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Role of Tenascin-C in the differential diagnosis of small round blue cell tumors

Küçük yuvarlak mavi hücreli tümörlerin ayırıcı tanısında Tenascin-C'nin rolü

Öz

Giriş: Bu çalışma; küçük yuvarlak mavi hücreli tümörlerde, serum tenascin-C düzeylerinin teşhis, tedavi ve takiplerdeki yerinin değerlendirilmesi amacıyla planlanmıştır. Gereç ve yöntem: Bu prospektif çalışmaya; 40 hasta ve kontrol grubu olarak 30 sağlıklı çocuk alındı. Hastalardan; tanı anında, tedavinin 3. ve 6. aylarında ve tedavi bittikten sonraki 3. ayda toplam dört defa, kontrol grubundan ise; bir defa serum örneği alınarak tenascin-C düzeyleri ölçüldü. Bulgular: Tüm hasta grubunun her dört ölçüm değeri de kontrol grubuna göre anlamlı derecede yüksekti (sırasıyla; p=0,013, p=0,009, p=0,011 ve p=0,015). Tüm hasta grubu alt gruplarına ayrılarak karşılaştırıldığında ise; sadece Wilms tümörü grubunun, ilk ölçüm değeri, kontrol grubuna göre anlamlı derecede yüksekti (p=0,026). Sonuç: Daha geniş hasta gruplarının katılımıyla yapılacak çalışmalara ihtiyaç olmakla beraber tenascin-C, Wilms tümörü grubunda olduğu gibi, bazı spesifik tümörlerin ayırıcı tanısında yol gösterici olabilir.

Anahtar kelimeler: mavi hücre, tenascin-c, tümör, yuvarlak

Abstract

Objective: The aim of this study was to evaluate the significance of serum tenascin-C levels in the diagnosis, treatment, and monitoring of small round blue cell tumors. Materials and methods: Forty patients and 30 healthy children (control group) were included in this prospective study. Serum tenascin-C levels were measured from blood drawn at the time of diagnosis, at the 3rd and 6th months of treatment, and at 3 months following the completion of treatment. While serum tenascin-C levels were measured a total of 4 times in the patient group, they were measured just once in the control group. Results: Each of the four measured serum tenascin-C levels in the patient group was significantly higher than that of the control group (p=0,013, p=0,009, p=0,011 and p=0,015, respectively). However, when the patient group was categorized into subgroups, only those with Wilms tumor had a significantly higher serum tenascin-C level at the time of diagnosis than did the control group (p=0,026). Conclusion: While larger, more thorough studies are needed, our data suggest that tenascin-C may be useful in the differential diagnosis of certain specific tumors, such as the Wilms tumor.

Keywords: blue cell, round, tenascin-c, tumor

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Introduction

The differential diagnosis of small round blue cell tumors includes neuroblastoma (NBL), Wilms tumor (WMS), non-Hodgkin lymphomas (NHL), rhabdomyosarcoma (RMS), Ewing sarcoma (EWG)/primitive neuroectodermal tumor (PNET), medulloblastoma (MBL), and retinoblastoma (RTB) (1).

Because immunohistochemical evaluations do not always allow for the differentiation of these entities, research is ongoing to determine new diagnostic markers for these diseases. Tenascin-C (Tnc-C) expression in healthy adults is often significantly reduced or nonexistent; however, it is re-expressed in cancer patients (2). This situation suggests that Tnc-C may have increased in body fluids of cancer patients. Therefore Tnc-C levels were analyzed in several malignant diseases (3).

Therefore, the aim of this study was to evaluate the significance of Tnc-C levels in the diagnosis, treatment, and monitoring of small round blue cell tumors in children.

Material and Method

Statement on Ethics: This study was approved by the ethics committee on scientific research in our university (decision nr: 2012-199). A voluntary participation form was signed by the family of each patient prior to his/her participation in the study.

Patients: A total of 40 patients and 30 healthy children (control group) between the ages of 0-18 years were included in this prospective study. The patients were diagnosed and treated for any of the small round blue cell tumors in our clinic between March 2012 and December 2014. All diagnoses were made histopathologically.

Study Plan: Those in the patient group had their blood drawn four times as follows: at the time of diagnosis, at the 3rd and 6th months of treatment, and at 3 months following the completion of treatment. Those in the control group had their blood sampled once, at no particular time. All of the blood samples used in this study were obtained from samples that had already been drawn for routine laboratory examinations. The serum separated from these samples was stored at -80°C until further analysis. Patients were excluded from this study if they had malignant diseases other than small round blue cell tumors, if they had concomitant health issues in addition to malignant disease, and if their treatment was initiated in another center and resumed in our clinic.

