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The level of total sialic acid, alfa-antitrypsin, ceruloplasmin in the serum of patients with choroidal melanoma

Melanoma of the choroid is the most frequent primary intraocular malignancy in adults. This neoplasm has several unique features including the unpredictable course of the disease, but there is a long delay between initial recognition and the evidence of metastatic disease, so that deaths due to the tumor occur 10 years and more after therapy. The metastases are usually present when tumor is visible during ophthalmoscopy. It is difficult to establish the stage of choroidal melanoma if evaluated exclusively on the basis of clinical examination and the results of routine biochemical tests.

The quest is ongoing for more reliable serum markers for detecting and staging ocular melanoma. Among various markers total sialic acid (TSA) levels in the serum have been recognized as a valuable non-specific marker of tumor burden in numerous diseases including melanoma (6, 7, 13, 14, 17). Since malignant disease may be considered an acute phase-state some of acute phase proteins such as alfa-1-antitrypsin (AAT) and ceruloplasmin (CER) could be useful in monitoring melanoma. The rise of plasmatic AAT and CER in diverse malignant tumors was described by many authors. The clinical stage of the disease was more important than the anatomical aspect of tumor in the values of the acute phase proteins in blood serum (1, 8, 16).

The aim of this study was to determine the total sialic acid (TSA), total sialic acid normalized to protein (TSA/TP), alfa-1-antitrypsin (AAT) and ceruloplasmin (CER) concentration in patients with choroidal melanoma,

as well as to evaluate the clinical applicability of this marker for diagnosis and monitoring of therapy.

MATERIAL AND METHODS

The blood serum was used for testing. The samples for the assay were defrosted just once, immediately before analyses. 61 patients with melanoma of the choroid (31 men, 30 women) at average age 53.36±13.86 years were investigated. Tumors were large (diameter>15mm, height>5 mm) in 59% of patients. In 41% of patients melanomas were smaller. 41% of patients were after brachytherapy and 59% were not treated this way. The patients with general diseases which could have influence on the level of the studied substances were excluded. None of the examined patients demonstrated clinical signs of metastases. Chest radiograph, CT scan, USG scans, routine blood tests were normal. The majority of patients had computerized axial tomography of the abdomen and brain. Routine studies at the time of protocol included the following: detailed history, physical examination, complete blood count. Control group consisted of Blood Bank donors, 84 healthy persons (42 men, 42 women). TSA level was determined by the method according to Svenerholm (15). TSA concentrations are expressed as mg/dl of sera. Radial immunodifusion was used to assess the concentration of AAT i CER (on Nor Partigen plates containing antibodies directed against the protein tested). AAT and CER concentrations are expressed as mg/dl of sera. Serum proteins were determined by the method of Lowry et al., using human serum albumin as standard (10). Protein concentrations are expressed as g/dl of sera.

Statistical analysis: Student's t test was used to determine if the mean values for TSA, TP, TSA/TP, AAT and CER were significantly different in the melanoma and normal control group. p values<0.01 were considered significant; p values>0.01 are indicated in the tables as NS (no significance), whereas all other p values are listed.

RESULTS

The TSA, TSA/TP values from the sera of melanoma patients were significantly increased ($p<0.001$) when compared to normal controls. No significant difference in AAT and CER values was observed between melanoma patients and normal controls (Table 1).

The TSA values from sera of patients with larger intraocular melanomas were significantly increased ($p<0.005$) when compared to patients with smaller intraocular melanomas. No significant difference in AAT and CER values was observed between these two groups (Table 2).

Table 1. Comparison of mean TSA, TSA/TP, CER, AAT values from the sera of intraocular melanoma patients and normal controls (mean±SD and p values)

	Patients with intraocular melanoma			Normal controls			
	number of individuals	mean concentration	SD	number of individuals	mean concentration	SD	
TSA		84.86	19.37		53.63	8.47	p<0.001*
TP	61	7.20	0.46	84	7.36	0.56	NS
TSA/TP		11.88	2.97		7.32	1.21	p < 0.001*
AAT		236.56	141.53		219.34	47.38	NS
CER		28.25	11.01		29.56	6.33	NS

TSA – total sialic acid, TP – total protein, TSA/TP – total sialic acid normalized to protein, AAT – alfa-1-antitrypsin, CER – ceruloplasmin, SD – standard deviation, NS – no significance, p>0.01.

