue endocan levels in pituitary adenomas.[11,13] A recent study showed that high levels of endocan levels on gliomas was associated with a degree of malignancy and with neovascularization.[16]

It is not reasonable to discuss our preliminary results with the studies that evaluated serum levels of endocan in tumors of other organs including liver, bladder, and colon, etc. However; we can make some comparisons with the studies including immunohistochemical analysis of endocan in brain tumors. Our preliminary results are in line with the studies assessing serum levels of endocan in other cancers that serum endocan levels are significantly higher compared to the controls. Regarding immunohistochemical studies including HGGs and LGGs, our results are different in some respects. The present study showed that endocan can be expressed in LGGs too, which is in contrast to immunohistochemical study, which showed no endocan expression in LGGs.[12] Serial serum levels after surgery in the current study showed higher levels during early period, but at the first month of surgery, serum levels decreased. We cannot explain these results depending solely on angiogenesis because angiogenesis is not a prominent pathological event in LGGs, as the case in HGGs. However, we can speculate that during the

early period after surgery, high levels of endocan can be due to the inflammation caused by the surgery itself. We expected high mean serum level obtained before surgery compared to the controls since it is obvious that LGGs also need neovascularization, which are stimulated by vascular endothelial growth factor and endocan.[3] However, these higher levels cannot be as high as in HGGs, in which necrosis and neovascularization are the *sine qua non*.

It is surprising that one of the highly vascularized organs, brain, does not contain endocan, although endocan is expressed from endothelium of vascularized organ.[1,12] Expression of endocan in the brain suggests that the molecule is expressed upon activation rather than resting state of the cell. Cancer researchers showed that endocan is involved in angiogenesis and tumor development. Angiogenesis-related factors, such as VEGF or proangiogenic molecules, are upregulated along with endocan, which promotes mitogenic and migratory activities of vascular endothelial growth factor-A and C, which are strong angiogenic molecules.[3] Several tumors showed high expression of endocan in the newly formed vessels,[1,7,12] however unfortunately, studies including brain tumors are very few and serum levels in gliomas have not been studied yet. Association of endocan levels with invasiveness and degree of malignancy has been shown in pituitary adenomas and gliomas by immunohistochemistry studies.[11,12] They suggest that further studies should assess serum levels of endocan in malignant glial tumors to predict prognosis and early detection of tumor recurrence.

By depending solely on our limited number of patients, we cannot speculate that serum endocan levels can be used as a marker for recurrence in LGGs because we did not observe serum at the long-term follow-up (last visit) after surgery. However, we underline that endocan can be expressed in LGGs and can be detected in serum. Thus, in the future, serum endocan levels should be measured at the long-term follow-up to speculate whether serum endocan levels are associated with recurrence or progression of residue or upgrading of LGGs.

Limitations

The authors contributed to the present study acknowledge some limitations. The first and the most important limitation is that we included less number of patients. This is because of our strict inclusion criteria, and the majority of patients operated during the study period did not give permission to the study. Second limitation is that long-term serum samples were not obtained; it would be interesting to see if there any association between long-term serum levels of endocan and recurrence of tumor.

CONCLUSION

In conclusion, considering our limited findings, we can state that endocan can be detected in the serum of LGG patients, which may be novel, but potentially not meaningful. Because there is no study reported so far to show serum levels of endocan in glial tumor, it is difficult to speculate the role of endocan in LGGs. Future studies should aim to assess serum endocan levels before and after surgery in HGGs, which recur in very short period of time so that we will be able to have an idea about the recurrence and response to the treatment. We in this study just wanted to show how serum endocan levels change before and after surgery on LGGs.

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Conflict of interest
The authors declare no conflict of interest. We would like to undertake the responsibility that the serum samples here are reused for the extension of our previous work.

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