Laboratory Analysis: All serum samples were thawed at room temperature on the day of analysis. Serum Tnc-C levels were analyzed using a Tenascin-C Elisa kit manufactured by Uscn Life Science Inc with the aid of a Rayto RT-2600+microplate washer and a Rayto RT-2100c microplate Elisa Reader device.

Statistical Analysis: Statistical analyses were performed using SPSS 21 software. The Kolmogorov-Smirnov Test was used to determine the normality of the data. The Mann Whitney U Test was used to compare parameters between groups and within groups that did not fit into a normal distribution, while parameters with a normal distribution were compared via an Independent T Test (for variables between groups) or a Paired Samples T Test (for variables within groups). Repeated Measures Analysis of Variance was used to determine differences between dependent groups with more than two measurements. Additionally, some descriptive features in this study are presented as "n" and mean values. Levels of $p<0,05$ were considered statistically significant.

Results

There were 20 males and 20 females in the patient group (total n=40) and there were 17 males and 13 females in the control group (total n=30).

The mean age of the patient group was 9,27±7,34 years (range=2-18) and the mean age of the control group was 9,38±5,45 years (range=3-17) (p=0,903).

In the patient group as a whole, the mean Tnc-C values were 1371,9±941,7 ng/L at diagnosis, 1364,5±867,2 ng/L at the 3rd month of treatment, 1347,6±856,6 ng/L at the 6th month of treatment, and 1375,8±951,7 ng/L 3 months after treatment. There were no significant differences between any of these values (p=0,761). However, each of these values were significantly different than the mean Tnc-C value of the control group, which was 833,2±715,5 ng/L (p=0,013, p=0,009, p=0,011, and p=0,015, respectively) (Table 1).

Table 1: Comparison of each tenascin-C measurement value in the whole patient group with those of the control group

All patient group	Control group	p
1st blood (1371,9±941,7)	833,2±715,5	0,013*
2nd blood (1364,5±867,2)	833,2±715,5	0,009*
3rd blood (1347,6±856,6)	833,2±715,5	0,011*
4th blood (1375,8±951,7)	833,2±715,5	0,015*

The patient groups were categorized into subgroups according to histopathological diagnoses as follows: 5 were RMS, 5 were WMS, 5 were EWG, 5 were NBL, 17 were NHL, 1 was PNET localized to central nervous system, and 1 was RTB.
There were no significant differences between any of the four Tnc-C measurements within any of the subgroups (Table 2).

Patient samples taken at the time of diagnosis and those taken 3 months after treatment were compared between each subgroup and the control group; it was found that the only significant difference occurred in the WMS group, in which the Tnc-C levels in the sample taken at diagnosis were significantly higher than those of the control group (p=0,026) (Table 3).

Table 2: Comparison of each of the tenascin-C values taken at four time points with each other within each disease subgroup

Blood sample	RMS (n=5)	p	WMS (n=5)	p	EWG (n=5)	p	NBL (n=5)	p	NHL (n=17)	p
1st-2nd	-11,9±296	0,893	276,2±810	0,489	485,6±462	0,079	112,5±941	0,803	-259,8±769	0,331
1st-3rd	219,6±370	0,225	329,3±601	0,288	247,3±1054	0,671	-123,1±1306	0,715	-160,8±439	0,363
1st-4th	-153,0±331	0,345	339,3±520	0,219	134,4±460	0,663	15,5±863	0,964	-106,0±546	0,701
2nd-3rd	231,5±580	0,423	53,0±230	0,686	-302,1±837	0,465	-155,2±321	0,273	251,1±664	0,179
2nd-4th	-141,0±284	0,345	63,0±443	0,767	-148,9±507	0,598	-16,5±444	0,715	110,4±656	0,382
3rd-4th	-372,6±688	0,293	9,9±321	0,948	-176,4±728	0,715	138,6±505	0,622	23,1±309	0,784

EWG: Ewing sarcoma, NBL: Neuroblastoma, NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma, WMS: Wilms tumor

Table 3: Comparison of the tenascin-C values of first and fourth samples in each disease subgroup with those of the control group