Table 2. Comparison of mean TSA, TSA/TP, CER, AAT values from the sera of patients with larger intraocular melanomas (diameter>15mm, height >5mm) and of patients with smaller intraocular melanomas (diameter<15mm, height <5mm) (mean±SD and p values)

	Patients with larger intraocular melanomas			Patients with smaller melanomas			
	number of patients	mean concentration	SD	number of patients	mean concentration	SD	
TSA	36	89.0	19.26	25	78.89	18.29	p < 0.005*
TP		7.18	0.48		7.23	0.44	NS
TSA/TP		12.48	2.91		11.02	2.89	NS
AAT		256.89	174.53		207.28	64.37	NS
CER		28.48	12.49		27.91	8.67	NS

TSA – total sialic acid, TP – total protein, TSA/TP – total sialic acid normalized to protein, AAT – alfa-1-antitrypsin, CER – ceruloplasmin, SD – standard deviation, NS – no significance, p>0.01.

The TSA/TP values from sera of intraocular melanoma patients treated with brachytherapy were significantly increased (p<0.005) when compared to melanoma patients not treated this way. No significant difference in AAT and CER values from sera was observed between these two groups (Table 3).

Table 3. Comparison of mean TSA, TSA/TP, AAT, CER, values from the sera of intraocular melanoma patients treated with brachytherapy and patients not treated this way (mean±SD and p values)

	Patients not treated with brachytherapy			Patients treated with brachytherapy			
	number of patients	mean concentration	SD	number of patients	mean concentration	SD	
TSA	36	81.76	16.36	25	90.30	22.68	NS
TP		7.27	0.50		7.06	0.39	NS
TSA/TP		11.28	2.24		12.94	3.60	$p< 0.005$*
AAT		237.97	176.97		244.04	66.73	NS
CER		26.86	13.74		32.52	8.64	NS

TSA – total sialic acid, TP – total protein, TSA/TP – total sialic acid normalized to protein, AAT – alfa-1-antitrypsin, CER – ceruloplasmin, SD – standard deviation, NS – no significance, $p>0.01$.

DISCUSSION

One of the contemporary problems of clinical oncology is the search for suitable markers of malignant diseases, for prognosis of the disease and evaluation of the tumor--host relationship. Serologic tumor markers have been valuable in the diagnosis, management and follow-up of a patient with cancer. There are relatively few reports in the literature concerning clinical laboratory studies on patients with metastatic uveal melanoma (2, 3, 4). Higher levels of sialic acid were found in the serum of patients with uveal melanoma and metastatic disease than in control group and in patients with choroidal melanoma without clinical evidence of metastases (3).

Sialic acids, a family of acylated derivatives of neuraminic acid usually occur as the terminal component of carbohydrate chains of glycoproteins and glycolipids (12). Melanomas have an increased concentration of sialic acid on the tumor cell surface. The altered carbohydrate compositions of malignant melanoma cell surface may contribute to aberrant cell-cell recognition, cell adhesion, antigenicity and the invasiveness demonstrated by malignant cells. Sialoglycoproteins and gangliosydes are shed or secreted by some of these tumour cells which increases the concentration of sialic acid in the blood (6, 7, 11, 14).