Subgroup	Blood sample	Control group	p
RMS (n=5)	1st (1249,9±918,6)	833,2±715,5	0,380
	4th (1402,9±1239,0)	833,2±715,5	0,149
WMS (n=5)	1st (1719,0±1252,9)	833,2±715,5	0,026*
	4th (1379,7±806,9)	833,2±715,5	0,099
EWG (n=5)	1st (1479,8±1585,6)	833,2±715,5	0,329
	4th (1481,9±1946,5)	833,2±715,5	0,730
NHL (n=17)	1st (1173,4±615,9)	833,2±715,5	0,156
	4th (1325,6±672,7)	833,2±715,5	0,068
NBL (n=5)	1st (1392,2±668,9)	833,2±715,5	0,125
	4th (1407,0±814,9)	833,2±715,5	0,119

EWG: Ewing sarcoma, NBL: Neuroblastoma,
NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma,
WMS: Wilms tumor

Further, each of the four Tnc-C levels was compared between subgroups, and there were no significant differences between any of the comparisons (Table 4, 5, 6, 7).

Discussion

Serum Tnc-C levels are more straightforward to determine than are immunohistochemical examinations. Further, the serum Tnc-C level can be a useful prognostic indicator, and can be used to determine the priority of treatment modalities, such as chemotherapy and surgery (4).
Tnc-C is a glycoprotein that is present in the extracellular matrix. It is encoded by a gene localized in the 9p33.1 region. Tnc-C is synthesized mainly by interstitial fibroblasts, and its expression is increased by inflammatory cytokines (e.g., tumor necrosis factor alpha, interleukin-1), growth factors (e.g., platelet derived and fibroblast derived growth factors), lipopolysaccharides, hypoxia, extracellular acidosis, and other conditions, such as oxidative and mechanical stress (5).
Tnc-C is a known regulator of embryogenesis, wound healing, neural regeneration and several cell functions (e.g., proliferation, differentiation, motility) (6). It is also

Table 4: Comparison of the tenascin-C values of the first samples in each disease subgroup with each other

	WMS 1st (1719,0±1552,9)	EWG 1st (1479,8±1585,6)	NHL 1st (1173,4±615,9)	NBL 1st (1392,2±668,9)
RMS 1st (1249,9±918,6)	p=0,754	p=0,570	p=0,965	p=0,602
WMS 1st (1719,0±1552,9)		p=0,570	p=0,458	p=0,754
EWG 1st (1479,8±1585,6)			p=0,245	p=0,291
NHL 1st (1173,4±615,9)				p=0,359
EWG: Ewing sarcoma, NBL: Neuroblastoma, NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma, WMS: Wilms tumor				

Table 5: Comparison of the tenascin-C values of the second samples in each disease subgroup with each other

	WMS 2nd (1442,7±1104,4)	EWG 2nd (1230,0±1283,0)	NHL 2nd (1497,4±709,5)	NBL 2nd (1279,7±678,1)
RMS 2nd (1261,8±1152,5)	p=0,778	p=0,715	p=0,248	p=0,754
WMS 2nd (1442,7±1104,4)		p=0,465	p=0,680	p=0,917
EWG 2nd (1230,0±1283,0)			p=0,238	p=0,273
NHL 2nd (1497,4±709,5)				p=0,563
EWG: Ewing sarcoma, NBL: Neuroblastoma, NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma, WMS: Wilms tumor				

Table 6: Comparison of the tenascin-C values of the third samples in each disease subgroup with each other

	WMS 3rd (1389,7±1098,8)	EWG 3rd (1665,5±1421,9)	NHL 3rd (1294,8±688,0)	NBL 3rd (1545,6±999,9)
RMS 3rd (1030,3±577,6)	p=0,740	p=0,690	p=0,256	p=0,624
WMS 3rd (1389,7±1098,8)		p=0,917	p=0,906	p=0,624
EWG 3rd (1665,5±1421,9)			p=0,875	p=0,806
NHL 3rd (1294,8±688,0)				p=0,720
EWG: Ewing sarcoma, NBL: Neuroblastoma, NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma, WMS: Wilms tumor				

Table 7: Comparison of the tenascin-C values of the fourth samples in each disease subgroup with each other

	WMS 4th (1379,7±806,9)	EWG 4th (1481,9±1946,5)	NHL 4th (1325,6±672,7)	NBL 4th (1407,0±814,9)
RMS 4th (1402,9±1239,0)	p=0,917	p=0,556	p=0,643	p=0,961
WMS 4th (1379,7±806,9)		p=0,624	p=0,926	p=0,806
EWG 4th (1481,9±1946,5)			p=0,442	p=0,564
NHL 4th (1325,6±672,7)				p=0,750
EWG: Ewing sarcoma, NBL: Neuroblastoma, NHL: Non Hodgkin Lymphoma, RMS: Rhabdomyosarcoma, WMS: Wilms tumor				

known to play a role in hepatitis, cirrhosis (7), tissue remodeling, inflammatory conditions, cardiac diseases, and cancer (6).

Tumor development and progress is dependent on genetic modifications (8) and cross interactions between tumor cells and tumoral stroma (9). Recently, it has been reported that cancer cells secrete certain soluble factors that can activate and modify their surrounding stroma (10). Tnc-C exerts its effects on cancer cells by triggering oncological signaling pathways (11) and by increasing angiogenesis, tumor growth and tumor metastasis (12).

Many benign and malignant neoplasms are reported to have Tnc-C immunoreactivity. Further, several solid cancers have been shown to have increased levels of Tnc-C, including cancer of the brain, breast, uterus, prostate, pancreas, colon, stomach, mouth, larynx, lung, liver, kidney, bladder, skin, bone, soft tissues, lymphomas, and ovaries (13).

One study reported that serum Tnc-C levels were significantly increased in stage 4 malignant melanoma (14), and another reported that serum Tnc-C levels were significantly higher in patients with recurrent squamous cell carcinoma of the head and neck with high tumor stage (15) when compared to a control group. In our current study, each of the four measurements in the patient group (when taken as a whole) was significantly higher than that of the control group. This result indicates that serum Tnc-C levels are generally increased in small round blue cell tumors. However, when the patient group was classified into subgroups, only the Tnc-C levels measured at the time of diagnosis in the Wilms tumor group were significantly higher than that of the control group. In addition, the Tnc-C level in the MBL group (1706,9 ng/L) was near the mean level of Tnc-C in the WMS group (1719,0±1252,9 ng/L); however, this could not be analyzed statistically, since there was only one patient in this group. The statistically significant result in the WMS group suggests that there may be a specific relationship between WMS and Tnc-C.

Similar to previously published results in non-small cell lung cancers (4), results from our current study also indicated no significant differences between the Tnc-C values in the EWG, NBL, RMS and NHL groups and the control group. Since the MBL, RTB and PNET groups had only one patient each, we could not perform statistical analyses for these groups; however, while the MBL case had a much higher Tnc-C value (1706,9 ng/L) than did the controls, patients diagnosed with RTB and PNET had values (588,3 and 522,7 ng/L, respectively) that were much lower than the mean Tnc-C level in the control group (833,2±715,5 ng/L).

High levels of Tnc-C have been evaluated from both a prognostic and diagnostic perspective. A relationship between increased Tnc-C expression and bad prognosis has been reported in glioma, breast, colon and lung cancers (13); however, its relationship with good prognosis has been reported in cervical (16), colon (17) and breast cancers (18). On the other hand, there are some studies in which no association could be made between the clinicopathological features of the tumor and patient characteristics (3). In our current study, a total of 8 patients (2 NHL, 1 EWG, 3 RMS, 1 RTB and 1 PNET) lost their lives during follow-up. Among these, the patient in the EWG group had the highest level of Tnc-C (4753,5 ng/L) of all of the patients. The three patients who passed away in the RMS group had the highest initial Tnc-C measurements in this group, which consisted of 5 cases in total. However, patients who passed away in the NHL, RTB and PNET groups had lower initial Tnc-C measurements when compared to the control group. Additionally, no patient was lost in the WMS group, which had significantly higher initial Tnc-C levels than did the control group. These results suggest that Tnc-C level at diagnosis is not a good prognostic indicator.

One study reported an association between high Tnc-C levels and increases in metastasis (19), while another found that a decrease in Tnc-C levels in mice dramatically reduced lung metastases in breast cancers (20). In our current study, we could not perform statistical analyses on all of the subgroups due to the small number of patients. However, there were patients with metastatic and advanced stage diseases with low initial levels of Tnc-C, and there were patients with non-metastatic and early stage diseases who had high initial Tnc-C levels. Nevertheless, among the patients who never achieved remission, there were patients whose Tnc-C levels increased, decreased, or did not change compared to their initial levels.

In our current study, both in the patient group as a whole and within the subgroups, there were no significant differences between the Tnc-C values obtained prior to the start of treatment and the values taken during and after treatment. This suggests that Tnc-C levels are not suitable for monitoring the response to treatment.

Conclusion

In conclusion, considering that high Tnc-C serum levels have been reported in various clinical conditions, such as in the WMS group in our current study, Tnc-C levels may be able to provide guidance in the differential diagnosis of certain specific tumors. However, further research, including a larger number of patient groups, is needed on this subject.

Conflict Of Interest: None declared

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