Reported elevations of TSA in melanoma patients may be a result of sheding tumor related membrane sialoglycoprotein and is caused by concomitant elevation of non-specific acute phase reactant glycoprotein level. Several investigators identified the importance of TSA in the staging, prognosis and early detection of recurrence of malignant diseases (14). No apparent correlation was observed between the values of sialic acid and specific organs affected with malignant disease occurrence (11) Silver et al. have

shown that there is a strong correlation between serum sialic acid and tumor burden in malignant melanoma (13,14). In hamsters with implanted Bomirski melanoma, TSA level reliably reflected the progression of the disease (17). This might explain the usefulness of exploitation of the total serum sialic acid estimation as a non-specific marker of melanoma development both in human and animal melanoma. Plucinsky showed that TSA and TSA normalized to TP (TSA/TP) were significantly increased and TP decreased in patients with melanoma. His results indicated that TSA/TP ratio was the most useful of the markers tested for detection of malignancies (11). In the present investigation TSA serum level and TSA normalized to TP in patients with intraocular melanoma was significantly higher than in normal controls. It was also higher in patients with larger primary intraocular melanomas than in patients with smaller melanomas. However, none of the patients demonstrated clinical signs of metastases. Silver et al. in their investigations acknowledged that serum sialic acid level is a nonspecific reflection of increased cycle activity (13). According to these authors significant sialic acid elevations in the group of patients who had no clinical evidence of metastatic disease at the time of sampling suggest that there is a more specific relationship. It is difficult to explain the appearance of sialic acid elevations in this group of patients unless we assume that small foci of inactive malignant cells remained after surgical treatment. The fact that metastases were not clinically manifested may be explained by an active immune system capable of dealing with a very small tumor burden. Subclinical metastases may be more common in intraocular melanoma patients than is currently suspected, particulary in patients with large tumors. In our study TSA/TP levels were higher in patients after brachytherapy (radioactive plaque placed on sclera above intraocular melanoma) while TSA concentration was not significantly higher. It is difficult to explain this fact. However, since the sialic acid level appears to be related to tumor mass and can reflect a very small tumor burden of melanoma this assay could provide a method for monitoring response to therapy in individual patients especially when sequential measurements are done (14). Combined use of multiple markers is found to be more useful than any simple marker. Alfa-1-antitrypsin and ceruloplasmin are significantly increased in the serum of patients with malignancies but similar modifications could be observed in a variety of pathological conditions including infectious diseases or cardiovascular disorders (1). In their study Silver et al. have shown that AAT correlated with melanoma burden, however increased serum sialic acid was more frequently seen than acute phase proteins elevations (13). Several studies have shown the ceruloplasmin concentrations are increased in various carcinomas including melanoma (8, 16). Vachtenheim detected elevated levels of ceruloplasmin in sera of hamsters with melanoma when compared to normal controls (16). Higher levels of ceruloplasmin found in cases of malignant tumors are probably due to resialilation of desialylated ceruloplasmin either on the surface of malignant cells or in the blood (5). The majority of patients who die of metastatic choroidal melanoma have liver metastases, which is often first indicated by abnormal serum liver enzyme levels and liver scans (4). In our study no significant difference in alfa-1-anti-

trypsin and ceruloplasmin levels were found in the group of patients with intraocular melanoma and normal controls. It seems that determining of TSA and TSA/TP values in blood serum is more sensitive than alfa-1-antitrypsin and ceruloplasmin levels. Tumor markers are not expected or needed to assist in the primary diagnosis of choroidal melanoma because there are better methods such as ophthalmoscopy, ultrasonography, computerized tomography and MRI. However, the intraocular lessions are not easily accessible to tissue sampling because of the possibility of dissemination of metastases. Undoubtedly, a reliable system of tumor markers to provide evidence of metastases or early warning of tumor growth would be of help in planning the treatment for the individual patient, in particular the necessity for, and the timing of adjuvant therapy. Sensitive nonspecific markers like TSA may be valuable in the long-term follow-up of patients at a risk of recurrence and the presence of occult metastases. Such markers may also be useful in evaluating patients after surgery and radiation therapy. However, more frequent serial studies that are more sensitive and more specific indicators of early metastatic disease are needed in view of changing concepts in the treatment of patients with uveal melanoma. Additional studies to assess the potential role of TSA in evaluating response to therapy and long-term follow-up of patients with melanomas and hamsters with Bomirski melanoma are ongoing.

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SUMMARY

Melanoma of the choroid is a fatal disease because of its metastases. The quest is ongoing for more reliable serum markers for detecting and staging ocular melanoma. Total serum sialic acid and acute phase proteins are valuable adjuncts in the management of malignancies, including melanoma. The aim of the paper was to assess the level of total sialic acid (TSA), total sialic acid to total protein (TP) ratio (TSA/TP) and the level of alfa-antitrypsin (AAT) and ceruloplasmin (CER) in patients with choroidal melanoma.

The concentrations of TSA, TP, AAT and CER were evaluated in 61 patients with choroidal melanoma and 84 healthy controls. 36 patients had larger tumors and 25 patients had smaller melanomas. 36 patients were treated with brachytherapy. The mean concentration of TSA in all intraocular melanoma patients was 84.86±19.37 mg/dl and was significantly higher than in control group 53.63±8.47 mg/dl ($p < 0,001$). TSA level was significantly higher in patients with large tumors than in those with smaller choroidal melanomas. There were not differences between groups of patients treated with brachytherapy and those not treated. TSA/TP in melanoma patients was 11.88± 2.97 and it was higher than in control group 7.32±1.21 ($p< 0.001$). AAT level was 236.56±141.53 mg/dl

in the group of melanoma patients and in the control group was 226.42±46.74 mg/dl but the differences were not statistically significant. The concentration of CER in the study group was 28.25±11.01 mg/dl and in the control group it was 29.56+6.33 mg/dl but the differences were not statisticaly significant.

The assessment of TSA in blood serum may be useful in evaluation of patients with choroidal melanoma.

Poziom całkowitego kwasu sialowego, alfa-antytrypsyny i ceruloplazminy w surowicy krwi chorych na czerniaka błony naczyniowej gałki ocznej

Czerniak błony naczyniowej oka jest chorobą o złym rokowaniu z powodu występowania przerzutów. Trwają poszukiwania odpowiednich markerów nowotworowych surowicy krwi, które mogłyby być pomocne w ocenie klinicznej chorych z czerniakiem błony naczyniowej. Celem pracy było zbadanie poziomu całkowitego kwasu sialowego (TSA), stosunku całkowitego kwasu sialowego do białka całkowitego (TSA/TP) oraz poziomu alfa-antytrypsyny (AAT) i ceruloplazminy (CER) u chorych na czerniaka błony naczyniowej gałki ocznej. Metoda: stężenia TSA, TP, AAT i CER były badane u 61 chorych na czerniaka błony naczyniowej. Grupę kontrolną stanowiły 84 zdrowe osoby. U 36 chorych stwierdzano obecność dużych guzów wewnątrzgałkowych, a u 25 mniejszych guzów. 36 chorych było leczonych brachyterapią. Wyniki: Średnie stężenie TSA u wszystkich chorych wynosiło 84,86±19,37 mg/dl i było wyższe w sposób istotny statystycznie niż w grupie kontrolnej grup 53,63±8,47 mg/dl ($p < 0,001$). Poziom TSA był wyższy u chorych z większymi czerniakami wewnątrzgałkowymi niż u chorych z mniejszymi guzami. Nie wykazano różnicy poziomów badanej substancji między grupą leczonych brachyterapią oraz nieleczonych w ten sposób. Średnie stężenie AAT wynosiło w grupie chorych 236,56±141,53 mg/dl, a w grupie kontrolnej 226,42±46,74 mg/dl, ale różnice nie były istotne statystycznie. Poziom CER w grupie chorych wynosił 28,25±11,01mg/dl, a w grupie kontrolnej 29,56+6,33 mg/dl, ale różnice nie były istotne statystycznie. Wnioski: badanie poziomu TSA w surowicy krwi u chorych na czerniaka błony naczyniowej może być użyteczne w ocenie klinicznej chorych.